RECEIVED AUG 1 5 2002

Ozone
Crops

Executive Summary

Ozone was petitioned for use as a gas that is injected into soil under plastic mulch for weed control. An additional request was made for use as an antimicrobial agent to clean irrigation lines. Ozone may also be used to treat soil for soil borne pathogens, and this was also considered in this review. In all these types of use ozone gas (O_3) is generated on-site using an electrically powered corona discharge ozone generator.

Ozone is a bluish explosive gas or blue liquid. It is found naturally in the atmosphere at sea level contains an ozone concentration at very low levels, but is also an air pollutant and a component of smog, reaching tenfold or higher levels in cities at times. Although it is a pollutant and health hazard in the lower atmosphere, naturally occurring ozone is produced in the outer atmosphere by the photoreaction of solar ultraviolet (UV) radiation on oxygen protecting the earth from excessive radiation.

Ozone decomposes spontaneously in water and is a very reactive oxidizing agent with a short half-life. It is used to disinfect water and to oxidize color and taste contaminants in water. It is also increasingly used for disinfection purposes of food and food contact surfaces and is permitted by the National Organic Standards for use in organic processing (including post harvest handling) with no restrictions.

Two reviewers felt that ozone should be permitted for use in organic crop production, though limited to use for cleaning irrigation lines, weed control and for soilborne pathogen control. One of the reviewers in favor of use found that this type of usage is a relatively new technique with unreliable results for pathogen control, and noted some reservations regarding possible surface crusting and loss of soil structure when used for weed control. One reviewer objected strongly to use of a "a known and problematic air pollutant" in organic farming and described hazards to workers and those downwind of application, negative impact on soil humic acid fraction, plant damage, and lack of evidence of effect on soil microorganisms. This reviewer did not object to use to treat irrigation water when ozone can be recaptured to prevent off-gassing into the environment.

Summary of TAP Reviewer's Analyses[1]

Synthetic/ Nonsynthetic	Allow without restrictions?	Allow only with restrictions?
Synthetic (3-0)	No (3) Yes (0)	Yes (2) No (1)

Identification

Chemical Names: Ozone, triatomic oxygen, O_3

Other Name: Trioxygen

Trade Names: SoilZone, Triox

CAS Number: 100028-15-6

Other Codes:
NIOSH RTECS #RS8225000

Characterization

Composition:
Ozone (O_3) is triatomic oxygen.

[1] *This Technical Advisory Panel (TAP) review is based on the information available as of the date of this review. This review addresses the requirements of the Organic Foods Production Act to the best of the investigator's ability, and has been reviewed by experts on the TAP. The substance is evaluated against the criteria found in section 2119(m) of the OFPA [7 USC 6517(m)]. The information and advice presented to the NOSB is based on the technical evaluation against that criteria, and does not incorporate commercial availability, socio-economic impact, or other factors that the NOSB and the USDA may want to consider in making decisions.*

Properties:

Ozone is a bluish, explosive gas or blue liquid. It has a characteristic pungent odor that is detectable at concentrations as low as 0.02 to 0.05 ppm. At greater concentrations it is irritating to eyes and the respiratory tract and at high concentrations ozone may be fatal. It is a strong oxidizing agent, mp -193° C, bp -111.9° C. It is sparingly soluble in water. At 20° C, solubility of 100 percent ozone is 570mg/L (Richardson, 1994).

Atmosphere at sea level contains an ozone concentration of about 0.05 ppm (Budavari, 1996). In cities with smog conditions ozone concentration may reach 0.5 ppm or higher at times. (Francis, 1997) Ozone decomposes spontaneously in water (US EPA, 1999). The reaction generates hydroxyl free radicals, which are very reactive oxidizing agents but have a half-life of microseconds. In aqueous solution, ozone can react by direct oxidation of compounds or can oxidize compounds by hydroxyl free radicals that are produced during ozone decomposition.

How Made:

Ozone is usually formed by combining an oxygen molecule with an oxygen atom in an endothermic reaction. Naturally occurring ozone is produced in the outer atmosphere by the photoreaction of solar ultraviolet (UV) radiation on oxygen. At ground level, ozone may be produced by reactions caused by changes in entropy, e.g. water falling on rocks in a waterfall. Ozone is also produced by photoreactions with nitrogen oxides (NO_x) and volatile organic compounds (VOC) from industrial emissions, vehicles and other sources (US EPA, 1999).

Because ozone is unstable it is generated at the point of use. It can be generated by irradiating oxygen-containing gas with UV light and other technologies but the primary industrial method is by the corona discharge method. The oxygen containing gas is passed through two electrodes separated by a dielectric and a discharge gap. When voltage is applied to the electrodes, electrons flow across the gap and provide energy for the disassociation of oxygen molecules, which leads to the formation of ozone (US EPA, 1999).

There are generally four system components to an ozone generating process: a power source or ozone generator, a gas source, an ozone delivery system and an off-gas destruction system. The gas source may be air, high purity oxygen or a combination of the two (US EPA, 1999). Air feed systems are more complicated than liquid oxygen feed systems because the air must be clean, dry, free of contaminants and with a maximum dew point of -60° C to prevent damage to the generator.

Specific Uses:

Ozone has been used in Europe to treat drinking water for more than 100 years (US EPA, 1999). Ozone in the United States has been used to disinfect water and to oxidize color and taste contaminants in water. It is increasingly used for disinfection purposes.

The petitioned use is for the use of ozone for weed control (Pryor 2001) with an additional request for use as an antimicrobial agent to clean irrigation lines as an alternative to chlorine (Herman 2002). In addition, the use of ozone for control of soil borne pathogens will be considered in this review. In all these types of use ozone would be generated on site.

Ozone gas for weed control is used in combination with plastic mulch and is applied in a gaseous form. The target treatment area is the space between the plastic mulch and either the drip irrigation tubing if it is buried or the soil surface if drip tubing is not buried. Ozone is applied under the mulch before the crop is planted. It has also been applied once the crop is in place (Pryor, 1999; Pryor, 2001). It may be applied through drip tape, which can later be used for crop irrigation. Ozone oxidizes plant tissue and weakens or kills emerging weeds. Ozone treatment for weed control may be used in combination with soil solarization. As described in the petition, ozone for weed control may be applied at rates of 2 lbs/acre with a total number of applications ranging from 7-30 depending on weed species.

Ozone uses for control of soil borne pathogens has been tested at rates ranging from 50-400 lbs per acre (Pryor, 1999). It can be applied through drip tubing under plastic mulch or by various methods of direct injection (Pryor 1996, 1997).

Ozone can be used to treat or prevent clogged drip irrigation systems by at least two methods. Recycled irrigation water can be treated with ozone before reuse. (NIDO, 1997) A requested additional use is to inject ozone into the irrigation lines to act as an antimicrobial agent (Herman 2002). This seems to be a fairly new use with little information to describe the method. One industry writer reports that the gas is generated on site in a closed system and dissolved in water under pressure, and that undissolved gas is collected and disposed of by means of a special separator to avoid accumulation of gas bubbles in the system (Hassan, undated).

Action:
Ozone is a strong oxidizing agent and very corrosive. In plants, it can cause membrane lysis and necrotic lesions. It may affect photosynthesis and generally represses various genes (Sandermann, 1996). It is germicidal against a wide range of organisms including bacteria, viruses and protozoa. In bacteria, it attacks the bacterial membrane, disrupts enzymes and affects nucleic acids (EPA, 1999). In viruses, ozone modifies the viral capsid and may break the protein.

Combinations:
Not sold in combinations.

Status

Historic Use:
Historically ozone has been used to disinfest and oxidize pathogens and contaminants from drinking water. It was first used in the Netherlands in 1893. Ozone was used in Los Angeles, California in 1987 to treat drinking water and by 1998, 264 water treatment plants in the U.S. were using ozone (US EPA, 1999). Since the implementation of the Surface Water Treatment Rule the use of ozone for primary disinfection of water has increased (EPA, 1999). Use as a soil treatment to kill living organisms is a relatively recent invention (Pryor, 1996).

OFPA, USDA Final Rule:
Ozone is listed for use in post-harvest handling and processing (7 CFR 205.605(b)(20). It could be considered a production aid under 7 USC 6518(c)(1)(B)(i).

Regulatory: EPA/NIEHS/Other Sources
The EPA sets standards for ozone levels under the National Ambient Air Quality Standards as required by the Federal Clean Air Act. EPA considers ozone producing equipment to be 'pesticidal devices.' Ozone generation is subject to pesticide worker safety requirements (40 CFR 170).

Ozone is subject to the National Primary Drinking Water Regulations under the Safe Drinking Water Act because it is used as a disinfectant in water treatment to kill pathogens. (40CFR 141.65)

FDA considers ozone to be GRAS as a direct food additive and allows the use of ozone as an antimicrobial agent for bottled water and food processing (21 CFR 184.1563). Bottled water maximum residual permitted ozone level is 0.4 mg/l at bottling.

OSHA: 29 CFR 1910.1000 Subpart Z
Transitional Limit: PEL-TWA 0.1 ppm
Final Limit: PEL-TWA 0.1 ppm; STEL 0.3 ppm
ACGIH: TLV-Ceiling Limit 0.1 ppm
NIOSH Criteria Document: None
NFPA Hazard Rating: Health (H): None
Flammability (F): None
Reactivity (R): None

Status Among U.S. Certifiers
California Certified Organic Farmers (CCOF) -CCOF Certification Handbook (rev. January 2000). Not specifically listed.
Maine Organic Farmers and Gardeners Association (MOFGA) -MOFGA Organic Certification Standards 2001. Not specifically listed.
Midwest Organic Services Association (MOSA) -MOSA Standards January 2001. Not specifically listed.
Northeast Organic Farming Association of New Jersey (NOFA-NJ) - NOFA-NJ 2000 Organic Certification Standards. Not specifically listed.
Northeast Organic Farming Association of Vermont (NOFA-VT) - 2001 VOF Standards. Not specifically listed.
Oregon Tilth Certified Organic (OTCO) - OTCO Generic Materials List (April 30, 1999). Not specifically listed.
Organic Crop Improvement Association International (OCIA) -OCIA International Certification Standards, July 2001. Not specifically listed.
Quality Assurance International (QAI) - QAI Program, Section 5.2 Acceptable and Prohibited Materials. Not specifically listed.

Texas Department of Agriculture (TDA) Organic Certification Program – TDA Organic Certification Program Materials List. Not specifically listed.
Washington State Department of Agriculture Organic Food Program – Chapter 16-154 WAC Organic Crop Production Standards. Not specifically listed.

International

CODEX – Not specifically listed.
EU 2092/91 – Not specifically listed.
IFOAM – Not specifically listed.
Canada – Not specifically listed.
Japan – Not specifically listed.

Section 2119 OFPA U.S.C. 6518(m)(1-7) Criteria

1. *The potential of the substance for detrimental chemical interactions with other materials used in organic farming systems.*
As a strong oxidizing agent, ozone has the potential to react with many different substances. Ozone oxidizes pesticides, organic matter, and reacts with iron and most other materials. Ozonation of water produces various by-products such as aldehydes, ketones, carboxylic acids, organic peroxides, epoxides, nitrosamines, N-oxy compounds, quininones, hydroxylated aromatic compounds, brominated organics and bromite ion. (Kirk-Othmer, 1996)

When ozone is used for weed control, it is applied directly to the space between the buried drip irrigation tubing or the soil and the plastic mulch. It is not clear how much ozone diffuses into the soil in this system but Qui, et al. (2001) found that the ozone mass transfer rate was influenced by soil moisture and texture. An early study found that ozone applied as gas at 0.5 ppm did not penetrate the soil to a statistically significant extent (Blum and Tingey, 1977). More recent work examined the effect of ozone on soil organic matter when ozone is used to decontaminate soil . In a system where a soil extract was ozonated, researchers found a decrease in the humic acid fraction, a reduction of the average molecular size, and an increase in the low molecular acid fraction. The low molecular acid fraction is readily degradable by microorganisms (Ohlenbusch et al., 1998).

In lab studies ozone caused reduction in respiration rates of ectomycorrhizal fungal mats. However when these fungi were associated with their host plant roots the ectomycorrhizal roots were more resistant to ozone than non-ectomycorrhizal roots (Garret et al., 1982). In laboratory studies soil nematode populations of Meloidogyne javanica and free living nematodes were significantly reduced by ozone treatment and were dosage and flow rate dependent (Qui et al., 2001). In other research, ozone treatment of Easter lily bulbs did not reduce nematode numbers (Giraud et al., 2001) although it did give a positive yield response. In field experiments with tomatoes, Pryor (2001b) found that ozone treatments did not significantly reduce nematode populations, but may have led to increased yields in some cases.

Ozone is used for water treatment because it oxidizes or disinfects many components that impact water quality. It will oxidize iron and manganese which precipitate as ferric and manganese hydroxides. This could result in crop iron deficiencies (von Broembsen, 2002.). It partially oxidizes organic matter to forms that are more easily biodegradable. Ozone is also germicidal against many types of pathogenic organisms including viruses, bacteria and protozoa (US EPA, 1999). Ozone itself does not remain as a residual in irrigation water because of its rapid decomposition. It does form a variety of byproducts in reaction with organic matter. It can also react with the bromide ion if present to form brominated disinfection byproducts (US EPA, 1999). The ozone will most likely oxidize any materials that a grower injects into the irrigation lines at the same time as the ozone. For example, if growers inject fertilizer such as fish emulsion or other material into the irrigation system, ozone will oxidize the material. The extent would depend on the concentration of the added material, the concentration of the ozone and the contact time.

2. *The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.*
Ozone is a strong oxidant and is inherently bioreactive. Given its reactivity and relative concentration, it is the oxidant of primary concern in photochemical smog (Klaasen, 2001).

Ozone is rated as a high irritant via inhalation and to skin, eyes and mucous membranes. It also affects the central nervous system and there are mutation data and reproductive concerns. (NTP 2002, NJ 1996) Higher exposure can cause headache, upset stomach, vomiting, and pain or tightness in the chest. Ozone can irritate the lungs causing coughing and/or shortness of breath. Higher exposures can cause a build-up of fluid in the lungs (pulmonary edema), with severe shortness of breath. Liquefied ozone on contact with skin or eyes can produce severe burns. There is

limited evidence that ozone causes cancer in animals. It may cause cancer of the lung, mutations (genetic changes) and may damage the developing fetus. (NJ 1996, Richardson 1994)

NTP Toxicity

Type of dose	mode	specie	amount	units
LC50	ihl	cat	34,500	ppb/3H
LC50	ihl	gpg	24,800	ppb/3H
LC50	ihl	ham	10,500	ppb/4H
LCLo	ihl	hmn	50,000	ppb/0.5 H
TCLo	ihl	hmn	100,000	ppb/0.016 H
TCLo	ihl	hmn	1,000	ppb

Source: NTP, 2001

Abbreviations
LC50 = lethal concentration 50 percent kill
LCL = lowest published lethal concentration
TCL = lowest published toxic concentration
H = hour
ihl = inhalation hmn = human gpg = guinea pig ham = hamster

Eco Toxicity (Richardson 1994):
Fish – LC 50 (96 hr) rainbow trout 9.3microg/l,
LC 50 (24 hr) bluegill sunfish 0.06 mg/l

Invertebrate – Bacteria species showed change in phospholipid levels after 30 sec. aeration with 1mg/l. *Euglena gracilis* had damaged plasma membranes. Enzyme deactivation in yeasts was found.

In plants, it can cause membrane lysis and necrotic lesions. It may affect photosynthesis and generally represses various genes (Sandermann, 1996). It is germicidal against a wide range of organisms including bacteria, viruses and protozoa. In bacteria, it attacks the bacterial membrane, disrupts enzymes and affects nucleic acids (US EPA, 1999). In viruses, ozone modifies the viral capsid and may break the protein.

When ozone is applied beneath plastic mulch for weed control its mode of action is in part by direct oxidation. It is taken up by the plant stomata where it is decomposed in the apoplast. Ozone effects chloroplast function and nuclear gene expression by mechanisms that are not understood at this time. Membrane lysis is thought to be a later effect of ozone (Sandermann, 1996). The ozone would also be in contact with soil. The amount of soil affected depends in part on the depth of the placement of the drip irrigation lines. Ozone oxidizes the soil humic acid fraction of organic matter (Ohlenbusch et al., 1998).

When ozone is applied under plastic the area of concentration is the zone between the drip irrigation tubing or soil surface and plastic mulch. When ozone is in contact with organic materials such as plants, its half-life is a few minutes. Potential concern would be for worker safety during the application of the ozone and any leaks in the system. The half-life of ozone in ambient air is 12 hours (Pryor 2001). Ozone's only decomposition product is oxygen.

In water there are two modes of action by ozone, direct oxidation and oxidation by hydroxyl free radicals. It oxidizes organic matter, attacks bacterial membranes, disrupts enzymatic activity, disassociates viral capsids and attacks RNA.

In water ozone decomposes rapidly and the only residual is dissolved oxygen. However decomposition by products may be present. If the bromide ion is present in water brominated decomposition products may remain. Formation of aldehydes has also been found as a result of ozone disinfection (Liberti and Notarnicola, 1999) Some of the disinfectant by products are potentially toxic or carcinogenic, however bioassay screening studies have shown that ozonated water induces substantially less mutagenicity than chlorinated water. (Kirk Othmer, 1996) Ozone does not form halogenated by products (trihalomethanes) when reacting with natural organic matter in water, unless bromide ion is present in the raw water. (US EPA 1999)

Disinfection and chemical oxidation rates by ozone are relatively independent of temperature (EPA, 1999). If recirculated irrigation water is treated with ozone, the excess ozone must be scrubbed to prevent release to the atmosphere and to protect workers from ozone exposure.

3. The probability of environmental contamination during manufacture, use, misuse, or disposal of the substance.

Ozone at ground level is considered a priority air pollutant by US EPA. Ozone would be generated on site both for use in soil treatment and as an antimicrobial agent in irrigation systems. Ozone is not stored on site. Because ozone is toxic care must be taken to avoid leaking of ozone from the system during generation. Levels of 1ppm for 30 minutes or more produce headaches. OSHA's maximum permissible exposure level (PEL) to ozone is not to exceed 0.1 mg/L by volume averaged over an 8 hour period.

During water treatment ozone gas is transferred to water. In treating recycled irrigation water, ozone that is not transferred to the water is released as off gas. The concentration of ozone in the off gas of these systems is above the concentration fatal to humans and may contain as much as 3,000 ppm ozone (US EPA, 1999). Off gas containing ozone should be captured and converted to oxygen before release into the atmosphere. Ozone systems that inject directly into the irrigation lines use much lower concentrations of ozone and do not treat off gas.

4. The effects of the substance on human health.

Ground level ozone may reach levels that are harmful to human health. Most of the studies regarding ozone as a threat to human health are related to ozone as an air pollutant generated by automobile exhaust and other fossil fuel generated sources (US EPA, 1999).

Acute Toxicity. High concentrations above 0.1 mg/L by volume average over an 8 hour period may cause nausea, chest pain, reduced visual acuity and pulmonary edema. Inhalation of > 20 ppm for at least an hour may be fatal.

Chronic effects. May have deleterious effects on the lungs and cause respiratory disease. See response to criterion number 1.

5. The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.

The effects are mainly the immediate result of ozone's strong oxidizing capacity. Ozone is a broad-spectrum biocide that can oxidize soil organic matter and other substances in soil (Ohlenbusch et al., 1998). Ozone does not persist in soil with either the weed control or water treatment system application. It is converted to oxygen within a short period of time. The issue is what, if any, are the remaining impacts of ozone use.

When ozone is used for weed control, the ozone is in contact with the soil, soil organic matter and microorganisms. It has been shown in the laboratory that ozone can oxidize the soil humic acid fraction into lower molecular weight fractions which are more biologically available to soil microorganisms (Olenbusch, 1998). This research found that bacterial regrowth increased with ozonation time. The effects on the populations of other soil microorganisms were not examined in this research.

Other research has shown that ozone does reduce populations of at least some other soil microorganisms such as some nematodes while other nematodes appear unchanged (Qui et al., 2001 and Giraud et al., 2001). Soil injection at 250 lb/acre rate resulted in increases of yield of tomatoes comparable to chemical fumigants in one year, although it did not statistically reduce root galling by nematodes. (Pryor 1999). Yield increases were theorized to have resulted from other biological effects, possibly increase in nutrient availability. Conventional farmers use soil fumigation with methyl bromide to achieve large increases in yield in crops such as carrots, tomatoes and strawberries although the increases are not linked to specific elimination of known pathogens. A study of the populations of the different strains of the fungi *Fusarium* in organic (treatments used cultural methods) and non-organic farming systems (treatments used the fumigant *Telone*) found that the greatest number of pathogenic strains were recovered from the organic farm, however no plants at the organic site showed any symptoms while plants on the conventional site did show symptoms. In addition, the organic site was found to exhibit more than twice the number of non-pathogenic strains of Fusarium which have been shown to reduce the incidence of Fusarium wilt (Bao, 2000).

The availability and form of soil organic matter affects a broad spectrum of soil chemical and microbiological reactions. Soil organic matter influences cation exchange capacity, soil buffering, soil microorganism population dynamics, and plant disease among other aspects of the soil environment (Brady, 1974, Engelhard, 1989).

If the crop is present when ozone is applied there can be physiological impacts such as burning on the crop (Pryor, 1999). It appears that when plants are exposed to ozone it elicits plant responses that are similar to plant responses to pathogens. These responses to ozone are just beginning to be understood (Sandermann, 1996). Ozone is a known air pollutant that causes crop damage (Mersie 1990, Hatzios 1983), and in event of a leak in application method can cause crop loss (Pryo 1999).

Ozone that is used to treat water before it is injected into the irrigation lines does not come in contact with the soil or crop plants. The ozone off gas is recycled or converted to oxygen so that it is not released to the atmosphere. The reaction of ozone with the bromide ion or organic matter that may be in the water can create decomposition by products. No information was found on the potential impact of these on the soil environment when irrigation water is used. The decomposition byproducts of ozone treatment appear to be of less concern than the decomposition byproducts of chlorine treatment although brominated decomposition byproducts may be of health concern (von Broembsen. 2002, EPA, 1999, Braghetta 1997).

When ozone is injected with water into irrigation lines to clean them, there is the potential that some ozone will move from the irrigation lines to the soil or air. No information has been found that examined this question. In actual practice the grower must monitor the system to determine that enough ozone has been injected to reach throughout the irrigation line before it has been completely consumed by oxidation reactions.

6. The alternatives to using the substance in terms of practices or other available materials.

There are various weed control methods available to organic growers and in general growers need to use a variety of techniques to achieve effective weed control. Some of the methods include: flame throwers, mulch, cultivation, water management, bioherbicides, steam treatment and soil solarization (Smith et al., 2000 and Boyette et al., 1999).

Soil solarization is a technique that could be used alone or in conjunction with ozone or other material like cabbage residue (Chellemi et al., 1997). It can be used both for weed and pathogen control. New heat-retentive films are more effective at raising soil temperatures during solarization (Chase et al., 1999). *Cyperus* spp. (nutsedge) are particularly difficult weeds to control. Recent research showed that soil temperature of 45° C was not lethal to *Cyperus spp.* tubers (Chase, Sinclair and Locascio, 1999). Temperatures of 50 - 55° C were 100% lethal to tubers. The new heat retentive films were more effective at killing *Cyperus rotundus.*

Alternatives for control of soil borne pathogens include crop rotation, solarization, use of disease suppressive compost, other organic nitrogen amendments, biocontrol, and IPM methods. A recent compendium of a 2000 EPA meeting report lists 117 papers on alternatives to methyl bromide, including many tests of biocontrols and cultural methods (US EPA 1997, 2000; Bull 2000). One-year rotations out of strawberries increased subsequent strawberry yields by 18-44% relative to continuous strawberries (Duniway 2000). Varieties more suited for organic production are also identifiable, for instance the 'Camarosa' variety is significantly more susceptible to *Verticillium* than 'Chandler' or 'Selva' (Duniway, 2000.) Existing organic production techniques are considered to adequately control soil borne pathogens, and result in slightly lower yields that are offset by higher prices (US EPA 1996). Use of strawberry plant plugs rather than bare root resulted in earlier production, less transplant wounding, increased vigor and offset problems from soil born pathogens (Sances, 2000.)

Current potential alternatives to the use of ozone as an antimicrobial in irrigation systems include chlorine, acetic acid, and citric acid (OMRI, 2001). Ozone is a stronger oxidizing agent than all of these. Ozone by itself and in water does not form trihalomethanes, which are carcinogenic (US EPA 1999) Chlorine treatment forms trihalomethanes.

If a grower wishes to removed pathogens and particulate from their water source, slow sand filtration would be an alternative (Wohanka, 1995). Slow sand filtration is a water treatment system that has been used for more than 100 years. Untreated water filters slowly through a fine sand bed. A skin of organic and inorganic material and microorganisms begins to form on the surface of the sand bed. The biological activity of this area extends through the upper region of the bed. This method has been effective against several pathogens including *Cylindrocladium spp.*, pythiaceaeous fungi, *Verticillium dahliae* and others (Wohanka, 1995).

There are certain situations where slow sand filtration would not be an alternative to ozone use. If a grower's irrigation lines are already clogged, sand filtration is not going to correct the situation. If a grower were applying a fertilizer such as compost tea or fish emulsion through the irrigation lines, the sand filtration process would not clean the irrigation lines or keep them from clogging due to biofouling. This is because the fertilizer would need to be injected after the sand filtration step. Otherwise the sand filtration would remove the desired nutrient content. The effectiveness of ozone injected into a drip irrigation system to prevent clogged emitters is not documented, and is questionable due to the rapid decomposition of ozone in the aqueous environment into oxygen. No supporting technical literature was found to substantiate this claim, it appears to be an experimental treatment.

7. Its compatibility with a system of sustainable agriculture.

To answer this question each use should be considered separately since the target organisms and methods and rates of application are different. In addition the mode of transport for each use is different. For weed control, ozone is injected into an air-water interface in the soil or on the soil surface. For use in cleaning of irrigation lines and water treatment, ozone is injected into the water either before or as it enters the irrigation line. In general the impacts of the

use of a material should be targeted rather than widespread. Potential non-target, unintended impacts need to be considered.

Ozone for weed or soil borne pathogen is not selective with regard to the plant species that it kills. It is toxic to all plants, however different species respond differently to the same dose of ozone (Hatzios and Yang, 1983, and Sandermann, 1996). It is applied in a defined space, the area between the buried drip irrigation tubing or the soil surface and the plastic mulch (Pryor, 1999). It is a very strong oxidant and will oxidize the soil surface that it contacts. It can oxidize soil organic matter and make it more biologically available (Ohlenbusch et al., 1998). It is unclear from the references found by the reviewer how deep ozone will diffuse into the soil under the conditions of the proposed use. It was also unclear what concentration of ozone the weeds and soil would be exposed to. The petitioner claims the impact will only reach 0.25 inches when applied at rates suitable for weed control. It is very reactive, has a short half-life and does not leave a residual effect. It is destructive to a wide range of microorganisms but not all (EPA, 1999; Giraud et al. 2001; and Qui et al., 2001).

The production of ozone from oxygen is due to an endothermic reaction, and requires a considerable input of energy. The patent documents mention the presence of a generator on the apparatus (Pryor 1996, 1997) but does not describe the power requirements needed, presumably supplied by diesel or gas engine. The EPA describes the voltage requirements for an air-fed corona discharge system as 5-7 kilowatts/hour/pound of O_3 produced. As much as 85% of the energy used in ozone production is lost as heat. (US EPA 1999)

When ozone is used to treat water it is reactive with a wide variety of chemicals and compounds in the water including iron, manganese and organic matter. It is also germicidal against many microorganisms such as protozoan cysts, viruses, and bacteria including *E. coli 0157:H7* (EPA, 1999 and Unal et al., 2001). It is applied to water before use in irrigation or directly injected into irrigation lines with irrigation water. When ozone is used treat water prior to irrigation, ozone concentrations are higher than when it is injected into irrigation lines to prevent biofouling. In the first instance, the system is enclosed and excess ozone is captured and recycled or converted to oxygen before it is released to the atmosphere. Typical concentrations of ozone found during water treatment are from <0.1 to 1 mg/L (EPA, 1999). When ozone is injected directly into the irrigation system, concentrations are lower. A potential problem with the second system from a purification point of view is that the ozone may be completely consumed by oxidation reactions with chemicals, microorganisms and organic materials in the line before it reaches the end of the irrigation line. Excess ozone is not captured in this system.

Additional Questions for the reviewers:

Note: The initial petitioner only requested review for purposes of weed control, and did not respond to questions requesting more information on other uses. NOSB advised that it also be reviewed for soil pathogen control.

1. Have you seen or can you find any specific mention of use of ozone injected in drip irrigation systems as a cleaning agent?
2. Does anyone have access to this reference, and can you report on it:
   Raub, L., Amrhein, C., and M. Matsumoto. 2001. The effects of ozonated irrigation water on soil physical and chemical properties. Ozone Science and Engineering. 23(1):65-76
3. Do you have any additional evidence on impact of ozone on the soil ecosystem, short or long term?
4. Have you seen any information on the effect of ozone application on soil organic matter and nutrient availability.
5. Please express your technical review, advice and conclusions distinctly on each of these uses of ozone. Is it possible to permit use for some purposes but not others? (e.g for weed control but not soil pathogens)

TAP Reviewer Discussion

Reviewer 1 [Ph.D. chemistry. Research entomologist advising growers and homeowners about pesticides and alternative pest control methods. Western US]

OFPA Criteria Evaluation

(1) *The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems*;
I agree with the criteria evaluation, with additional comment:
Since ozone is such a powerful oxidizing agent, it might attack the plastic irrigation tubing and destroy it over time. Seems like plasticizers such as dioctylphthalate in tubing would be destroyed. However, this is speculation, and no one seems to have observed this with limited ozone applications in the field.

(2) *The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment*;

I agree with the criteria evaluation.

(3) The probability of environmental contamination during manufacture, use, misuse or disposal of such substance;

I agree with the criteria evaluation, with additional comment:

The possibility of a problem increases with the size of the ozone generator. For soilborne pathogen control, amounts generated and release volumes would be higher than with the other two applications, and thus might be riskier.

If the generator is set up properly, leaks in the ozone supply line, torn or compromised plastic sheeting, and the possibility of fire are the only risks that I can think of.

(4) *The effect of the substance on human health*;

Ozone has actually been used in medicine. Amounts in plasma higher than 80 μg/ml of gas per ml of blood are detrimental (Bocci et al. 2001).

(5) *The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock*;

Ozone seems to have very little effect on soil nematodes. It seems to have more of an effect on soil bacteria than soil fungi. Treatment of strawberry fields with high rates of ozone improved colonization of *Trichoderma* when this microbial was used subsequently as an inoculant, so there must have been either an initial knockback of competing microbials or releases of nutrients favorable for *Trichoderma* sp. growth (Pryor 2001b).

(6) *The alternatives to using the substance in terms of practices or other available materials*; and

For nursery operations, steam is a practical alternative for management of pathogens. Suppressive composts are especially valuable in containerized production. Crop rotation is probably the most practical alternative for field crops (see Quarles and Daar 1996).

(7) *Its compatibility with a system of sustainable agriculture.*

One possible problem is destruction of soil organic matter. Raub et al. (2001) believed that oxidation of organic matter on the soil surface could lead to surface crusting and loss of soil structure. They suggested longterm studies to explore this possibility. Surface effects would be most likely with weed control. For weed and pathogen control there are several applications throughout a 30-day period. Amounts applied for pathogen control are 10-fold or more greater, but the ozone is applied about 3 inches deep, rather than directly on the surface. Cleaning of irrigation lines should not lead to any problem with soil structure because most of the ozone would be contained in the irrigation tubing.

Another consequence of ozonation could be release of copper ion, which is bound to organic matter. Lin et al. (2001) found that ozonation of humic acids in water degraded them to smaller molecules that were unable to chelate copper ion. In soils where Cu has been overapplied, ozonation could lead to phytotoxicity due to excess free copper.

RESPONSE TO ADDITIONAL QUESTIONS

(1) *Have you seen or can you find any specific mention of use of ozone injected in drip irrigation systems as a cleaning agent?*

I talked to [owner of a well known west coast organic farm supply company.] She has not heard of anyone cleaning irrigation lines by direct injection of ozone. She has heard of farmers treating irrigation water with ozone before it is applied to the irrigation system.

(2) *Can you find and report on this reference?:* Raub, L., Amrhein, C. and M. Matsumoto. 2001.

To check the effect of ozone on soil structure, Raub et al. (2001) applied ozonated water at 10mg/liter to 20 cm glass columns containing various California soils. They found that the ozone reacted with the humic acids and other organic material, degrading it to smaller molecules. Degradation of the organic matter released cations such as Ca+2. The organic acids and cations lowered pH of the applied water and caused clay in the soil to coagulate. Coagulation of the clay particles increased the water infiltration rate and allowed the soil columns to drain quicker. In soils with high sodium content (> 15%) the improved drainage was not observed.

Positive results other than improved drainage was improved oxygenation, and probably increased microbial activity, since the humic acid was degraded to smaller molecules that could be metabolized by microbes. Anecdotal information was presented that soil ozonation might "improve crop vigor, reduce insect and disease, enhance water penetration, and reduce fertilizer needs."

Raub et al. (2001) felt, however, that longterm studies were needed to see if oxidation of organic matter on the soil surface would lead to surface crusting and loss of soil structure.

(3) Do you have any additional evidence on impact of ozone on the soil ecosystem, short or long term?
See Larson (1999), Lin and Klarup (2001), Hayes (2000) and Pryor (2001b).

(4) Have you seen any information on the effect of ozone application on soil organic matter and nutrient availability?
Ohlenbusch et al. (1998), Raub et al. (2001) and Lin and Klarup (2001) show humic acid breakdown into smaller molecules. Pryor (2001b) showed improved soil colonization of *Trichoderma* after soil ozonation. This fact could indicate that ozone treatment made more nutrients available. Earlier reports (Larson 1999) also speculated that the ozone soil treatment increased nutrients available for crops.

(5) Please express your technical review, advice and conclusions distinctly on each of these uses of ozone. Is it possible to permit use for some purposes but not others? (e.g for weed control but not soil pathogens)
1. Use of ozone to clean irrigation lines.
Cleaning irrigation lines with ozone seems a reasonable use of the material. Ozone is already being used to treat irrigation water. It does not seem to be much of a jump to use it to clean the irrigation system.

However, if it is injected directly into the tubing and flushed with water, care must be taken to do it safely and effectively.

2. Use of ozone to control soil pathogens.
Using ozone in this manner is probably safe enough, and data presented by Pryor (2001a) shows that there will probably be few impacts on soil microflora.
However, I could not find any information on effects on earthworms.

My major concern is that the technology has not yet been optimized and may be somewhat unreliable. The problem for pathogen control is soil penetration. Best results have come in sandy soils that were irrigated with water before fumigation. Perhaps because of patchy field coverage, published field trials on ozone pathogen control give inconsistent results. When yield increases do occur, they are not directly related to the dose of ozone used. Larger application rates often give lower yields. It may be that any yield increases are due to improved nutrient availability and better biocontrol. Both of these factors could vary considerably.

In the 1997 field trials reported at a methyl bromide alternatives conference, ozone was applied through drip tubing buried about 3 inches deep to sandy pre-irrigated soil. This placed the ozone very near the root zones. With these best-case conditions there were significant yield increases with tomatoes, carrots and strawberries (Pryor 1999).

California 1998 field trials were published in Larsen (1999). Ozone soil treatment reported here gave increased yields of tomatoes, carrots, strawberries and other crops. Applications were made through drip irrigation tubing to sandy soils. Large emitters (4 gallons/hr) were used to get a large flow rate. Strawberry fields that were treated were under heavy attack of *Verticillium*. Strawberry yields increased 51% as a result of ozone treatment. Ozone application rates were 400 lb/acre.

Hayes (2000) treated strawberry fields with ozone plus the biocontrol organism *Trichoderma*. The combination treatment generally gave increased yields over controls. However, increases were smaller compared to earlier trials because standard 0.5 gallon/hr irrigation drip emitters were used. According to the author, higher ozone flow rates with the larger 4.0 gallons/hour emitters give better results, especially if you are not dealing with sandy soil.

In field trials conducted in 2000, Pryor (2001b) tried treating tomatoes with ozone for nematode control and strawberries with ozone for pathogen control. Tomatoes were treated with ozone alone, ozone +biocontrol organisms, and standard nematicides (Telone). The highest application rate of ozone gave yields lower than the controls. Modest application rates of ozone plus biocontrol microbials gave yields similar to the standard chemical Telone. Best yields were shown with biocontrol microbials alone. Only Telone gave any nematode control, but yields with Telone were lower than with microbials alone.

Strawberries were treated with ozone alone, ozone plus microbials, and microbials alone. None of the treatments significantly increased yields over controls. This report, though, was for a year when the pathogen challenge was low.

Combination of ozone plus *Trichoderma* did, however, lead to increased colonization rates of the microbial (Pryor 2001b).

Despite my concerns about reliability, the technology should be allowed. Perhaps continued use will lead to more reliable treatments.

3. Use of ozone for weed control.
Laboratory data supplied by Pryor (2001a) show that ozone should only have minor non-target impacts on the soil ecosystem. The field test by Pryor and Bayer (2001) seems to establish efficacy. If oxidation of soil organic matter causes negative longterm impacts on soil structure (Raub et al. 2001), NOSB can suspend its use.

Reviewer 1 Conclusion *- Summarize why it should be allowed or prohibited for use in organic systems.*

a. Ozone should be allowed in organic agriculture for cleaning irrigation lines. Use in this manner should not violate any of the Section 2119 Criteria. Excessive amounts should not be used so there is no appreciable off-gassing and air contamination.

b. Ozone should be allowed in organic agriculture for weed treatments. Publications cited show that it is generally effective for this purpose, and use in this manner should not violate any Section 2119 Criteria. If long term use leads to problems with soil structure, the NOSB can determine that this use should be suspended.

c. Application for pathogen control should not violate Section 2119 Criteria. I have some reservations, however, that the technique has not yet been optimized for reliable pathogen control in the field.

Reviewer 1 Recommendation Advised to the NOSB:

The substance is Synthetic

Though a case can be made for non-synthetic, since ozone is already classified synthetic in Section 205.605 of the Final Rule, it should be classified as synthetic for the cases below.

For Crops, the substance should be

Added to the National List.

Suggested Annotation, including justification:

Ozone should be added to the National List for the following applications:

1. For cleaning irrigation lines
2. For weed control
3. For soilborne pathogen control

Reviewer 2 *[Ph.D. exposure assessment-toxicology, M.S. chemistry. Certification review committee member, Eastern U.S.]*

Comments on Database

The following information needs to be corrected or added to the database:

The photochemical production of ozone in the troposphere, and the difficulties associated with minimizing its impact are not adequately represented in this document. Most ozone in the troposphere is anthropogenically-generated, and is often above 0.80 ppm in prolonged afternoon and evening episodes (Lioy and Dyba, 1989). At this concentration, decreased pulmonary function and athletic performance, increased airway reactivity and decreased (respiratory) particle clearance were found in non-smoking adults (Hobbes and Mauderly, 1991). Significant reductions on respiratory function are proportional to tropospheric ozone concentration, which is alarming, as a large segment of the US population resides in locations where the National Ambient Air Quality Standards (NAAQS) are violated for more than 100 days per year (McDonnell *et al.*, 1993).

OFPA Criteria Evaluation

(1) The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems;

I agree with the criteria evaluation

(2) The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment;

The criteria evaluation needs to be corrected or amended as follows:

I don't follow the NTP table very easily, as I don't use LC data alone.

Long-term exposure studies indicate that the primary target tissues are the nasal epithelium and the centrianinar region of the lung ((Hobbes and Mauderly, 1991). In the lower regions of the lung, where lining fluid is thin, damage to cells may be due directly to O_3 (Pryor, 1992). In higher regions, aldehydes and peroxides, which result from reactions in the lipid bilayers of the mucous lining with O_3, may be inciting damage (*ibid.*, 1992). See the section on human health (number 4) for additional human toxicity.

(3) the probability of environmental contamination during manufacture, use, misuse or disposal of such substance;

I agree with the criteria evaluation.

(4) the effect of the substance on human health;

The criteria evaluation needs to be corrected or amended as follows:

A correlation has been drawn between tropospheric summer ozone concentration and emergency room hospital visits for asthma, in four different regions of the North American continent (Cody, 1992). Healthy individuals at risk included those who exercise outdoors and who occupationally remain outdoors for much of the day, and also children, particularly in summer, when temperatures are comfortable for outdoor activities and ozone levels are at their highest. (See Database section for related comments.)

(5) the effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock;

Here is additional supporting information or comments.

A three year study of Scots pine seedlings led to the conclusion that in a relatively O_3 tolerant species, the chronic effects of O_3 exposure include growth reduction, increased needle abscission and changes in C allocation that are influenced by plant N availability (Utriainen and Holopainen, 2001).

Response to ozone in ponderosa pine was greatest when there was low nutrients supplied (Andersen and Scagel, 1997). Significant effects on below-grown respiratory activity were apparent before any reduction of total plant growth was found.

(6) the alternatives to using the substance in terms of practices or other available materials; and

I agree with the criteria evaluation

(7) its compatibility with a system of sustainable agriculture.

I agree with the criteria evaluation.

RESPONSE TO ADDITIONAL QUESTIONS

1. *Have you seen or can you find any specific mention of use of ozone injected in drip irrigation systems as a cleaning agent?*
   No
3. *Do you have any additional evidence on impact of ozone on the soil ecosystem, short or long term?*
   No.
4. *Have you seen any information on the effect of ozone application on soil organic matter and nutrient availability ?*
   See Ohlenbusch et. al 1998... I was unable to get more than the citation of the following. Also, see criterion (5).
   Anderson, C.P. Ozone stress and changes below-ground: linking root and soil processes. *Phyton.* 2000,*40*: 7-12.

5. See Conclusion.

Reviewer 2 Conclusion *- Summarize why it should be allowed or prohibited for use in organic systems.*

The use of ozone may be seriously detrimental to the health of humans who work with it, and those exposed indirectly, downwind of exposure. The use of a known and problematic air pollutant would make its consideration as a tool in organic farming questionable. One argument that is commonly submitted, utilizes that characteristic odor of O_3 as an early detection signal for avoidance. However, rapid olfactory fatigue is being overlooked, as is the tendency for workers to ignore minor, acute irritations, in order to achieve the work goal. Long-term and cumulative effects can not be ignored.

Additionally, the references provided and which I have obtained make little reference to long term effects of ozone in the soil characteristics. The effects of altering the humic acid fraction and precipitating iron oxides are significant to ban its use in soil applications, as an organic treatment. Damage to plants also is of concern, as even ozone-tolerant species are affected by ozone exposure. Further, I encountered no references in peer-reviewed work to impacts to beneficial soil organisms.

The use of ozone for (1) control of soil borne pathogens, (2) weed control, (3) to treat livestock waste for either control of pathogens or (4) to ozonate for fertilizer, should not be allowed, as the ecological and human health impact may be too high to warrant its use. Cleaning irrigation lines without recapture, should not be allowed for latter reason. However, water purification of recycled nursery or hydroponic and aquaculture systems, using the stipulation of off-gas recapture, may be reasonable, since other options for this goal often add unwanted by-products into the water stream.

Reviewer 2 Recommendation Advised to the NOSB:
The substance is Synthetic
For Crops the substance should Not Be Added to the National List.

Reviewer #3 *[Organic farmer, organic inspector, works with organic certifier. Western U.S.]*

OFPA Criteria Evaluation

For OFPA Criteria 1-3, 5-6:
I agree with the criteria evaluation

(4) the effect of the substance on human health;

I agree with the harmful effects discussed in the criteria section

I believe amendments should be added which discuss the claimed positive effects on human health. These effects fall roughly in three categories; water purification, use as a residential and office air cleanser, and use in alternative and conventional medicine. ... The health claims [made by manufacturers of ozone generating] residential air purification systems are discounted, and [consumers are] warned against their use by the American Lung Association. (ALA, 2002) [Alternative medical publications describe] the use of ozone therapy in some human diseases and in medical therapy. (Bocci, 1996, Figueras undated; Bocci et al 1994)

RESPONSE TO ADDITIONAL QUESTIONS

1. *Have you seen or can you find any specific mention of use of ozone injected in drip irrigation systems as a cleaning agent?*
Internet search turned up very few references concerning use of ozone in drip lines (Hassan, undated; Von Broembson 2002; Del Ag.2002)

3. Do you have any additional evidence on impact of ozone on the soil ecosystem, short or long term?
4. Have you seen any information on the effect of ozone application on soil organic matter and nutrient availability?
3 and 4. Discussion in criteria evaluation is sufficient. Some minor additional discussion is included in attached references.

5. *Please express your technical review, advice and conclusions distinctly on each of these uses of ozone. Is it possible to permit use for some purposes but not others? (e.g. for weed control but not soil pathogens)*
I think it is possible but difficult to separate soil application of ozone for weed control but not for soil pathogens control. The primary difference is the pounds per acre used. Appropriate record keeping may be able to track this, but since ozone is generated on site, tracking could be more difficult. Assuming honesty and integrity on the part of the producer, I believe it is difficult to justify limiting the amount of ozone used for these primary reasons:

The primary detrimental effects are how much ozone escapes into the atmosphere and how deeply the soil is sterilized. The atmospheric problem is dealt with by system design and monitoring. It is also in the producer's best interest to not waste the costly ozone. A poorly designed or maintained system for weed control could leak more than a well designed and maintained system for destroying soil pathogens. If both systems are well designed, the pollution of the atmosphere would be minimal. In practice, it is an identical technique and practice being used. The problem of how deeply the soil is sterilized is reflected in two concerns. One concern is what residues or breakdown products are left and the other concern is the effects on the soil microorganisms. Some data indicates that the breakdown products

of ozone in the soil are beneficial to the microorganisms and subsequently to the crops. The concern of how quickly microorganisms recolonize is dependent of the effects of the residues. Ozone itself does not have significant residues and its breakdown products may actually encourage both the growth and diversity of microorganisms.

Ozone treatment for soil pathogens is a possible replacement for far more toxic materials (which, ironically deplete atmospheric ozone) and its use should be encouraged from the environmental perspective. The environmental perspective is an important element of the organic industry both in producer's intention and in market expectations.

Ozone's use in the soil is a technique as well as a material that affects both weeds and microorganisms at all levels of use. If it is approved for weed control but not soil pathogen control, it will be hard to specify what level will be allowed. In some regions for some weeds, the application rate needed to be effective may also be effective for controlling some soil pathogens. On what basis should it be decided which weeds and pathogens are allowed to be controlled by this technique (and which aren't) since the technique is the same and the residues similar at all levels?

For these reasons, I think if Ozone is approved for weed control, it should also be allowed for soil treatment.

Reviewer 3 Conclusion *- Summarize why it should be allowed or prohibited for use in organic systems.*

Ozone is a highly reactive oxidizer, that leaves little residue and fewer decomposition products than other oxidizers such as chlorine. It requires a high energy input and specialized equipment to produce. It does not have a history of being used in organic agriculture. No major certification agencies make reference to it nor is it mentioned in organic production guides. Ozone's use in conventional agriculture is relatively recent and still in research and development stage, though some commercial scale farms have begun to use it. The decision to use ozone by conventional growers is based on weighing these factors; the increased costs, increased efficacy and environmental regulations. Ozone is an alternative to materials that have higher undesirable residuals such as chlorine or are being phased out such as methyl bromide.

Being highly reactive, ozone exhibits many conflicting properties depending on the concentration and on which trace materials are present. It is.a major pollutant with severe negative health effects. It is used both in alternative and conventional medicine in therapy and also in large scale water purification systems designed for human consumption.

As a TAP reviewer with a farmer's perspective, my approach is to look primarily at the material itself, what it would replace and how it would be used in organic production. Since the material is not currently used in organic agriculture; the questions that need to be answered are: why would it be needed? What organic production problems might it solve? Are the effects of using the material compatible with organic agriculture's goals? I will also address the environmental effects of producing the material.

The environmental effects of producing ozone are primarily related to the energy required to produce it (85% of which is lost as heat). The cost of equipment and the effort needed to maintain it limit ozone's use to medium and large scale operations. The high energy cost is a potential reason to not permit its use in organic agriculture due to energy related pollution. On the other hand, if a more efficient method of ozone production were developed, this objection would disappear. Therefore, the high use of energy is not sufficient reason to support its ban from organic agriculture.

The more important question is on what basis should a new, synthetic material be introduced to organic agriculture. The only reasons for inclusion I can support are:

1. If the material being introduced replaces materials that are less desirable to use because of environmental, safety, residue or health considerations. In short, if the new material fits the idealized organic criteria more closely than existing materials. This concept envisions an evolving organic production system that continually changes toward the idealized criteria as both new materials and new knowledge become available. This is true for some uses of ozone.

2. The material fits the criteria for use in organic agriculture except for being synthetic AND is an effective solution for an organic production problem or contributes to the expansion of organic production systems. This concept allows the methods and techniques of organic production to evolve and handle new situations and reach further into mainstream society.

In the current organic climate, concerns about contamination from use of manures and compost products are new threats to organic agriculture. An effective sanitizer or disinfectant without residues may be needed to meet changing USDA and HAACP regulations and still be acceptable to the organic market. Ozone has already been accepted in organic food processing for direct contact with food. Current ozone technology may not be sufficient to meet crop

production problems, but if more efficient ozone production or techniques were developed, the material itself may be able to provide a partial solution.

Reviewer 3 Recommendation Advised to the NOSB:

Ozone should be considered as a Synthetic allowed only with annotations

1. Restricted to use as weed and disease control with appropriate environmental controls and monitoring AND only after other methods have been tried. This method must be considered as a last resort

   Comment- There are many approved organic alternatives for weed and disease control in soils. These should be tried first. The potential for ozone to develop into an alternative to extremely high polluting materials is important to explore. If shown to be effective and clean, it should be allowed as a tool for organic farmers.

2. Allowed for use in cleaning drip irrigation lines with appropriate environmental controls and monitoring
   Comments- The efficacy of using ozone in this manner has not been shown but there is potential that it may be an alternative to chlorine or hydrogen peroxide.

Conclusion - Ozone for organic crop production:

Two out of three reviewers felt that ozone should be permitted for use in organic crop production, with use limited to:

1) cleaning irrigation lines,
2) weed control and
3) for soilborne pathogen control.

One suggested further restrictions limiting weed and pathogen control use to that of "last resort." If approved for use, this requirement is already established under 7CFR 205.206(d-e). A possible further restriction on use in irrigation as suggested by one reviewer, could be stated at 205.601(a)(5) "ozone, injected in irrigation lines in a method to prevent off-gassing."

These two reviewers did not find a compelling reason to reject usage, despite a lack of data in some areas such as effect on soil structure or earthworm populations. They did find some benefits to use and generally felt further experimentation might yield more data on effectiveness and impact.

The third reviewer found that health and safety reasons are a strong argument to prohibit use, along with the known effects on soil humic acid fraction, and the unknown long-term effects on soil and beneficial soil organisms.

This use is not permitted under current regulatory language of CODEX, the EU, or Japan and may require further consultation over equivalency issues if approved in the US.

References

*= *included in packet*

*ALA 2002. Ozone Generators- What is Ozone Air Pollution? American Lung Association of Washington. http://www.alaw.org/air_quality/information_and_referral/indoor_air_quality/ozone_generatiors.html

* Anderson, C.P. and C.F. Scagel, 1997. Nutrient availability alters belowground respiration of ozone-exposed ponderosa pine. *Tree Physiology*, 1997, *17*: 377-387. (abstract)

* Bao J., D. Fravel, G. Lazarovits, D. Chellemi, P. van Berkum, and N. O'Neill. 2000 Population Structure Of Fusarium Oxysporum In Conventional And Organic Tomato Production In Florida. In: *2000 Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions,* US EPA and USDA http://www.epa.gov/ozone/mbr/airc/2000/7fravel.pdf

*Barth, G. 1995. The potential for slow sand filtration for recirculating hydroponic systems in Australia. *South Australian Research and Development Institute.* Http://www.sardi.sa.gov.au/hort/floricul/barth3.htm.

*Blum, U., and D. Tingey. 1977. A study of the potential ways in which ozone could reduce root growth and nodulation of soybean. *Atmospheric Environment.* 11:737-739.

* Bocci, V., F. Corradeschi, Silva Silvestri, E. Luzzi and L. Paulesu. 1994. Further Evaluation of the Therapeutic Index of Ozone Used in Autohemotherapy. *From the Proceedings, Ozone Application in Medicine, September 1, 1994, Zurich Switzerland,* no organization named. Excerpted on internet at http://www.o3therapy.com/further.htm

* Bocci,V. 1996. Ozone as a bioregulator: Pharmacology and toxicology of ozone therapy today. *Journal of Biological Regulators and Homeostatic Agents* Vol. 10 number 2 pg. 31-53 abstract available at http://www.o3therapy.com

* Bocci, V., C. Aldinucci, E. Borrelli, F. Corradeschi, A. Diadori, G. Fanetti and F. Valacchi. 2001. Ozone in medicine. *Ozone Science and Engineering* 23:207-217.

*Boyette, C.D., Abbas, H.K., and H.L. Walker. 1999. Bioherbicides as alternatives to methyl bromide foe weed control in tomato. In: *Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions.* US EPA and USDA.

Brady, N.C. 1974. Ed. 8th Edition. *The Nature and Properties of Soils.* McMillan Pub. Co. NY pp .639.

Braghetta A., J Jacangelo, R Rhodes Trussell, J Meheus, M. Watson. 1997 The practice of chlorination: application, efficacy, problems and alternatives. *Internationl Water Supply Association Blue Pages.* http://www.iwahq.org.uk/pdf/bp0004.pdf

*Budavari, S. 1996. *The Merck Index* 12th Ed. Whitehouse Station NJ, Merck and Co.

* Bull, C. T. , K. G. Shetty, and K. V. Subbarao . 2000 . Interactions Between Myxobacteria, Plant Pathogenic Fungi and Biocontrol Agents In: *2000 Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions. US EPA and USDA* http://www.epa.gov/ozone/mbr/airc/2000/94bull.pdf

*Chase, C.A., Sinclair, T., Chellemi, D., Olson, S., Gilreath, J. and S. Locascio. 1999. Heat-retentive films for increasing soil temperatures during solarization in a humid, cloudy environment. *Hortscience* 34(6):1085-1089.

*Chase, C.A., Sinclair, T. and S. Locasio. 1999. Effects of soil temperature and tuber depth on Cyperus spp. Control. *Weed Sci.* 47:467-472.

*Chellemi, D., Olson, S., Mitchell, D., Secker, I., And R. McSorley. 1997. Adaptation of soil solarization to the integrated pest management of soilborne pests of tomato under humid conditions. *Phytopathology.* 87(3)250-258.

Cody, R.P. 1992, *Environmental Research, 58*:184-194.

* Del Agricultural. 2000 Complete Understanding of Ozone Use and Technology http://www.delozone.com/Pages/agozonefacts.html

* Duniway, J. M., J. J. Haoa, D. M. Dopkinsa, H. Ajwab, and G. T. Brownec. 2000. Some Chemical, Cultural, And Biological Alternatives To Methyl Bromide Fumigation Of Soil For Strawberry. In: *2000 Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions. US EPA and USDA.* http://www.epa.gov/ozone/mbr/airc/2000/9duniway.pdf

Engelhard, A.W. 1989. Ed. *Soilborne Plant Pathogens: Management of Diseases with Macro and Microelements.* APS Press, St. Paul Minn. Pp. 217.

* Figueras MD José Turrent, . Antonio A. Ramírez de Arellano Llovet MD. (no date) Ozone vs. Ozone Therapy: The Paradox" Ozone Research Center, Havana Cuba. http://www.o3therapy.com/PARADOX.htm

* Francis, A. W. 1997. Ozone. In *McGraw-Hill Encylclopedia of Science and Technology 8th Ed.* v. 12: 683-686. McGraw-Hill, NY.

*Gilreath, J., Noling, J., Locascio, S. and D. Chellemi. 1999. Efficacy of methyl bromide alternative in tomato and double cropped cucumber. In: *Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions.* US EPA and USDA.

*Giraud, D.D., Westerdahl, B., Riddle, L., Anderson, C., and A. Pryor. 2001. Hot water and ozone treatments of Easter lily for the management of lesion nematode, Pratylenchus penetrans. *Phytopathology.* 91(6 supplement) S134.

* Hassan, F. H. (undated) Cleaning of Drip Lines. *The Microirrigation Forum*. Downloaded Aug. 2002 from http://www.microirrigationforum.com/new/archives/cleandlines.html

*Hatzios, K., amd Y. Yang. 1983. Ozone-herbicide interatctions on sorghum (Sorghum bicolor) and velvetleaf (Abutilon theophrasti) seedlings. *Weed Science* 31:857-861.

* Hayes, C. 2000. Ozone biocidal properties and stimulation of *Trichoderma harzianum* (strain T-22) when applied in combination as an environmentally benign alternative for methyl bromide. EPA Grant 68D99035, Bioworks Inc.

* Herman, M. Feb 13, 2002. electronic mail: Ozone as an antimicrobial agent, crop production aid. *Sent to NOSB members and NOP.*

Hobbes C.H. and J.L. Mauderly. 1991, *Clinical Toxicology, 29*:375-384.

*Hoitink, H., and M. Krause. 1999. New approaches to control of plant pathogens in irrigation water. In: *Ornamental Plants - Annual Reports and Research Reviews 1999, special Circular 173-00.* Ohio State University. Http://ohioline.osu.edu/sc173/sc173_13.html.

*Kirk-Othmer 1996. *Encylopedia of Chemical Technology*, 4th Ed. Vol. 17. J. Kroschwitz, ed. pp 987-994. John Wiley, NY. Price

* Larson, L.E. 1999. *Integrated Agricultural Technologies Demonstrations.* Public Interest Energy Research (PIER) Rpt. No. P600-00-012, California Energy Commission, Sacramento, CA. 100 pp.

* Liberti, L. and M. Notarnicola. 1999. Advanced treatment and disinfection for municipal wastewater reuse in agriculture. *Water Sci. Tech.* 40(4-5):235-245.

* Liew, Chiam L.; R. Prange. 1994. Effect of ozone and storage temperature on postharvest diseases and physiology of carrots (Daucus carota L.). *J. Amer. Soc. Hort. Sci.* 119(3):563-567.

* Lin, M. and D.G. Klarup. 2001. The effect of ozonation of humic acids on the removal efficiency of humic acid-copper complexes via filtration. *Ozone Science and Engineering* 23:41-51.

Lioy, P. and Dyba, R. 1989. *Toxicology and Industrial Health, 5*:493-504.

Klaasen, C.D. 2001. *Casarett & Doull's Toxicology* (6th ed.) New York: McGraw-Hill.

*Locascio, S., Olson, S., Chase, C.A., Sinclair, T., Dickson, D. Mitchell, D. and D. Chellemi. 1999. Strawberry production with alternatives to methyl bromide fumigation. In: *Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions. US EPA and USDA.*

*Mathers, H. 2000. Weed Control: Root rots, recirculated water and disinfectants. Part 2. Ohio State University. Http://hcs.osu.edu/basicgreen/diseases/rootrot2.htm.

McDonnell, W.F., Zenick, H. and C. Hayes 1993.*J. Air Waste Man. Assoc., 43*:950-954.

*Mersie, W., T. Mebrahtu, and M. Rangappa. 1990. Response of corn to combinations of atrazine, propyl gallate and ozone. *Environmental and Experimental Botany.* 30(4):443-449.

* NIDO 1997 National NIDO Project. Water disinfestation:- Chloro-bromination and ozone systems get the thumbs up! Nursery Paper No. 8-97. Http://www.ngia.com.au/np/np97_8.html.

*Ohlenbusch, G., Hesse, S., and F. H. Frimmel. 1998. Effects of ozone treatment on the soil organic matter on contaminated sites. *Chemoshpere* vol. 37 (8):1557-1569.

*Ozonators. Http://www.greenair.com/ozonat.htm.

Pryor, W.A. 1992. *Free Radical Biology and Medicine, 12*:83-8.

*Pryor, A. 1996. Method and apparatus for ozone treatment of soil to kill living organisms. US Patent #5,566,627.

_____ 1997. Method and apparatus for ozone treatment of soil. US Patent #5,624,635.

* _____.1999. Results of 2 years of field trials using ozone gas as a soil treatment. In: *1999 Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions,* G. L. Obenauf, ed. US EPA and USDA.

* _____.2001. Petition For the Inclusion of Ozone Gas Used for Weed Control in the National List. Submitted to National Organic Program, USDA.

* Pryor, A. 2001b. Field trials for the combined use of ozone gas and beneficial microorganisms as a preplant soil treatment for tomatoes and strawberries. *Pest Management Grants Final Report*. Contract No. 99-0220 California Dept. Pesticide Regulation. 18 pp.

Quarles, W. and S. Daar. 1996. *IPM Alternatives to Methyl Bromide.* Bio-Integral Resource Center, Berkeley, CA 94707.60 pp.

*Qui, J.J., Westerdahl, B., Pryor, A., and C.E. Anderson. 2001. Reduction of root-knot nematode, M. javanica, in soil treated with ozone. (abstr.) *Phytopathology* 91(6 supplement) S141.

* Raub, L., Amrhein, C., and M. Matsumoto. 2001. The effects of ozonated irrigation water on soil physical and chemical properties. *Ozone Science and Engineering*. 23(1):65-76.

* Richardson, M. L. 1994. *The Dictionary of Substances and their Effects*. P 388-391. Royal Soc. Chemistry, Cambridge UK

*Sances, F., and E. Ingham. 1999. Conventional and organic alternatives to methyl bromide on California strawberries. In: Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions. US EPA and USDA.

* Sances F. 2000. Conventional and organic alternatives to methyl bromide on California strawberries. *In : 2000 Annual International Conference on Methyl Bromide Alternatives and Emissions Reductions. US EPA and USDA.* http://www.epa.gov/ozone/mbr/airc/2000/24sances.pdf

*Sandermann, H. 1996. Ozone and Plant Health. *Annual Review of Phytopathology*. 1996. 34:347-366.

*Smith, R., Lanini, W.T., Gaskell, M., Mitchell, J., Koike, S. and C. Fouche. 2000. Weed management for organic crops. Univ. of California, Div. Agriculture and Natural Resources, Publication 7250.

*Unal, R., Kim, J., and A. Yousef. 2001. Inactivation of Escherichia coli O157:H7, Listeria monocytogenes, and Lactobacillus leichmannii by combination of ozone and pulsed electrical field. *J. of Food Protection*. 64(6):777-782.

* US EPA 1996. Methyl Bromide Alternatives Case Studies Vol. II. Organic Strawberry Production As An Alternative to Methyl Bromide http://www.epa.gov/ozone/mbr/casestudies/volume2/orgsber2.html

US EPA 1997. Methyl Bromide Alternatives Case Studies Vol. III. Disease Suppressive Compost as an Alternative to Methyl Bromide. http://www.epa.gov/ozone/mbr/casestudies/volume3/compost3.html

* United States EnvironmentalProtection Agency, 1999. *Alternative Disinfectants and Oxidents Guidance Manual.* Office of Water. EPA 815-R-99-014, April 1999. http://www.epa.gov/safewater/mdbp/alternative_disinfectants_guidance.pdf

US EPA 2000. *Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions* http://www.epa.gov/ozone/mbr/airc/2000/index.html

* Utriainen, J. and T. Holopainen. 2001. Nitrogen Availability modifies the ozone responses of Scots pine seedlings exposed inan ope-filed system. *Tree Physiology. 21*:1205-13.

*Von Broembsen, S. 2002. Disease management and water recycling. Oklahoma Cooperative Extension Service. http://zoospore.okstate.edu/nursery/managing/disease/index.html and http://zoospore.okstate.edu/nursery/managing/treat/ozonation.html

*von Broembsen, S. Capturing and recycling irrigation water to protect water supplies. In: E-951, *Water Quality Handbook for Nurseries.* Oklahoma Extension Service. Http://okstate.edu/OSU_Ag/agedcm4h/pearl/e951/e951ch7.htm.

*Wohanka, W. 1995. Disinfection of recirculating nutrient solutions by slow sand filtration. *Acta Horticulturae* 382:246-251.

This TAP review was completed pursuant to United States Department of Agriculture Purchase Order # 43-6395-2900A.

AMERICAN LUNG ASSOCIATION® of Washington

When You Can't Breathe, Nothing Else Matters®

Most smokers start in their youth: almost 90% of current smokers started before the age of 19.

- Childhood Asthma
- Tobacco Control
- Air Quality
  - Information and Referral
    - Healthy House
    - Indoor Air Quality
    - Master Home Environmentalist
    - Outdoor Air Quality
- Lung Disease
- Support ALAW

Quicklinks:

- Donate
- Master Home Environmentalist
- Climb for Clean Air
- Research
- Trek Tri Island

Member Sessions:

- Login

Ozone Generators

What Is Ozone Air Pollution?

Ozone is a potent lung irritant and exposure to elevated levels is a contributor to the exacerbation of lung disease; it is especially dangerous for persons with asthma and other chronic lung diseases, children, and the elderly. Residential indoor ozone is produced directly by ozone generators and indirectly by ion generators and some other electronic air cleaners. There is no difference, despite some manufacturers' claims, between outdoor ozone and ozone produced by these devices.

The Federal Trade Commission (FTC) took action in 1995 against two manufacturers of ozone generating devices. The FTC charged that they made unsubstantiated claims about the ability of their products to clean air of various indoor air pollutants and to prevent or relieve allergies, asthma and other conditions. Under the FTC's settlement, the manufacturers are prohibited from making marketing claims that ozone is effective in cleaning indoor air, that their products do not create harmful by-products, and that they prevent or provide relief from allergies, asthma, and other specified conditions, unless the claims are supported by reliable and adequate substantiation (FTC, 1995).

Consumer Reports (1992), the National Institute of Occupational Safety and Health (NIOSH) (Boeniger, 1995), and the U.S. EPA (1995) concluded that tabletop and room unit ozone generators are not effective in improving indoor air quality. Studies have found that while some indoor air pollutant concentrations decline in the presence of ozone, other pollutants increase. In fact, upon reaction with ozone, some previously undetected, toxic chemicals emerge in indoor air, including formaldehyde and other alehydes (Boeniger, 1995). There is a lack of evidence in the scientific literature that would support the effectiveness of ozone at low concentrations in removing organic contaminants from indoor air (Boeniger, 1995). A recent study by the U.S. EPA demonstrates that ozone is not effective for killing airborne molds and fungi even at high concentrations (6-9 ppm) (U.S. EPA, 1995). At higher concentrations, especially above 0.08 ppm, ozone is a potent irritant that can bring about diminished lung function, cough, inflammation associated with biochemical changes, and increased responsiveness to allergens (Horstman, et al., 1990). Current evidence of the health effects of ozone suggests that there is no "safe" threshold concentration for the onset of health responses due to exposure above background ozone concentrations (Burnett, et al., 1994; U.S. EPA CASAC letter, 1995). Also, simultaneous exposure to ozone and other compounds may produce additive or synergistic effects (Last, et al., 1984; Peden, et al., 1995). In addition, persons with asthma have increased

susceptibility to ozone and exposure to low concentrations results in increased symptoms, medications use and hospitalizations.

The FDA has set a limit of 0.05 ppm of ozone for medical devices. A small percentage of cleaners that claim a health benefit are listed by the FDA and these devices conform to FDA regulations. However, ozone generators, negative ion generators, and certain other electronic air cleaners that are not listed by the FDA, or cannot otherwise prove that their ozone emission levels are lower than 0.05 ppm, may produce levels of ozone recognized as unsafe for humans and are not recommended for use in occupied spaces because of the risk of generation of ozone. For similar reasons, the American Lung Association does not suggest the use of these products.

For more information, email the American Lung Association of Washington at **alaw@alaw.org** or call us at (206) 441-5100, or 1-800-732-9339. No matter where you live in the United States, you can call **your local American Lung Association** at 1-800-LUNG-USA.

ALA of Washington **Contact Information** **ALA National Website**

Author **Andersen, C.P. Scagel, C.F. 1997**

U.S. EPA National Health and Environmental Effects Research Laboratory, Corvallis, OR.

Title Nutrient availability alters belowground respiration of ozone-exposed ponderosa pine.

Tree Physiology. [Victoria [B.C.] Canada : Heron Pub] June 1997. v. 17 (6) p. 377-387.

Abstract

Exposure to ozone (O3) and changes in soil fertility influence both the metabolism of plant roots and their interaction with rhizosphere organisms.Because one indication of altered root metabolism is a change in below ground respiratory activity, we used specially designed measurementchambers to assess the effects of O3 and nutrient availability on belowground respiratory activity of potted three-year-old ponderosa pine (Pinus ponderosa Dougl. ex Laws.). Seedlings were exposed to a factorial combination of three O3 treatments and three fertilization treatments in open-top O3 exposure chambers. Ozone exposure decreased and high nutrient supply increased total plant dry weight, but root/shoot ratios were not affected. In general, exposure to O3 increased rates of belowground O2 uptake and CO2 release and the respiratory quotient (RQ, CO2/O2), although seasonal differences were detected. In October, following the second season of O3 exposure, rates of belowground O2 uptake and CO2 release and RQ were increased in trees in the high-O3 exposure treatment by 22, 73 and 32%, respectively, over values in control trees in charcoal-filtered air. Increasing nutrient supply resulted in decreasing rates of belowground O2 uptake and CO2 release but it had little effect on RQ. In the high-nutrient supply treatment, rates of belowground O2 uptake and CO2 release were decreased by 38 and 39%, respectively, compared with rates in the low-nutrient supply treatment. At the end of the second growing season, the high-nutrient supply treatment had decreased lateral root total nonstructural carbohydrates by 22% compared with the low-nutrient supply treatment. Nutrient availability altered the belowground respiratory response. to O3, such that the response to O3 was greatest in the low-nutrient supply treatment. Significant O3 effects on belowground respiratory activity were apparent before any reduction in total plant growth was found, suggesting that roots and rhizosphere organisms may be early indicators of physiological dysfunction in stressed seedlings.

POPULATION STRUCTURE OF FUSARIUM OXYSPORUM IN CONVENTIONAL AND ORGANIC TOMATO PRODUCTION IN FLORIDA

J. Bao[1], D. Fravel[1*], G. Lazarovits[2], D. Chellemi[3], P. van Berkum[4], and N. O'Neill[4]

Fusarium wilt in tomatoes, caused by *Fusarium oxysporum* f. sp. *lycopersici* is currently managed through fumigation with methyl bromide. Nonpathogenic Fusaria have been demonstrated to reduce Fusarium wilt on several crops, including tomato (Larkin and Fravel, Plant Dis. 82:1022-1028; Phytopathology 89:1152-1161). Currently, the only way to distinguish pathogenic Fusaria from beneficial or saprophytic Fusaria is through a plant bioassay. This research was undertaken to determine the population structure of Fusaria in soil and on tomato roots, as well as to identify genetic markers for pathogenicity and biocontrol ability.

Roots and soil were collected from tomato plants at two sites in Osceola County, FL representing conventional and organic farming systems. Samples were dilution plated onto Komada's medium with each of the following plated separately: nonrhizosphere soil, soil in the root zone but not attached to roots, rhizosphere soil, fine roots, long lateral roots, and main roots. A total of 21,054 Fusaria were recovered, 26.6% of which were 5610 *F. oxysporum* (Table 1). Soil near tomato roots or rhizosphere soil had approximately 10-fold larger Fusarium population sizes than non-rhizosphere soil (Table 1). Fine roots (either attached to or not attached to the plant) had population sizes 2 to 7 fold greater than long lateral roots and main roots. This is likely associated with greater nutrient availability, as well as the volume:surface root surface ratio of fine vs larger roots. Soil amended with Telone alone had the largest fungal population of any site tested.

Pathogenicity assays using tomato cultivar Bonny Best were performed on all 406 *F. oxysporum* recovered (Table 2). Of these, 63 (15.5%) were pathogenic with 34 expressing symptoms as *F. o. lycopersici* and 29 as *F. o. radicis-lycopersici.* All pathogenic isolates were tested on differential tomato cultivars to determine race of the pathogen. All of the 34 pathogenic *F. o.. lycopersici* recovered were from the organic farm. Of these,15 were Race 1, 6 were Race 2 and 5 were Race 3. However, when samples were originally collected, no plants on the organic site showed symptoms, while several plants at the conventional site showed symptoms. Race 3 *F. o. lycopersici* was recovered from these plants from the conventional farm.

One hundred and twenty-nine *F. oxysporum* isolates were further characterized using ITS1-5.8S-ITS2 rDNA regions. Fungal mycelia were harvested from 5-day cultures in PDB and DNA were isolated from mycelia using a DNA isolation kit (Qiagen). ITS1-5.8S-ITS2 region was amplified using ITS4 and ITS5 primers on a thermalcycler, digested using *Msp*I restriction enzyme, and then separated on a agarose-gel Twin gel. At least 5 groups were identified based on polymorphism of the ITS1-5.8S-ITS2 rDNA regions. Group II, containing one *Msp*I cut site, was the largest containing 82 isolates, including almost all the pathogenic strains. Group III, with 2 cuts, contained 17 isolates; and Group V, with 3 cuts, had 4 isolates. Results indicated that nonpathogenic strains had higher diversity in the population.

[1]Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD; [2]Agriculture and Agri-Food Canada, London, ON; [3]Subtropical Plant Pathology Research, USDA, ARS, Ft. Pierce, FL; [4]Soybean and Alfalfa Research Laboratory, USDA, ARS, Beltsville, MD.

Table 1. Total Fusaria and *F. oxysporum* (*F.o.*) populations recovered from a conventional and an organic farm in Florida.

Sampling location	Total Fusaria [a]	*F.* o. (%)	Organic farm (CFU counted)				Conventional farm (CFU counted)			
			Solarized		Intercropped beans [b]		Telone+solarized		Telone alone	
			Total	*F.o.* (%)	Total	*F.o.* (%)	Total	*F.o.* (%)	Total	*F.o.* (%)
Non-rhiz. soil	955	408 (42.7)	100	8 (8.0)	160	7 (4.4)	90	10 (11.1)	389	331 (85.1)
Root-near soil	4482	1431 (31.9)	2303	178 (7.7)	633	553 (87.4)	108	15 (13.9)	810	540 (66.7)
Rhiz. soil	2893	921 (31.8)	591	170 (28.8)	1452	460 (31.7)	47	24 (51.1)	230	173 (75.2)
Root surface										
Fine root not attached	4730	978 (20.7)	2244	168 (7.5)	1581	436 (27.6)	97	6 (6.2)	336	285 (84.8)
Fine root attached	2219	308 (13.9)	1334	84 (6.3)	245	57 (23.3)	--	-- (--)	145	60 (41.4)
Long lateral root	3607	804 (22.3)	1477	94 (6.4)	940	399 (42.4)	434	225 (51.8)	120	40 (33.3)
Main root	2168	760 (35.1)	669	168 (25.1)	388	68 (17.5)	99	40 (40.4)	197	49 (24.9)

[a]Per g of soil or per g of root.
[b]Not solarized. Planted into bean stubble and intercropped with beans.

Table 2. Pathogenicity of *F. oxysporum* collected from organic and conventional tomato fields in Florida.

Farming system	No. of plants	Origin of *F. o.* isolate		Total *F. o.* isolated	Pathogenic (# of isolates)		Non-pathogenic (# of isolates)
		Root	Soil		*Fol* [a] (%)	*Forl* [b] (%)	
Organic	7	69	221	290	30 (52.6)	27 (47.4)	233
Conventional	3	16	100	116	4 (66.7)	2 (33.3)	110
Total	10	85	321	406	34 (54)	29 (46)	343

[a]*F. oxysporum* f. sp. *lycopersici.*
[b]*F. oxysporum* f. sp. *radicis-lycopersici.*

SARDI HORTICULTURE
Ornamental & flower crops

THE POTENTIAL FOR SLOW SAND FILTRATION FOR RECIRCULATING HYDROPONIC SYSTEMS IN AUSTRALIA

Gail Barth, South Australian Research and Development Institute

Throughout the world's greenhouse and nursery industries there is increased awareness of the need for water recycling and effluent control as governments legislate against environmental pollution and water is recognised as a limited or expensive commodity. To control the spread of water-borne plant pathogens, recycled water in nurseries or greenhouses can be subjected to a range of disinfestation methods, both chemical and physical. In Holland, Runia (1993) reports that more that 500 nurseries (greenhouses) used heat treatment, ozonisation or UV-radiation to disinfect water against fungi, bacteria and viruses. These methods require high capital investment in equipment, need to be used in conjunction with traditional sand filters to remove particulate matter, and are not always effective at removing persistent pathogens. Considering investment costs and the high energy consumption of heating, these techniques can be used economically only in large production units of more that 1 ha of greenhouses.

In Australia, container nurseries with water recycling programs are most commonly using chlorination or bromination to treat their run off (Rolfe et al, 1994). With the provision of correct slopes and drains to collect and channel water in the nursery, media filters to screen out particulate matter and solids and storage tanks for treatment, such systems can be quite effective in disinfesting water of the most serious nursery crop pathogens such as phytophthora and pythium. Often collection ponds or dams are integrated into the system if they can be incorporated into the site. This alleviates some of the filter requirements and reduces the number of holding tanks.

There are currently active research programs in Europe investigating the biological activity of ponds and water plants in reducing plant pathogens and cleaning irrigation waters. On a smaller scale, slow sand filtration has been adapted for recycling water in greenhouse crops and has demonstrated biological activity in suppressing and controlling pathogens (Wohanka, 1993).

Slow sand filtration is an adaptation of traditional systems used for treating drinking water. Flow rates through the filters are on the order of 100 to 300 L/ hr per m2 of surface area (compared to rates of 600-900L/min/m2 used in media filters for screening water used in micro or drip irrigation systems). Dr Walter Wohanka of Geisenheim Research Centre in Germany has been investigating the development of such filters for several years and is leading the pathological investigations of the efficacy of sand filters. In 1991, I visited Dr. Wohanka's trials and was impressed with the potential for application of sand filters in Australia's' floriculture industry where production units are often small and most systems were running all their nutrient solutions to waste. At the time of my visit, the sand filters at

Geisenheim were effectively filtering *Phytophthora* from water circulating between an infected, symptomatic gerbera crop and an isolated clean crop. Later investigations have focused on *Pythium, Rhizoctonia* and *Fusarium spp.* On the basis of the potential of this work we applied to the Hydroponics Association and HRDC for funding to undertake investigations of sand filters at our new research facility at the Waite campus and to encourage the trialing of such filters by Australian producers. We have established an ebb and flow recirculating system attached to slow sand filters in our greenhouses, where crops can be isolated and inoculated with pathogens.

The filtration system is a slow sand filter with a reservoir above the sand and a water inlet below the sand head to avoid disturbing a skin that forms on the surface of the filter soon after the filter is put in use. This skin consists of organic and inorganic material and a range of biologically active microorganisms which break down organic matter. The filter appears to have biological activity in the top 40 cm of sand, thus it is recommended that the filter thickness should be a minimum of 50-60 cm. With time, some cleaning of the filter bed may be necessary and it is recommended that an initial thickness of 80-120 cm is more appropriate to allow for scraping off of a few cm during cleaning.

The efficiency of a sand filter is dependent on the particle size distribution, analytical data of which is given by Wohanka (1994).We have found that the particle size of the sands can be modified considerably to allow for the use of locally available components to reduce costs. Beneath the sand are three layers of graded gravel to prevent the sand from blocking the outlet (polyester fiber materials can be used to replace some gravel layers). The casing for the sand filter can be designed of many common materials, including large plastic drums or water tanks. In our trials in Adelaide we have joined 200 litre plastic drums in tandem, adding more as the need for higher flow rates (greater surface area) is needed.

Experimental sand filters have been inoculated with suspensions of plant pathogens to test their short and long term effectiveness in eliminating various fungi and bacteria. Early investigations demonstrated that the filters were reliable in eliminating phytophthora and pythium from recirculating nutrient solutions or drainage water. High efficiency was observed against *Cylindrocladium, Verticillium dahliae, Thielaviopsis,* and *Xanthomonas* bacteria. There is also a report of high efficacy against a virus, pelargonium flower break (Berkelmann et al, 1994).

Pathology work with *Fusarium spp.* has demonstrated a 99.9% reduction rate of microconidia (small resting spores) which were poorly filtered by early designs of sand filters. It is assumed by researchers that this level of efficacy is sufficient to prevent serious problems with distribution of fusarium through recirculating filtered water. Fusarium microconidia are more resistant to heat and UV treatment than other pathogens and are most likely to be the pathogen most poorly controlled by any disinfestation method.

Current work at the SARDI Plant Research Centre in Adelaide is concentrating on improved design characteristics of filters and on the efficacy of filters in controlling *Fusarium oxysporum*. Suspensions of fusarium isolated from carnations, gerberas and ixodia have been inoculated onto a range of susceptible species. The activity and distribution of fusarium spores has then been monitored within the filters and throughout the recirculating hydroponic system. To date fusarium presence has been established in the hydroponic system, to depths of 20 cm within the filter, with no detectable presence at greater depths or

within the filtered water which is recirculated onto a 'clean' crop.

Future work will concentrate on understanding the biological activity occurring in the filters that destroys the plant pathogens. This is a complex problem as previous examinations of the bacterial flora of hydroponic systems have shown that high levels of bacteria (105 to 106 cfu) develop within 24 hours of plants being introduced into the nutrient solutions. Over 160 separate bacterial strains have been characterised in a tomato hydroponic system (Berkelmann et al, 1994), many of which represent groups successfully implicated in biological control systems. A greater understanding of the biological processes occurring within the filters could allow for improved management of filters and possible targeted inoculations with control agents for increased pathogen control.

REFERENCES

Berkelmann, B., W. Wohanka and G. Wolf. 1994. Characterisation of the bacterial flora in circulating nutrient solutions of a hydroponic system with rockwool. Acta Horticulturae 361:372-381.

Fox, Roger. 1995. Slow sand filtration: report on South Australian research at the Australian Hydroponics Conference. Practical Hydroponics and Greenhouses Sept/Oct Issue p.14-16.

Rolfe, C., A. Currey and I. Atkinson. 1994. Managing water in plant nurseries. NSW Agriculture and HRDC 163 p.

Roberts, B.W. and C.W. O'Hern. 1993. Inexpensive sand filters for drip irrigation systems. Horttechnology 3(1): 85-89.

Runia, W. T. 1993. A review of possibilities for disinfestation of recirculation water from soilless cultures. Acta Hort. New Cultivation systems in Greenhouse (in press).

Wohanka, W. 1993. Slow sand filtration and UV radiation: Low-cost techniques for disinfestation of recirculating nutrient solutions or surface water. Pro. 8th Conf. Int Soc for soilless Culture, South Africa p.497-511.

Wohanka, W. 1994. Disinfestation of recirculating nutrient solutions by slow sand filtration. Proc. Sino-Int. Colloq. on Soilless Culture p.168-176.

E-mail: sardi@saugov.sa.gov.au URL: http://www.sardi.sa.gov.au/
Head Office: Plant Research Centre. Gate 2b. Hartley Grove. Urrbrae 5064. South Australia.
Postal Address: GPO Box 397. Adelaide 5001. South Australia.
Ph: +61 8 8303 9400. Fax: +61 8 8303 9309

This page last updated: 02 February 2000

BACK

FURTHER EVALUATION OF THE THERAPEUTIC INDEX OF OZONE USED IN AUTOHEMOTHERAPY

V.BOCCI, F. CORRADESCHI, SILVA SILVESTRI, E. LUZZI AND L. PAULESU
INSTITUTE OF GENERAL PHYSIOLOGY OF THE UNVERSITY OF SIENA 53100 ITALY

On the basis that ozone is a very rective and potenially toxic gas, it has been a common wisdom to use a fairly narrow range of ozone concentration anong 5 and 40µg/ml of blood.

This was based on empirical data, that low ozone concentrations are immunostimulatory while high concentration are suppressive. After having clarfied that an important mechanism of action of ozone is to induce cytokine productions by mononuclear cells, we could define a reliable end-point and correlate ozone concentration and cytokine levels after a suitable incubation of blood. The main aim of this research was to achivev an effective immunostimulation with the least toxic effects by measuring the level of ozone-induced hemolsis (below 3.5%), possible formation of metahemoglobin (always absent), morphologic damage (absent below 80µg/ml of ozone) as evaluated by electron microscopy, plasma level of lipid hydroperoxides (increasing 3 fold after ozonization with 90µg/ml ozone/ml of blood but rapidly returning to base line values) and intraerythrocytic reduced glutathion levels never below 10 % and repidly restored. Unexpectedly and contrary to ozone dosage usually used in autohemotherapy (from 5 to 40µg/ml O_3/ml of blood), we found that we could raise the ozone level and the most effective concentrations without toxicity are ranging between 50 and 80µg/ml depending upon individual plasma levels of anti-oxidant compounds.

The concept of correlating the production of cytokines of blood mononuclear cells versus ozone concentration has thus represented a crucial advantage and it has become an indispensable end-point

(From the Proceedings, Ozone Application in Medicine, Thursday, September 1, 1994, Zurich Switzerland)
(page 16)

---

eMail Prof. Velio Bocci

BACK

1948

Atmospheric Environment Vol. 11, pp. 737–739. Pergamon Press 1977. Printed in Great Britain.

A STUDY OF THE POTENTIAL WAYS IN WHICH OZONE COULD REDUCE ROOT GROWTH AND NODULATION OF SOYBEAN*

UDO BLUM and DAVID T. TINGEY

Associate Professor of Botany, North Carolina State University, Raleigh, N.C. 27607, and Plant Physiologist, Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, Oregon 97330, U.S.A.

(*First received* 21 *June and in revised form* 6 *December* 1976)

Abstract—The possible mechanisms by which ozone reduces root growth and nodulation of soybean were investigated. Ozone did not appreciably penetrate the plant growth substrates nor did it oxidize soil organic matter to form compounds inhibitory to *Rhizobium*. When ozone was excluded from the plant foliage, but not from the soil, root growth and nodulation were not reduced. However, when plant tops were directly exposed to ozone, root growth and nodulation were reduced. These results indicated that observed reductions in root growth and nodulation did not occur by way of the soil, but resulted from an effect of ozone on the plant foliage.

INTRODUCTION

...te and chronic ozone exposures induce foliar in-... suppress nodulation and inhibit root growth ...re than top growth in legumes (Engle and Gabel-... 1967; Manning *et al.*, 1971; Tingey and Blum, ...; Tingey *et al.*, 1973b). Ozone could suppress root ...wth and nodulation either directly by a) diffusing ... the soil to inhibit growth and nodulation, b) by ...dizing soil organic matter to form toxins, c) or ...rectly by altering foliar metabolism and reducing ...quality and/or quantity of photosynthate translo-...d to the roots. The objective of this study was ...determine which mechanism or combination of ...chanisms could explain ozone suppression of root ...wth and nodulation.

METHODS AND MATERIALS

...e penetration into plant growth substrates

...lindrical chambers each consisting of a soil hold-... area and an air sampling area separated by a ...rated plate were constructed. The soil holding ... was constructed of 2 × 14.6 cm (14 cm i.d.) Plexi-...rings stacked on top of one another and held ...ce with bolts (outside of the rings) through top ...se plates (20 × 20 cm). The top plate had a single circular opening (154 cm^2). The air sampling area was located between the base plate and a perforated plate at the bottom of the soil handling area. The air sampling area consisted of a solid base plate to which a 2 × 14.6 cm dia. ring topped with a perforated plate containing 28 holes (0.4 cm dia.) was fixed. Air was sampled from this area through a 0.63 cm ID sampling port and the ozone content of the air exiting the base of the chambers was measured using the alkaline potassium iodine method (U.S. Department of Health, Education, and Welfare, 1965). The depth of substrates was varied in 2 cm increments for each study by changing the number of rings in the soil holding area. Before the 2 h ozone exposure, the outsides of the chambers were covered with plastic film and aluminum foil. Air containing either 0 or 0.5 ppm ozone was drawn through the soil columns at a linear velocity of 12.7 cm min^{-1}. The penetration of ozone into several substrates was studied.

The substrates and their mean water content (no water was added to substrate) over the experimental periods were: gravel (3.7%), sand (1.8%), Jiffy mix† (27.1%), and Jiffy mix + gravel (1:2:v:v) (6.1%). Conclusions were based on 3 replicates per substrate per ozone concentration.

The diffusion of ozone into soil substrate was measured by the oxidation of dichlorophenol–indophenol. The Jiffy mix + gravel substrate was saturated with a solution of dichlorophenol–indophenol (1.7 mM) and allowed to drain overnight yielding a mean water content of 19%. The dye coated substrate was exposed to either 0 or 0.5 ppm ozone for 1, 2, or 4 hr. Immediately after exposure, 1 cm layers of substrate were removed; the dye was extracted with water and the amount of unoxidized dye was measured spectrophotometrically at 600 nm.

...operative investigations of the Environmental Pro... Agency, North Carolina State University, and the ...Department of Agriculture, Raleigh, N.C. Paper No. ... the Journal Series of the North Carolina Agr. Exp. ...Raleigh, N.C.

† ...s mix is a trade mark of a product containing peat ... and vermiculite. Mention of a trade mark or pro... product by North Carolina State University, the ...mental Protection Agency does not imply approval ... exclusion of other products that may be suitable.

Ozone and the production of inhibitors in substrates

Jiffy mix + gravel in 1800 cm^3 (10 cm dia.) plastic pots was exposed in two different experiments to either 0 and 0.5 ppm ozone for 4 hr or to 0 or 0.18 ppm ozone for 8 hr/day for 30 days. Water was added only to the 30 day experiment. Mean water content for the 4 hr experiment was 8.7% and 18% for the 30 day experiment. Following the completion of exposure, the upper 2 cm of substrate were removed from each of 4 pots and combined into a single sample. A 25 g aliquot was extracted for 24 hr with acetone in a Soxhlet extractor. The extracts were concentrated by flash evaporation and adjusted to a final volume (5 ml) with acetone. The concentrated extracts were incorporated in sensitivity discs (Difco Laboratories, 1953) and tested for toxicity to *Rhizobium japonicum* (5–7 day cultures*, $3I_1b$ 59 NC 1946) innoculated on yeast mannitol agar. Zones of inhibition around the sensitivity discs were measured daily for 4 days. Conclusions were based on 4 replicates of 4 discs per ozone treatment. Water extracts were also obtained from both studies and tested for toxicity.

Ozone effects on plant growth and nodulation

Soybean (*Glycine max* (L.) Merr.) cv'Dare'seeds (5/pot) were planted in Jiffy mix + gravel in 10 cm diam. pots and innoculated with 20 ml of yeast mannitol broth containing 5–7 day old cultures of *Rhizobium japonicum* ($3I_1b$ 59 NC 1946) and covered with 1–2 cm of substrate. The plants were grown in the Southeastern Plant Environmental Laboratories as previously described (Kramer *et al.*, 1970; Tingey *et al.*, 1973a). Plants were watered twice daily—once with a complete nutrient solution and once with distilled water (Raper and Johnson, 1971). One week after planting the seedlings were thinned to one plant per pot. Two weeks after seeding the plants were divided into 2 equal groups. The foliage of 1 group was enclosed in plastic bags and the foliage of the other group was not enclosed. Both bagged and unbagged plants were exposed to ozone as previously described (Heck *et al.*, 1968; Tingey *et al.*, 1973a). Immediately following exposure, the plastic bags were removed and all plants were returned to the growth environment.

Plants were harvested, separated into tops and roots 4, 8, and 16 days following exposure and the dry weights were measured.

Data analysis

Top and root relative growth rates were calculated as described by Radford (1967). Significant differences among treatments were determined using analysis of variance procedures.

* Culture ($3I_1b$ 59 NC 1946) was obtained from B. E. Caldwell Soybean Investigations, Plant Industry Station, Beltsville, MD. 20705.

RESULTS AND DISCUSSION

Ozone penetration into plant growth substrate

When a total of 120 μg ozone (0.5 ppm for ... was applied to either 2 or 4 cm thick colum... gravel, sand, Jiffy mix, or Jiffy mix + gravel, no ... was detected in the air exiting the columns. ... diffusion into Jiffy mix + gravel as measured ... oxidation of dichlorophenol–indophenol, alt... not significant, occurred in the first cm of su... after 2 and 4 hr of treatment (Table 1). These ... suggest that ozone does not penetrate into the ... strates used to any appreciable extent. This is ... tent with the reports that ozone is unstable ... presence of moisture, organic matter or roug... faces and supports the concept that the soil ... ozone sink (Alder and Hill, 1950; Bohn, 1972; ... Dowell, 1974; Turner *et al.*, 1973).

Ozone and the production of inhibitors in sub...

Ozone oxidizing the organic portion of the ... strate could form substances inhibitory to *Rhizo...* In the sensitivity disc assays of the acetone and ... extracts from substrates exposed for a short time ... or 0.5 ppm for 4 hr) or for a long time (0 or 0.1... ... for 8 hr/day for 30 days), the zones of inhibition ... not significantly different from the control. This ... gests, at least for the substrates tested, that ozone does not induce the formation of substances inhibitory to *Rhizobium*.

Ozone effects on plant growth and nodulation

The relative growth rates of the plant tops, roots and nodulation (increase in nodule number) the ozone exposed plants were significantly less than either of the controls or the ozone + bag treatment (Table 2). This supports the previous reports that ozone suppresses top and root growth and nodulation (Engle and Gabelman, 1967; Manning *et al.*, 1... Tingey and Blum, 1973; Tingey *et al.*, 1973b). The lack of significant differences between the ozone + bag (where ozone was excluded from the foliage) and the two types of controls supports the concept that

Table 1. Depth of ozone diffusion into Jiffy mix ... gravel*†

Duration of treatment (hr)	Unoxidized dichlorophenol–indophe... recovered from soil after treatment... expressed as a % of control (depth in cm) 0–1	1–2	2–4
1	100 ± 10‡	100 ± 14	104 ± ...
2	88 ± 6	107 ± 7	94 ± ...
4	84 ± 11	91 ± 10	100 ± ...

* No statistically significant differences were found ... the 5% significance level.

† Substrate was subjected to charcoal filtered air (con... trol) or 0.5 ppm of ozone. Each mean is an average ... 4 observations.

‡ Standard error of the mean.

Table 2. The effect of ozone on ... of bagged and unbagged soy...

Treatment	Relative growth rate ($days^{-1}$) Top growth	Root growth
Control	0.11	0.09
Control + bag	0.11	0.10
Ozone	0.08	0.07
Ozone + bag	0.11	0.10

*Two week old soybean plants ... ppm of ozone for 4 hr. During ... were enclosed in plastic bags. ... 4, 8, and 16 days after exposure. E... from 9 observations. The ozone treat... were than the other 3 treatments a... level.

...does not penetrate the s... effect on root growth or nod... reductions in root growth and no... directly from ozone induced alter... fixation and translocation.

Acknowledgements—The authors ... their appreciation to Hans H... North Carolina State Expe... with the experimental design and s... This research were support... by the Southeastern Plant E...

REFERENCE...

... M. C. and G. R. Hill (1... ...m of hydroxide ion cat... ...in aqueous solution. *A*... 1886.

Table 2. The effect of ozone on the growth and nodulation of bagged and unbagged soybean plants*

Treatment	Relative growth rate (days^{-1}) Top growth	Root growth	Increase in nodule numbers (nodules days^{-1})
Control	0.11	0.09	6.8
Control + bag	0.11	0.10	7.4
Ozone	0.08	0.07	2.7
Ozone + bag	0.11	0.10	6.1

* Two week old soybean plants were exposed to 0 or ppm of ozone for 4 hr. During exposure half of the were enclosed in plastic bags. Plants were harvested 4, 8, and 16 days after exposure. Each value was derived m 9 observations. The ozone treatment was significantly er than the other 3 treatments at the 0.05 significance el.

one does not penetrate the soil to cause a direct ect on root growth or nodulation. Rather, the ductions in root growth and nodulation resulted in- ectly from ozone induced alterations in foliar meta- ism and translocation.

nowledgements—The authors would like to express r appreciation to Hans Hamann, Associate Statistician, rth Carolina State Experiment Station, for assisting the experimental design and statistical analyses. Por- s of this research were supported by NSF Grant No. st to the Southeastern Plant Environmental Labora- y.

REFERENCES

r, M. C. and G. R. Hill (1950) The kinetics and echanism of hydroxide ion catalyzed ozone decompo- ion in aqueous solution. *Am. Chem. Soc. J.* **72**, 4-1886.

Bohn, H. L. (1972) Soil absorption of air pollutants. *J. Environ. Qual.* **1**, 372–377.

Difco Laboratories (1953) Bacto-sensitivity Disks pp. 311–336 in *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures.* 9th ed. Difco Laboratories, Detroit, Michigan.

Engle, R. L. and W. H. Gabelman (1967) The effects of low levels of ozone on pinto beans *Phaseolus vulgaris* L. *Am. Soc. Hort. Sci. Proc.* **91**, 304–309.

Heck, W. W., J. A. Dunning and H. Johnson (1968) Design of a simple plant exposure chamber. DHEW National Center of Air Pollution Control Publication APTD-68-6.

Kramer, P. J., H. Hellmuth and R. J. Downs (1970) SEPEL: New Phytotrons for environmental research. *BioScience* **20**, 1201–1208.

Manning, W. J., W. A. Feder, P. N. Papia, and I. Perkins (1971) Influence of foliar ozone injury on root development and root surface fungi of pinto bean plants. *Environ. Pollut.* **1**, 305–312.

MacDowall, F. D. H. (1974) Importance of soil in the absorption of ozone by a crop. *Can. J. Soil Sci.* **54**, 239–240.

Radford, P. J. (1967) Growth analysis formulae—their use and abuse. *Crop Science* **7**, 171–175.

Raper, C. D., Jr., and W. H. Johnson (1971) Factors affecting the development of flue-cured tobacco grown in artificial environments. II. Residual effects of light duration, temperature, and nutrition during growth on curing characteristics and leaf properties. *Tob. Sci.* **15**, 75–79.

Tingey, D. T., and U. Blum (1973) Effects of ozone on soybean nodules. *J. Environ. Qual.* **2**, 341–342.

Tingey, D. T., R. C. Fites and C. Wickliff (1973a) Foliar sensitivity of soybeans to O_3 as related to several leaf parameters. *Environ. Pollut.* **4**, 183–192.

Tingey, D. T., R. A. Reinert, C. Wickliff, and W. W. Heck (1973b) Chronic ozone or sulfur dioxide exposures, or both, affect the early vegetative growth of soybeans. *Can. J. Plant Sci.* **53**, 875–879.

Turner, N. C., S. Rich and P. E. Waggoner (1973) Removal of ozone by soil. *J. Environ. Qual.* **2**, 259–264.

U. S. Department of Health, Education and Welfare (1965) Determination of oxidants (including ozone): alkaline potassium iodine method. In *Selected Methods for the Measurement of Air Pollutants.* Public Health Service Publication No. 999-AP-11. D1–D5.

Home Page

OZONE vs OZONE THERAPY: THE PARADOX

FURTHER EVALUATION OF THE THERAPEUTIC INDEX OF OZONE USED IN AUTOHEMOTHERAPY

Contact

Favorite Links

About Page

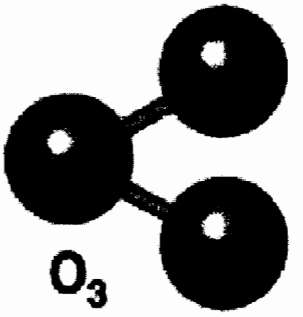
Custom Page

Source:

Journal of Biological Regulators and Homeostatic Agents, 1996; Volume 10, Number 2, Pages 31 - 53

Ozone as a bioregulator:

Pharmacology and toxicology of ozonetherapy today.



Ozone - Triatomic Oxygen (O_3) is gas, created from diatomic oxygen (O_2) atoms.

Velio Bocci

Institute of General Physiology of the Unversity of Siena, Siena - Italy

Abstract:

The disinfectant activity of ozone is well recognized and ozone is used worldwide for sterilization of water. The use of ozone as complementry medical approach is less known, because it has mostely been used in an empirical fashion without a rational basis and appropriate controls. In spite of this drawback, the use of judicious and standardized ozone dosages can elicit the formation of ROS acting as natural physiological activators of several biological functions. There is now a reasonable understanding of a few mechanisms of actions and, using classical pharmacological concepts, it appera possible to formulate a rational for optimizing clinical applications. A further exciting development is that ozone, being an oxidizer,can upregulate the intracellular anti-oxidant enzymes eventually inhibiting the constant, life-long oxidative stress responsible for degenerative diseases and aging. Among various routes for administration of ozone, the autohemotransfusion procedure, consisting in exposing blood to ozone, i.e. to a calculated and brief oxidative stress, appears safe, simple, inexpensive and amenable to be adjusted to different pathological states. It is hoped that this review will help to dispel prejudices, to clarify that ozone toxicity can be tamed, to show that ozone can act a a bioregulator and to encourage controlled clinical investigations to evaluate definitively the validity of ozonetherapy

Send an email

(*Journal of Biological Regulators and Homeostatic Agents, 1996; Volume 10, Number 2, Page 31)*

CAN OZONETHERAPY BE USEFUL IN SOME HUMAN DISEASES?

There are several areas where O_3 - AHT* has been used and examining Table III it seems that ozonetherapy is almost a panacea but actully it is not: the fact that it can be useful in unrelated pathologies is simply due to the fact that ozone can activate distinct blood cells which express different functions. There are five main patholigic areas, namely: 1) infectious states, 2) immune depression, 3) ishemic conditions, 4) neurodegenerative diseases and 5) acute and chronic articular diseases including discal hernias, where, in spite of striking advances, conventional medicine is still unable to provide a definitive improvement. It seems reasonable and ethically correct to take advantage of ozonetherapy when the best orthodox-treatment fails; as an example why patients with either hide-limb ishemia (III-IV grade) facing amputation or chronic hepatitis patients, who do not tolerate IFN, should not try autohemotherapy?

(*Journal of Biological Regulators and Homeostatic Agents, 1996; Volume 10, Number 2, Page 45)*

* AHT=autohemotherapy

---

eMail Prof. Velio Bocci

Back

OZONE vs OZONE THERAPY: THE PARADOX

José Turrent Figueras MD.
Antonio A. Ramírez de Arellano Llovet MD.

Ozone Research Center
PO Box 6880, Havana City, Cuba
Phone Number - 53 7 21 2089

Received by OMRI
AUG 12 2002

OZONE

Ozone is recognized as a very powerful oxidizing agent (1,2) capable of polluting the environment and producing adverse effects when inhaled by humans (3). It has been established that short periods of ozone exposure through the airways, produces reactions that include: reduction in ventilatory function; increased permeability and reactivity of the respiratory tree; an increase of the endogenous mediators and inflammatory cells, and a decrease of neumocytes Type 1, in the alveoli (4,5,6). Bronchoalveolar lavage fluids from humans exposed to ozone exhibit increased neutrophil infiltration and increased content of inflammatory mediators and cytokines (1). Some investigators correlate the neutrophil infiltration with high levels of interleukin 8 (IL-8) that are found in these fluids (3). Also, it has been reported that the damage that ozone causes, when inhaled, is directly related to the release of arachidonic acid of the cellular membrane of the lungs, producing an increase in Leukotrienes levels, the first responsible for the chemotaxis process. As a result, neutrophils are attracted toward the pulmonary tissue causing local damage (6).

In animals that inhaled ozone for hours or days, alterations in the biochemistry and the pulmonary morphology were observed, as was a potential for bacterial respiratory infection. The morphological changes were seen in the terminal bronchus and in the alveoli, accompanied by damage to the ciliated cells and the alveolar epithelium Type I. These structures are replaced, later, by a proliferation of Clara-cells and epithelial cells Type II, respectively (2). It has been reported that the presence of tumoral nodes (adenomes) on the pleural surface in animals exposed to ozone through the airways, showed a significant statistical difference from the control group (not exposed to ozone). Other cellular alterations include hyperplasia and metaplasia, especially those which can be considered as inflammatory reactions (2). In this sense it is reported that animals, exposed to ozone by inhalation, show a dose dependent effect, generating inflammation in the centriacinar region of the lung with ulterior fibrosis at that level (7). It is also reported that ozone exposition promotes or causes DNA damage (8).

While considering all these aspects, much is still unknown about ozone and its harmful effects. Research has produced differential and sometimes paradoxical results. For example, it has been seen that ozone inhalation did not produce carcinogenic effects nor did it increase the incidence of pulmonary neoplasia in rats of both sexes (7). Schulz et al. report anticarcinogenic effects in NMRI mice treated with urethane and ozone (9).

A phenomenon has also been reported of tolerance to ozone in experimental animals that have inhaled

a low dose, during long periods of time. This effect has been related to an increase in the antioxidant enzyme level, namely glutathione S-transferase, glutathione peroxidase, catalase and superoxide dismutase (10,11). Other studies have found a decrease in the damage done to the pulmonary tract in rats which were exposed previously to low doses of the gas for seven days followed by high ozone concentrations. This suggests that initially low doses may reduce the permeability of the lower airways and causes them to face out thus providing protection later when greater ozone concentrations are administered (12).

These discrepancies in ozone research data, the well-know adverse effects when ozone is inhaled, as well as the paradoxical effects found after the utilization of the gas, give us some reasons for reflection.

From the epidemiological point of view, it is seen that only some projects have been used to study the effects of ozone exposure by airways and that more studies are needed to evaluate and distinguish between acute and passing effects of ozone. Also, further research is needed to determine the extended effects of this gas on premature pulmonary aging, and on the symptomatology and the mortality of human beings. Future studies should investigate a wider range of variables in the effort to obtain a more comprehensive interpretation of the phenomenon described above (13).

OZONE THERAPY

Ozone employed for medical purposes is a gas constituted by an ozone/oxygen mixture it is obtained by means of an electrical discharge through pure oxygen, achieving concentrations between 0,05 and 5 in percent of volumes. Chemically it is a triatomic molecule and an allotropic form of oxygen (14).

After the discovery of ozone, by Christian Friedrich Schöumlnbein in 1840, many decades passed without any interest in its uses in medicine. It was not until the beginning of World War I when Albert Wolf used the gas for the first time for therapeutic purposes, in particular for the healing of infected wounds. Wolf also employed ozone, using its deodorant property, in patients with rectal and gynecological cancer (15).

Thereafter, the lack of plastic materials for the application of the gas, the discovery of new antibiotic drugs (namely sulphonamides and penicillins), and a certain skepticism that always has been associated with the applications of ozone in the field of medicine have impeded development of medical applications (16).

Dr. Joachim Hänsler from Germany, in the late 1950s, invented and designed the first therapeutic ozonizer with the use of plastic materials. This opened new perspectives for the application and extension of ozone therapy (16). Unfortunately, the scarce studies of the biological bases of ozone therapy and the clinical experience, although vast, have been limited to private practice and have produced in mainly anecdotal material which was not published in peer-reviewed journals. Moreover the general knowledge that ozone is a serious pollutant that can generate oxidizing compounds has comprehensibly prejudiced the public against its use (17).

BIOLOGICAL ACTIONS AND THERAPEUTIC PROPERTIES OF OZONE

As ozone is an extremely reactive and unstable gas, it has been postulated that the mechanisms through which it acts are directly related to the products that it generates (18) through selective interaction with organic compounds that are present in the plasma and in the cellular membranes. For this selectivity, the reaction of ozone with lipids occurs in the carbon-carbon double bond which is present in polyunsaturated fatty acids, thereby generating organic peroxides and ozonides (19). All

these products, in a controlled and appropriate quantity, can exert different biological actions, namely those which confer on ozone a series of therapeutic properties (20-26). These are shown in Fig.1.

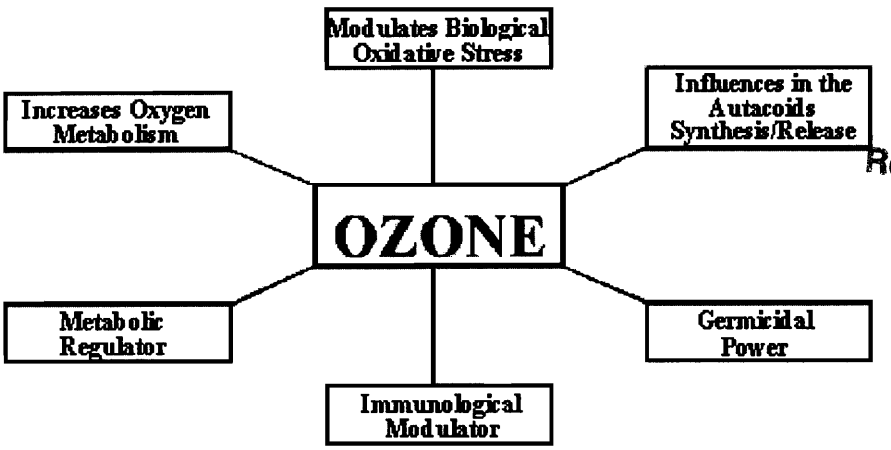


Figure 1. Biological actions of ozone

Figure 1. Biological actions of ozone

These biological effects produce beneficial results when ozone is applied therapeutically in appropriate doses without producing any adverse reactions (27), especially genotoxic damage (28). The wide range of effects thus generated make possible its application in a diversity of medical specialties, and within these, different pathological processes.

ck

References

1. Wright DT. Ozone stimulates release of platelet activating factor and activates phospholipases in guinea pig tracheal epithelial cells in primary culture. Toxicology and applied Pharmacology 1994;127: 27-36.
2. Victorin K. Review of genotoxicity of ozone. Mutation Research 1992; 277: 221-238.
3. McBride DE, Koenig JQ, Luchtel DL, Williams PV, Henderson WR. Inflammatory effects of ozone in the upper airways of subjects with asthma. Am J Respir Crit Care Med 1994; 149:1192-1197.
4. Morton L. Use of human lung tissue for studies of structural changes associated with chronic ozone exposure: Opportunities and critical issues. Environ Health Persp Supp 1993; (102)Supp.4: 208-213.
5. Madden MC, Eling TE, Dailey LA, Friedman M. The effect of ozone exposure on rat alveolar macrophage arachidonic acid metabolism. Exp Lung Res 1991;17:47-63.
6. Doelman CJ. Reactive oxygen species and airway. Amsterdam: Febodruk Ed. 1991:7.
7. Boorman GA. Ozone and ozone-4 (N-nitrosomethylamino-1-3(3-pyridyl)-1-butanone in Fisher-344/ N rats. Tox and Pathol 1994;(22)5: 545-553.
8. Cajigas A, Mitchell G, Beam C, Steinberg JJ. Ozonation of DNA forms adducts: A 32P-DNA labeling and Thin-Layer Chromatography technique to measure DNA environmental biomarkers. Arch of Environ Health 1994; (49)1: 25-36.
9. Schulz S. Anticarcinogenic effect of inhaled ozone/oxygen in urethan-treated NMRI-mice. Proceedings Ninth Ozone World Congress, New York 1989: 69-76.
10. Plopper CG, Duan X, Buckpitt AR, Pinkerton KE. Dose-dependent tolerance to ozone. IV. Site-

specific elevation in antioxidant enzymes in the lung of rats exposed for 90 days or 20 months. Toxicol Appl Pharmacol 1994;127: 124-131.
11. Duan X, Buckpitt AR, Plopper CG. Variation in antioxidant enzyme activities in anatomic subcompartments within rat and rhesus monkey lung. Toxicol Appl Pharmacol 1993;123: 73-82.
12. van der Wal WA, van Bree L, Marra L, Rombout PJ. Attenuation of acute lung injury by ozone inhalation. The effect of low level pre-exposure. Toxicol Lett 1994; (72)1-3: 291-298.
13. Muñoz A. Design and analysis of studies of the health effects of ozone. Environ Health Persp Supp 1993; (101)Supp.4: 231-235.
14. Rilling SH. The basic clinic applications of ozone therapy. OzoNachrichten 1985; Heft 1/2: 7-17.
15. Viebahn R. The use of ozone in Medicine. 2nd. Rev. Germany: Haugh Pub Ed., 1994: 7, 22, 100.
16. Rilling SH. 30 years of ozone-oxygen therapy: A historical perspective. Proceedings Eleventh Ozone World Congress. Ozone in Medicine. San Francisco 1993: M-1-3 to M-1-6.
17. Bocci V. Ozone therapy today. Proceedings 12th World Congress of the International Ozone Association. Ozone in Medicine. Lille, France 1995: 13-27.
18. Gabrielson EW, Yu XY, SpannhakeWE. Comparison of the toxic effects of hydrogen peroxide and ozone on cultured human bronchial epithelial cells. Env Health Persp 1994; (102)11: 972-974.
19. Pryor WA, Uppu RM. A kinetic model for the competitive reactions of ozone with amino acid residues in proteins in reverse micelles. The J of Biolog Chem 1993; (268) 5: 3120-3126.
20. Viebahn, R.: The biochemical process underlying ozone therapy. OzoNachrichten 1985; Heft 1/2: 18-22.
21. Bocci V. Ozonization of blood for the therapy of viral diseases and immunodeficiencies. A hypothesis. Medical Hypotheses 1992;39: 30-34.
22. Bocci V. Autohemotherapy after treatment of blood with ozone. A reappraisal. The J of Intern Med Res 1994; 22:131-144.
23. Bocci V. A reasonable approach for the treatment of HIV infection in the early phase with ozonetherapy (autohemotherapy). How "inflammatory" cytokines may have a therapeutic role. Mediators of inflammation 1994;3: 315-321.
24. Carpendale MT, Griffiss J. Is there a role for medical ozone in the treatment of HIV and associated infections? Proceedings Ozone in Medicine. Eleventh Ozone World Congress. San Francisco 1993: m-1-32 to m-1-45.
25. Menéndez S, Iglesias O, Bidot C, Puga A, Carballo A. Application of ozone therapy in children with humoral immunity deficiency. Proccedings 12th World Congress of the International Ozone Association. Ozone in Medicine. Lille, France1995: 271-274.
26. Basabe E, Menéndez S, Segarra F, Ponce de León M. Ozone therapy like a favoring element in the rehabilitation of children with hearing loss. Proccedings 12th World Congress of the International Ozone Association. Ozone in Medicine. Lille, France, 1995: 275-278.
27. Jacobs MT. Zwischenfalle und typische komplikationen in der Ozon-saverstoff-therapie. Atti Congresso sull'ozono. Baden-Baden 1981; (11)20: 5-6.
28. Díaz S, Menéndez S, Eng L, Fernández I. No increase in sister chromatid exchanges and micronuclei frequencies in human lymphocytes exposed to ozone in vitro. Proceedings 12th World Congress of the International Ozone Association. Ozone in Medicine. Lille, France 1995: 43-51.

Back

3780

TAP Review for Ozone

Additional Questions

1. Internet search turned up very few references concerning use of ozone in drip lines. These are attached to the report

a. "Cleaning of Drip Lines" by Farouk A. Hassan
b. "Ozonation" Oklahoma State University
c. "Complete Understanding of Ozone Use and Technology" Del Agricultural

2. Could not find requested reference

3 and 4. Discussion in criteria evaluation is sufficient. Some minor additional discussion is included in internet documents.

5. I think it is possible but difficult to separate soil application of ozone for weed control but not for soil pathogens control.

The primary difference is the pounds per acre used. Appropriate record keeping may be able to track this, but since ozone is generated on site, tracking could be more difficult. Assuming honesty and integrity on the part of the producer, I believe it is difficult to justify limiting the amount of ozone used for these primary reasons:

a. The primary detrimental effects are how much ozone escapes into the atmosphere and how deeply the soil is sterilized. The atmospheric problem is dealt with by system design and monitoring. It is also in the producers best interest to not waste the costly ozone. A poorly designed or maintained system for weed control could leak more than a well designed and maintained system for destroying soil pathogens. If both systems are well designed, the pollution of the the atmosphere would be minimal. In practice, it is an identical technique and practice being used.

The problem of how deeply the soil is sterilized is reflected in two concerns. One concern is what residues or breakdown products are left and the other concern is the effects on the soil microorganisms. Some data indicates that the breakdown products of ozone in the soil are beneficial to the microorganisms and subsequently to the crops. The concern of how quickly microorganisms recolonize is dependent on the effects of the residues. Ozone itself does not have significant residues and its breakdown products may actually encourage both the growth and diversity of microorganisms.

b. Ozone treatment for soil pathogens is a possible replacement for far more toxic materials (which, ironically deplete atmospheric ozone) and its use should be encouraged from the environmental perspective. The environmental perspective is an important element of the organic industry both in producers intention and in

Additional Questions pg 2 of 2

market expectations. Organic producers and consumers are both trying to move agriculture in a more environmentally sound and sustainable direction. A material which does this should be given consideration for use in organic agriculture.

c. Ozone's use in the soil is a technique as well as a material that affects both weeds and microorganisms at all levels of use. If it is approved for weed control but not soil pathogen control, it will be hard to specify what level will be allowed. In some regions for some weeds, the application rate needed to be effective may also be effective for controlling some soil pathogens. On what basis should it be decided which weeds and pathogens are allowed to be controlled by this technique (and which aren't) since the technique is the same and the residues similar at all levels?

For these reasons, I think if Ozone is approved for weed control, it should also be allowed for soil treatment.

Additional Consideration

This reviewer also considered post harvest treatment of fruits and vegetables along with the specific uses petitioned for and described on lines 77 - 79 of Ozone TAP Review. It is already approved for processing by the USDA National List and clarification for post harvest seems appropriate in this review.
See attachment "Ozone May Stop Bacteria on produce Better Than Food Irradiation and Current Washing Methods, Says UF Expert"

OZONE SCIENCE & ENGINEERING
Vol. 23, pp. 207-217
Printed in the U.S.A.

0191-9512/01 $3.00 + .00
International Ozone Association

Ozone In Medicine

V.Bocci[4], C.Aldinucci, E.Borrelli,[1] F.Corradeschi, A.Diadori[2], G.Fanetti[3] and G.Valacchi

Institute of General Physiology, University of Siena, Via A. Moro, 53100 Siena, Tel: 0039 0577 234226;
Fax: 0039 0577 234219 ; email: Fisgen@unisi.it
[1)] Institute of Thoracic and Cardiovascular Surgery
[2)] Department of Ophthalmology of the University of Siena
[3)] Servizio Trasfusionale, Azienda Ospedaliera Senese, Siena, Italy, Tel: 0039 577 585070, Fax: 0039 577 586167
[4)] Corresponding author

Received for Review: 9 March 2000
Accepted for Publication: 6 December 2000

Abstract

Ozone therapy has been used as a complementary medical approach for half a century but it has encountered skepticism by orthodox medicine because, particularly in the past, it has been used by practitioners and others without a rational basis and appropriate controls. With the advent of modern medical ozone generators incorporating a photometer, it has become possible to obtain precise ozone concentrations and to evaluate some mechanisms of action and possible toxicity. In contrast with the respiratory tract, human blood exposed to appropriate ozone concentrations is able to tame its strong oxidant properties and neither acute, nor chronic side effects have ensued in millions of patients treated with ozonated autohaemotherapy (O_3-AHT). This review summarizes our studies aimed at clarifying biological effects, defining any possible damage, the therapeutic window and suitable doses able to express a therapeutic activity. A very interesting and promising aspect is the induction of the so-called heat stress proteins (HSP) leading to adaptation to a chronic oxidative stress. The use of ozone in human therapy has been reviewed but so far very few controlled clinical studies have been reported. Mostly on the basis of anecdotal results, ozone therapy appears useful in infectious diseases, immune depression, vascular disorders, degenerative diseases and orthopedics.

Key Words

:one; Medical Applications; Reactive Oxygen Species; Antioxidants; Hemotherapy; Ozone Tolerance;

Introduction

though ozone has been used as a potent infectant since the first World War (1), its idity in medicine still remains controversial, even ıugh the National Health Institutes of several ıntries, namely Germany, Italy, Austria, Russia l some of the United States now include ozone rapy and bio-oxidative therapy among the ırmacological approaches of complementary c . In most of the United States, the problem ozone, as one of the worse pollutants in large cities, has acquired such a preeminent consideration that it practically denies its use in medicine. Studies in vitro and in vivo (2-5), confirming its toxicity for the respiratory tract have led to the conclusion that ozone is "always" toxic for humans, animals and plants. The authors believe that the generalization of this conclusion is, at least in part, unjustified because we have demonstrated that judicious use of ozone can be therapeutically useful and atoxic (6-10). There is no doubt that ozone is intrinsically toxic (11), but as any other drug, when used properly, has a definite therapeutic window. Moreover, every year millions of

patients all over the world undergo some sort of ozone therapy and minimal, if any, side effects have been noted. Some charlatans, mostly without any medical qualifications, have caused a few deaths because they inject directly the gas intravenously, a procedure prohibited since 1986 in Europe (7;8;10). It is unfortunate that even today a few physicians and many naturopaths and others, owing to the fact that they cannot practice the classical hemotherapy, predicate that intravenous injection of oxygen-ozone is "the only effective way". This crucial problem will be discussed in order to clarify the danger and its basic irrationality.

The purpose of this brief review is four fold: firstly, to present data from our Laboratory that show how ozone, coming in contact with biological fluids, decomposes and generates reactive oxygen species (ROS), secondly, to define how ozone's messengers can activate biochemical and immunological mechanisms leading to biological effects, thirdly, to show that we are now able to determine a therapeutic window or, in other words, a range of biologically active concentrations below which ozone is practically inactive and above which can be toxic. Fourthly, we will attempt to analyze the results regarding therapeutic efficacy in five main areas: infectious diseases, immune depression, vascular disorders, degenerative diseases and orthopedics. The breadth of ozone therapy, rather than arising the suspicion of a "panacea", ought to be envisaged as due to the multiform action of ozone on cells with different functions.

The knowledge recently acquired allows one today to plan rational clinical applications in different diseases and to evaluate the therapeutic activity and side effects. Future breakthroughs can be achieved only if we are able to grasp firstly, the biological activity of lipid oxidation products (LOPs), secondly, the practical implications of the ozone tolerance by clarifying the role of heat-stress proteins (HSP) and, thirdly, if we will be able to carry out randomized, double blind clinical trials possibly performed in several medical centers.

The present paper intends to give a general overview of the results so far achieved and therefore technical details can be found in previous papers (12-15).

Ozone Mechanism

Progress in this field is expected only if we are able to clarify precisely the mechanisms of action because it will allow defining the therapeutic dose and possible toxicity. We will examine separately the possible mediators broadly defined as reactive oxygen species (ROS) and the cell targets that are ultimately responsible for the therapeutic response.

Ozone's active messengers

Both oxygen (about 97%) and ozone (no more than 3%) dissolve in biological fluids according to their solubility, relative concentrations, partial pressure and temperature (1). However, there is a critical difference between these two gases because oxygen is fairly stable in solution while ozone decomposes immediately by avidly reacting with polyunsatured fatty acids (PUFA) (11). This implies that ozone does not obey Henry's law and therefore an extremely dynamic equilibrium arises between the ozone in the gas phase and the ozone reacting and disappearing in the aqueous solution. Thus, we can envisage a continuous flow of ozone into the solution from the gas phase until the latter is exhausted. It is felt that this crucial instability has not been fully appreciated by cell biologists, who examine ozon toxicity in tissue cultures maintained in a gas phase where concentration of ozone, although very low (0.2 - 1 ppm), remains stable for several hours or days of incubation. The final results are misleading because it is obvious that overall cell toxicity cannot be simply attributed to the low ozone concentration, but to the uncalculated total sum of ozone that during every millisecond has passed into the solution. In other words, a cell layer in culture exposed to an ozone concentration as low as 0.1 ppm may not be damaged if the exposure lasts only ten min whereas total cell death may ensue after 60 min exposure because ozone will continue to dissolve during the following 50 min reaching the lethal amount.

It has been shown (11) that the reaction between a mole of an unsaturated fatty acid containing a cis-double bond and O_3 in water generates two moles of aldehyde and one mole of hydrogen peroxide (H_2O_2).

$$R - HC = CH - R^1 + O_3 + H_2O \rightarrow R - COH + R^1 - COH + H_2O_2$$

H_2O_2 is also generated when O_3 reacts with physiological saline.

The reduction potential is smaller (0.682 volt) in the semireaction towards H_2O_2 than directly towards H_2O (1.229 volt). Indeed we have demonstrated (14) that after ozonation of either saline or human plasma, H_2O_2 is formed in both liquids with the important difference that H_2O_2 in the plasma has a very short half-life (about 2.5 min) due to the presence of traces of enzymes such as glutathione peroxidase (GSH-Px) and catalase, which are able to degrade H_2O_2. Appropriate enzyme inhibitors are able to prolong the lifetime of H_2O_2 while addition of catalase, as it was expected, accelerates its decay (14). The other important reaction is that O_3, by reacting with PUFA, will generate a number of LOPs such as hydroperoxides, isoprostanes, platelet-activating factor (PAF) and terminal products such as malondialdehyde and 4-hydroxynonenal (HNE) (16-21). The latter compound is becoming particularly interesting because, depending upon its final concentration (>10 μM or < 1 μM), may either be harmful or act as a physiological messenger, respectively (22;23). Owing to the wealth and heterogeneity of PUFA, several types of LOPs may be generated and their biological activities, including potential toxicity, remain to be explored *in vivo*. Once again, results obtained in vitro by using apparently toxic LOPs may not be applicable in vivo owing to their rapid turnover and, as an example, enzymes such as glutathione transferases and aldehyde dehydrogenases are involved in the metabolism of HNE (16). Phospholipases and sphyngomyelinase are likely to be activated by LOPs and this may lead to an amplification of some biological processes. Furthermore LOPs have a short half-life but, upon reinfusion of ozonated blood, may reach specific sensors situated in critical organs such as bone marrow, spleen, liver and other sectors of the immune system. If this is true, LOPs may be responsible for transmitting the information of peroxidative stress and possibly inducing the upregulation of antioxidant enzymes, hence the tolerance to O_3. This phenomenon dubbed as "oxidative stress adaptation" or oxidative preconditioning (7;24-26) is extremely interesting because it could allow a reversal of chronic oxidative stress typical of degenerative diseases. We have already demonstrated an increase of antioxidant enzymes (7) and we are examining levels of heme oxygenase (HO) activity. The isoform 1 of the latter enzyme (HO-1), also known as heat shock protein 32 (hsp 32), is inducible and is responsible for the conversion of heme into biliverdin, carbon monoxide (CO) and free iron (27-29). Cytochrome P450 constitutes another source of heme undergoing degradation via HO-1. We would like to emphasize that the above products, until recently regarded as toxic waste destined only for excretion, are compounds with great physiological and a possible therapeutical role: bilirubin (via biliverdin reductase) is a crucial lipophylic antioxidant and CO may function as a gaseous regulator of endothelial tone in synergy with nitric oxide (NO). Indeed we have just demonstrated that human endothelial cells exposed to ozonated plasma increase the release of NO (30). Nitrosothiols such as S-nitrosocysteine, S-nitrosoglutathione and S-nitrosoalbumin, formed in human plasma to buffer NO's concentration, have physiological significance because function as a reservoir for NO (31). Another important mechanism of activation that has been partly clarified (13) is the opening of Ca^{2+} channels somehow related to ROS acting on the external part of the cell membrane leading to a sudden increase of intracellular Ca^{2+} concentration with consequent enzymic activation. So far we have only indirect evidence of this phenomenon by either chelating the extracellular Ca^{2+} with citrate used as a blood anticoagulant or by adding from 5 up to 25 mM Ca^{2+} in heparinized blood (13) but obviously it will be important to measure the actual intracellular increase of Ca^{2+}

Effectors and the biochemical targets

It is now clear that ozone works indirectly in different ways: owing to the fact that H_2O_2 is an unionized molecule and its passage through the cell membrane is free, its sudden increase in the extracellular water is immediately transferred into the intracytoplasmic water, but the intracellular environment counteracts this potentially toxic increase by quenching it with reduced glutathione (GSH) coupled to GSH peroxidase. This causes an increase of oxidized glutathione (GSSG) and a decrease of the GSH/GSSG ratio, which is rapidly reconstituted by the action of GSH reductase in turn exploiting the NADPH/NADP reservoir. Lowering the NADPH level enhances the activity of glucose 6 phosphate dehydrogenase (G6PD) that, particularly in the erythrocytes, leads to the activation of the hexose monophosphate shunt. When necessary an excessive increase of intracytoplasmic H_2O_2 is also double checked by catalase. There is a concomitant activation of glycolysis with increased ATP and a still controversial increase of 2-3 diphosphoglycerate (2-3

DPG) production as key enzymes involved in this process have not yet been shown to be activated (32;33). Obviously the shift to the right of the HbO_2 dissociation curve would favor an increased oxygen delivery to hypoxic tissues (7;10). It has also been claimed (34) that the erythrocytic membrane becomes more fluid and more negatively charged, that blood viscosity decreases due to hypofibrinogenemia and to a decreased level of low density lipoproteins (LDL). However Morgan et al. (35) found that erythrocytes from ozone-exposed mice exhibited decreased deformability and therefore all of these claims must be controlled because we must be sure if indeed ozone can improve, blood rheology in ischemic diseases.

As far as the activation of cytokine synthesis in leukocytes is concerned, it is now well accepted that the sudden surge of intracytoplasmic H_2O_2 is finally responsible for the activation of the nuclear transcription factor (NF-kB). Briefly, H_2O_2, by activating specific protein kinases, would phosphorylate the I-KB subunits that detach from the NF-kB complex. The free heterodimer (p50-p65 proteins) can then move into the nucleus where, after binding to DNA control elements, activates gene expression and the successive synthesis of interferons and interleukins as shown by us (12-15;36) and others (37;38).

The transient rise of intracytoplasmic H_2O_2 prompts a few considerations: the first one is that the O_3 concentration must be adequate to allow a sufficient H_2O_2 generation for the activation of transducer molecules and to counteract the simultaneous degradation, and the second is that H_2O_2 concentration must reach a critical threshold. If it is below the liminal value, activation will not occur but if it is excessive, damage may result implying the relevance of having identified the therapeutic window between about 20 and 80 µg/ml of gas per ml of blood. If the O_3 concentration is below 20 µg/ml, most of the oxidant power of O_3 will be quenched by the natural antioxidants (between 1.28 and 1.83 mM plasma) (39) and therefore the necessity of measuring precisely the O_3 concentration to avoid either a placebo or a toxic effect is of crucial importance. On the experimental basis of progressively increased hemolysis, ozone concentrations higher than 80 µg/ml are more likely detrimental than beneficial.

Little is known about the biological activity of LOP such as hydroperoxides, isoprostanes, malondialdeh and 4-hydroxyalkenals produced during b ozonation. Aggregation of platelets, as we have observed in platelet rich plasma anticoagulated with heparin (40), is at least in part attributable to released PAF (21). While some of these can act as physiological messengers (18;20-23) they appear to be, particularly in vitro, very toxic (17;22;23). Their production and consequent plasma levels are somewhat related to the ozone dose and it is conceivable that in vivo a low ozone dose may express a more favorable activity/toxicity ratio than a higher ozone dose. Thus, once again, we should aim to define in different pathologies the optimal dose that may be either in the low (20-40 µg/ml per ml of blood), or in the medium-high range (30-80 µg/ml per ml of blood).

Moreover LOPs may exert the overlooked and yet crucial function for transmitting the information of on ongoing peroxidative stress to distant organs with the purpose of inducing the "oxidative stress adaptation" or ozone tolerance (24-26;41-48). This can be achieved only by slowly activating gene expression towards the synthesis of heat-shock proteins, antioxidants enzymes (GSH-Px, catalase, superoxide dismutases etc), DNA repair enzymes and, most important, heme-oxyge (27-29). This may lead to increased bilirubin levels (49) and local release of CO that, associated to increased endothelial production of NO (30) may well explain the vasodilation and consequent clinical improvemen observed in limb ischemia treated with O_3 AHT. It is almost needless to say that upregulating the production of antioxidant enzymes in patients with degenerative diseases (favored or caused by a life-long oxidative stress) is the simplest way to readjust the redox balance possibly leading to a stabilization of the disease Administration of antioxidant compounds may be helpful (50;51) but, most likely, not so effective for neutralizing ROS as the intracellular increase of antioxidant enzymes.

Applications of Ozone Therapy in Medicine

Today, a better understanding of the basic reactions of ozone able to activate different biological functions allows the dispelling of skepticism surrounding ozone therapy. Although its application is extremely versatile there are two important limitations: firstly, ozone should never be inalated as the fluid film lining the tracheo-bronchial mucosa is too thin to protect it from oxidative insult (11) and secondly, the gas mixture O_2/O_3 should never be injected intravenously (IV) either

because it can cause oxygen embolism and because no meaningful blood /ozone ratio can be ever calculated. We will never get tired of repeating that human organism, although composed of almost 66% water should not be compared and treated as a water sterilization plant. Were we to allow the IV gas administration, it would cause severe side effects and many deaths each year.

On the other hand the approach consisting in the exposure of a precisely measurable volume of the patient's blood (200-250 ml) to an equal volume of gas (1 to 1), of which the ozone concentration can be accurately measured in real time by photometry, is by far the most scientific, simple, inexpensive and side-effects free procedure. Most of the merit goes to Wolff (52) who applied the ozonated autohemoterapy (O_3-AHT) in the late 70s. The optimized procedure that must be carried out in neutral glass and ozone-resistant tubing where the inlet is separated from the outlet equipped with a standard blood filter has been recently described in detail (53). Standard autotransfusion bags made of polyvinyl chloride (PVC) additioned with about 40% additives have been banned by the Italian Ministry of Health after our demonstration (53) that ozone causes the release of significant amounts of plastic microparticles and phthalates into the blood.

Other routes of administration of ozone can be allowed for selected applications: the subcutaneous (SC) route for treating lipodistrophy; the intramuscular (IM) route into the paravertebral muscles after locating the point(s) triggering low back pain; the intradiscal- intraforaminal and/or the epidural route for treating a herniated disc; the intraarticular or periarticular route for treating acute and chronic arthrosis. Knoch et al. (54), Carpendale et al. (55) and ourselves (56) have evaluated pros and cons of the rectal insufflation of O_2-O_3 as a possible option when O_3-AHT cannot be used for difficult venous access. This route has been used in human immunodeficiency virus (HIV) infection (55), chronic hepatitis, ulcerative colitis and Crohn's disease with apparently satisfactory results (54) using up to 800 ml of O_2-O_3 at a maximal O_3 concentration of 40 µg/mL administered within 5 minutes. In the case of chronic bacterial and parasitic infections becoming resistant to antibiotics, low O_3 concentration (3-5 µg/mL) have been also insufflated into the oral, nasal, tubal (during 30 sec apnea), vaginal, urethral, vesical, pleural and peritoneal cavities. Obviously the technique of gas insufflation is a very empirical and approximate one but it can be useful, as ozone does not allow bacterial resistance.

Which are the diseases likely to benefit from the application of ozone therapy? It appears reasonable and ethical to use ozone especially when conventional therapies are ineffective or not available as too often occurs in poor countries. Obviously, by considering the potent disinfectant action of O_3, top priority goes to all sorts of bacterial, viral and fungal infections. Either gas, or ozonated water, or ozonated oil display a cleansing and disinfectant effect (1;57-63).

Moreover O_3-AHT, combined with topic therapy, can be helpful because, as previously discussed, it activates cell metabolism and the immune system. Indeed various immunodeficiencies associated with chronic viral diseases and metastatic cancer, particularly after high-intensity chemotherapy, may benefit from a long cycle (about 50 treatments, twice weekly for six months) of O_3-AHT that, in comparison to interferon, highly active antiretroviral therapy (HAART) and cytostatics does not procure acute or chronic side effect (64;65). Actually the majority of patients reports an unusual feeling of well-being that should not be neglected.

Unfortunately, for the time being, we have to rely on anecdotal reports (65). One clinical study in HIV infection (66) yielded doubtful results because blood was badly mistreated by heat, UV irradiation and O_3 in unknown concentration.

In western countries several circulatory disturbances (hind-limb ischemia, heart-brain-retinal ischemia) due to atherosclerosis, diabetes, smoking, aging and a too intense lifetime oxidative damage represent a formidable medical problem that cannot be entirely coped by orthodox medicine.

O_3-AHT has shown therapeutic effects particularly in patients refractory to conventional treatments because, as it has been mentioned, expresses multiple actions such as vasodilation, increased delivery of oxygen in hypoxic tissues and release of wound healing factors (67). Clinical results in acute cerebro-vascular disorders, chronic ischemic cardiopathy and even in the III-IV stages of hind-limb ischemia have been remarkable, particularly, when a systemic treatment was combined with a topical one on torpid ulcers and incipient necrosis (33; 68-70). Two randomized, placebo controlled (O_2-AHT) cross-over studies have been performed to evaluate the efficacy of O_3-AHT in patients with age-

related macular degeneration (ARMD) (33) and with mild hypertension (71). Significant clinical improvement was achieved in both trials although it faded 2-4 months after the end of the treatment. However, as it happens with other medications, this is to be expected and can be minimized by continuing the treatment at a slow pace.

As far as degenerative diseases are concerned, preliminary studies by using O_3-AHT and O_3 rectal insufflation carried out in patients with cardiac infarction (72), neurodegenerative disease (73) and ARMD (33) have shown clinical improvement and interestingly a progressive increase in GSH Px, glucose-6-phosphate dehydrogenase and superoxide dismutase in erythrocytes. However there is an urgent need for programming controlled studies in order to show that ozone therapy can induce a state of oxidative stress adaptation, possibly capable of stabilizing the disease.

Finally injections of small volumes of O_2-O_3 at a O_3 concentration below 30 μg/ml are being used in orthopedic pathology, via peri, or intrarticular, or intradiscal injection (74; 75). It appears that the treatment that is occasionally painful for a few minutes has no side effects and in about 70 % of patients allows pain relief, decongestion, reabsorption of edema and improved mobility (74; 75). How ozone works remains hypothetical: after intradiscal injection, ozone generates hydroxyl radicals ($OH^{\bullet}$) measured by electron spin resonance (Bocci et al, manuscript in preparation) that can degrade proteoglycans in the degenerate nucleus pulposus leading to its reabsorption with consequent reduction of herniated material responsible for radicular pain. In the synovial membrane ozone therapy may either induce the release of immunosuppressive cytokines and/or proinflammatory cytokine antagonists as well as the over-expression of antioxidant enzymes able to block excessive ROS formation. In regard to the injection of 5-10 mL O_2 -O_3 (15-20 μg/ml) into the trigger points of paravertebral muscles correspondent to the metamers of the hernial disc, we have proposed (76) that the "chemical acupuncture" due to the needle and ozone inhibits amyelinic nociceptors fibers and activate the antinociceptive system. This explanation appears plausible because the successive analgesia permits muscle relaxation and vasodilation with consequent improvement of local muscular physiology and disappearance of pain. Brayda-Bruno and Cinnella (77) have reported that about 70 % of patients improve after a few session of this easy, risk free procedure. It worth noting that lower back pain syndrome is very common and it is advantageous to try this minimally invasive treatment. However, as it was proposed in 1998 (76), it is impellent to compare this procedure against a wait-list control, two placebo controls (one with O_2 alone and another without any gas) and a standard-treatment control.

Conclusions and Perspective

On the basis of experimental results obtained in the last decade (6;7;9;12-15;24-26;36-38;40), we have selected a range from 20 up to 80 μg/ml of ozone per ml of blood to be used for different pathologies, within which, no damage to blood components has been noticed. An orientative scheme of dosages has been previously reported for different diseases (10) depending upon whether the therapeutic activity is mainly exerted by either erythrocytes or leukocytes (7; 8;10). In order to avoid toxicity and allow oxidative stress adaptation, we are applying the "start low, go slow" principle: that is O_3-AHT is performed starting with very low ozone concentrations (20-25μg/mL per ml of blood) to be increased in single steps of 5 μg/mL to the highest level between 40 and 80 μg/mL depending upon the disease and the state of the patient (10).

Although we do not yet have unequivocal clinical data based upon controlled double-blind studies, we have encouraging evidence suggesting that ozone therapy can be useful in vascular, infectious and degenerative diseases (1;7;10;34;55;57-63;68-73). Whether ozone therapy can be useful in metastatic cancer (65) and surprisingly in orthopedics (74-77), respiratory and immune diseases remains to be seen and it should be ascertained starting with cautious and controlled experimentation.

Even if, theoretically, ozone therapy implies always an oxidative insult, this must be carefully calculated on the basis of a precise ozone dose and brief time of exposure. Luckily this is possible owing to the large antioxidant potential of blood (39;40;50;51) that is practically impossible to overwhelm with the indicated ozone concentrations. Moreover, during the course of therapy the total antioxidant status must be sustained by daily administration of antioxidant vitamins (0.5 g of vit C, 10 mg vit E, Se, etc and at least 0.6 g of N-acetylcysteine as a precursor of GSH) accompanied by a diet rich in fresh vegetables and fruits.

concept of "oxidative stress adaptation" must thoroughly evaluated because it is expected to to great improvements. If this idea will prove to be correct against all the most pessimistic views of ozone as a therapeutic agent (11), we will have demonstrated that ozone is indeed a paradoxical molecule and that prejudices are the worst foes of biology and medicine. In order to achieve a suitable and smooth adaptation, the best strategy seems to start with low and slowly increasing ozone dosages. Two to three weeks may be necessary before measuring a substantial increase of antioxidant enzymes in erythrocytes. One must also take into account that erythrocytes have a fairly slow turnover (78) and therefore it takes a few weeks before the newly "super-gifted" erythrocytes, released from the bone marrow, can progressively substitute the old ones. Thus the application of the "start low, go slow" principle (10) appears reasonable for demonstrating the validity of the concept.

In conclusion, in spite of our efforts during the last decade to give a solid scientific basis to ozone therapy, much work remains to be done. Ozone therapy is in the middle of a schizophrenic situation: on one hand, if one reads the weekly reports in the lists, one remains appalled by wonderful apeutic achievements obtained in most cases by charlatans without any medical qualification. This is very detrimental for the real progress of ozone therapy as desperate patients searching a hopeful treatment are not in the position to distinguish between the truth and the fake. On the other hand, in the age of molecular medicine and gene therapy, ozone therapy appears at best as an obsolete, empirical and still doubtful approach. It reminds the well-known Indian story about the blind men and the elephant. We touch it, we smell it but we still do not see it. However, as it happens in Science, even gene therapy that seemed so promising present great problem (79). Against all the odds, I firmly believe that if we can continue with an appropriate biological and clinical experimentation, ozone can become an important therapeutic agent because it can reactivate a variety of biological functions crucial for regaining health and is very cheap, easy to use, versatile and atoxic, if used properly.

Acknowledgements

This work was partly supported by MURST grants (ex-40%). The editorial assistance of Mrs. Helen Carter and Patrizia Marrocchesi is gratefully acknowledged.

References

1. R.Viebahn, The use of ozone in medicine (Iffezheim: ODREI Publishers, 1999), p.1-148.

2. M.G.Mustafa, "Biochemical basis of ozone toxicity", Free Radic. Biol. Med. 9: 245-265 (1990).

3. R.B.Devlin, W.F.McDonnell, R.Mann, S.Becker, D.E.House, D.Schreinemachers, H.S.koren, "Exposure of humans to ambient levels of ozone for 6.6. hours causes cellular and biochemical changes in the lung", Amer. J. Respir. Cell Molec. Biol. 4: 72-81 (1991).

4. W.A.Pryor, "How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts?", Free Radical Biol. Med. 12: 83-88 (1992).

5. W.H.M.Stevens, P.D.Conlon, P.M.O'Byrne, "Ozone-induced oxygen radical release from bronchoalveolar lavage cells and airway hyper-responsiveness in dogs", J. Physiol. 486.1: 257-265 (1995).

6. V.Bocci, E.Luzzi, F.Corradeschi, L.Paulesu, "Studies on the biological effects of ozone: 5. Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy.", Biotherapy 7: 83-90 (1994).

7. V.Bocci, "Ozone as a bioregulator. Pharmacology and toxicology of ozone therapy today", J. Biol. Regulat. Homeost. Agent. 10: 31-53 (1996).

8. V.Bocci, "Is ozone therapy therapeutic ?", Perspect. Biol. Med. 42: 131-143 (1998).

9. V.Bocci, N.DI Paolo, G.Garosi, C.Aldinucci, E.Borrelli, G.Valacchi, F.Cappelli, L.Guerri, G.Gavioli, F.Corradeschi, R.Rossi, F.Giannerini, P.DI Simplicio, "Ozonation of blood during extracorporeal circulation. I. Rationale, methodology and preliminary studies", Int. J. Artif. Organs 22: 645-651 (1999).

10. V.Bocci, "Biological and clinical effects of ozone. Has ozone therapy any future in medicine ?", Brit. J. Biomed. Sci. 56: 270-279 (1999).

11. W.A.Pryor, "Mechanisms of radical formation from reactions of ozone with target molecules in the lung", Free Radical Biol. Med. 17: 451-465 (1994).

12. V.Bocci, E.Luzzi, F.Corradeschi, L.Paulesu, R.Rossi, E.Cardaioli, P.DI Simplicio, "Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes.", J. Biol. Regulat. Homeost. Agent. 7: 133-138 (1993).

13. V.Bocci, E.Luzzi, F.Corradeschi, L.Paulesu, A.DI Stefano, "Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines", Lymphokine Cytokine Res. 12: 121-126 (1993).

14. V.Bocci, G.Valacchi, F.Corradeschi, C.Aldinucci, S.Silvestri, E.Paccagnini, R.Gerli, "Studies on the biological effects of ozone: 7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone", J. Biol. Regulat. Homeost. Agent. 12: 67-75 (1998).

15. V.Bocci, G.Valacchi, F.Corradeschi, G.Fanetti, "Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production", Mediat. Inflamm. 7: 313-317 (1998).

16. H.Esterbauer, R.J.Schaur, H.Zollner, "Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes", Free Radic. Biol. Med. 11: 81-128 (1991).

17. A.Del Corso, M.Dal Monte, P.G.Vilardo, I.Cecconi, R.Moschini, S.Banditelli, M.Cappiello, L.Tsai, U.Mura, "Site-specific inactivation of aldose reductase by 4-hydroxynonenal", Arch. Biochem. Biophys. 350: 245-248 (1998).

18. G.Leonarduzzi, A.Scavazza, F.Biasi, E.Chicrpotto, S.Camandola, S.Vogl, R.Dargel, G.Poli, "The lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates transforming growth factor β1 expression in the macrophage lineage: a link between oxidative injury and fibrosclerosis", FASEB J. 11: 851-857 (1997).

19. J.D.Morrow, L.Jackson Roberts, "The isoprostanes: unique bioactive products of lipid peroxidation", Prog. Lipid Res. 36: 1-21 (1997).

20. C.N.Serhan, J.Z.Haeggström, C.C.Leslie, "Lipid mediator networks in cell signaling: update and impact of cytokines", FASEB J. 10: 1147-1158 (1996).

21. T.M.McIntyre, G.A.Zimmerman, S.M.Prescott, "Biologically active oxidized phospholipids", J. Biol. Chem. 274: 25189-25192 (1999).

22. M.U.Dianzani, "4-Hydroxynonenal and cell signalling", Free Rad. Res. 28: 553-560 (1998).

23. M.Parola, G.Bellomo, G.Robino, G.Barrera, M.U.Dianzani, "4-Hydroxynonenal as a biological signal: molecular basis and pathophysiological implications", Antiox. Redox Signal. 1: 255-284 (1999).

24. V.Bocci, "Does ozone therapy normalize the cellular redox balance?", Med. Hypotheses 46: 150-154 (1996).

25. E.Barber, S.Menéndez, O.S.León, M.O.Barber, N.Merino, J.L.Calunga, E.Cruz, V.Bocci, "Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia.", Mediat. Inflamm. 8: 37-41 (1999).

26. O.S.León, S.Menéndez, N.Merino, R.Castillo, S.Sam, L.Pérez, E.Cruz, V.Bocci, "Ozone oxidative preconditioning: a protection against cellular damage by free radicals", Mediat. Inflamm. 7: 289-294 (1998).

27. R.Galbraith, "Heme oxygenase: who needs it?", Proc Soc. Exp. Biol. Med. 222: 299-305 (1999).

28. F.Amersi, R.Buelow, H.Kato, B.Ke, A.J. X.D.Shen, D.Zhao, J.Zaky, J.Melinek, C.R.Lass J.K.Kolls, J.Alam, T.Ritter, H.D.Volk, D.G.Farme R.M.Ghobrial, R.W.Busuttil, J.W.Kupiec-Weglinsk "Upregulation of heme oxygenase-1 protect genetically fat Zucker rat livers from ischemia/reperfusion injury", J. Clin. Invest 10 1631-1639 (1999).

29. M.Suematsu, Y.Ishimura, "The heme oxygenase carbon monoxide system: a regulator of hepatobiliar function", Hepatology 31: 3-6 (2000).

30. G.Valacchi, V.Bocci, "Biological effects of ozone o human endothelial cells", Mediat. Inflamm. (2000 In press.

31. A.R.Butler, F.W.Flitney, D.L.Williams, "N nitrosonium ions, nitroxide ions, nitrosothiols and iron-nitrosyls in biology: a chemist's", Trends Pharmacol. Sci. 16: 18-22 (1995).

32. R.Viebahn-Hänsler, "Einfluss auf die erythrozytenstoffwechsel". In: Ozon-Handbuch Grundlagen. Prävention. Therapie (Landsberg Lech Ecomed, 1999).

33. A.Diadori, V. Bocci, F. Carraro, A. Ni Corradeschi, G. Ferrari, L. Sabatini, S. Silvest R. Frezzotti, "Ozone therapy and age related macula

degeneration: a pilot study". In: F.CECCHERELLI and F. GIRON (Eds.), L'Ozonoterapia nel 2000 (Torino: Edizioni Libreria Cortina, 1999), pp.33-45.

34. O.Rokitansky, "Klinik und Biochemie der Ozontherapie.", Hospitalis 52: 643-647 (1982).

35. D.L.Morgan, A.F.Dorsey, D.B.Menzel, "Erythrocytes from ozone-exposed mice exhibit decreased deformability", Fundam. Appl. Toxicol. 5: 137-143 (1985).

36. V.Bocci, L.Paulesu, "Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes", Haematologica 75: 510-515 (1990).

37. K.Arsalane, P.Gosset, D.Vanhee, C.Voisin, Q.Hamid, A.B.Tonnel, B.Wallaert, "Ozone stimulates synthesis of inflammatory cytokines by alveolar macrophages in vitro", Amer. J. Respir. Cell Molec. Biol. 13: 60-68 (1995).

38. E.B.Haddad, M.Salmon, H.Koto, P.J.Barnes, I.Adcock, K.F.Chung, "Ozone induction of cytokine-induced neutrophil chemoattractant (CINC) and nuclear factor-kappa b in rat lung: inhibition by corticosteroids", FEBS Lett. 379: 265-268 (1996).

39. N.J.Miller, C.Rice-Evans, M.J.Davies, V.Gopinathan, A.Milner, "A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates", Clin. Sci. 84: 407-412 (1993).

40. V.Bocci, G.Valacchi, R.Rossi, D.Giustarini, E.Paccagnini, A.M.Pucci, P.DI Simplicio, "Studies on the biological effects of ozone: 9. Effects of ozone on human platelets", Platelets 10: 110-116 (1999).

41. M.F.Christman, R.W.Morgan, F.S.Jacobson, B.N.Ames, "Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in Salmonella typhimurium", Cell 41: 753-762 (1985).

42. W.J.Mckinney, R.H.Jaskot, J.H.Richards, D.L.Costa, K.L.Dreher, "Cytokine mediation of ozone-induced pulmonary adaptation", Amer. J. Respir. Cell Molec. Biol. 18: 696-705 (1998).

43. I.Rahman, L.B.Clerch, D.Massaro, "Rat lung antioxidant enzyme induction by ozone", Amer. J. Physiol. 260: L412-L418 (1991).

44. H.Sandermann, JR., D.Ernst, W.Heller, C.Langebartels, "Ozone: an abiotic elicitor of plant defence reactions", Trend. Plant Sci. 3: 47-50 (1998).

45. Y.K.Sharma, K.R.Davis, "The effects of ozone on antioxidant responses in plants", Free Rad. Biol. Med. 23: 480-488 (1997).

46. D.R.Spitz, W.C.Dewey, G.C.Li, "Hydrogen peroxide or heat shock induces resistance to hydrogen peroxide in chinese hamster fibroblasts", J. Cell. Physiol. 131: 364-373 (1987).

47. M.H.varcgas, L.Romero, B.Sommer, P.Zamudio, P.Gustin, L.M.Montaño, "Chronic exposure to ozone causes tolerance to airway hyperresponsiveness in guinea pigs: lack of SOD role", J. Appl. Physiol. 84: 1749-1755 (1998).

48. P.Wang, H.Chen, H.Qin, S.Sankarapandi, M.W.Becher, P.C.Wong, J.L.Zweier, "Overexpression of human copper, zinc-superoxide dismutase (SOD1) prevents postischemic injury", Proc. Nat. Acad. Sci. USA 95: 4556-4560 (1998).

49. M.Minetti, C.Mallozzi, A.M.M.Di Stasi, D.Pietraforte, "Bilirubin is an effective antioxidant of peroxynitrite-mediated protein oxidation in human blood plasma", Arch. Biochem. Biophys. 352: 165-174 (1998).

50. B.Halliwell, "Antioxidants in human health and disease", Annu. Rev. Nutr. 16: 33-50 (1996).

51. B.Halliwell, "Antioxidant defence mechanisms: from the beginning to the end (of the beginning)", Free Radic. Res. 31: 261-272 (1999).

52. H.H.Wolff, Dasmedizinische ozon. Theoretische grundlagen, therapeutische anwedungen (Heidelberg: Verlag für Medizin, 1979).

53. V.Bocci, "Proposal of a new methodology for performing a safe and atoxic ozonated autohemotherapy (AHT)". In: F.Ceccherelli and F. Giron (Eds.), L'Ozonoterapia nel 2000 (Torino: Edizioni Libreria Cortina, 1999), pp.11-24.

54. H.G.Knoch, W.Roschke, W.Klug, "Ozone/Oxygen therapy in proctology", OzoNachrichten 6: 51-70 (1987).

55. M.T.Carpendale, J.Freeberg, J.Mcleod Griffiss, "Does Ozone alleviate AIDS diarrhea?", J. Clin. Gastroenterol. 17 : 142-145 (1993).

56. V.Bocci, E.Borrelli, F.Corradeschi, G.Valacchi, "Systemic effects after colorectal insufflation of

oxygen-ozone", Z. Naturforsch. C. (2000). Submitted.

57. E.Payr, "Über Ozonbehandlung in der Chirurgie", Münch. med. Wochenschr. 82: 220-291 (1935).

58. P.Aubourg, "Ozon in der Chirurgie", Mem. Acad. Chir 65: 1183-1192 (1940).

59. S.J.Miroshin and C. N. Kontorshikova, "The use of ozone therapy technology in the treatment of modern war surgical trauma". In: The ozone in biology and medicine. 2nd all Russian scientific-practical conference, Septmber 6-8, 1995. Russian association of ozone therapy, Reshetnikovskaya street 2, Nizhni Novgorod, 603006 Russia1995), pp. 16.

60. N.M.Shaschova, T. S. Kachalina, and A. L. Nevmjatullin, "Application of ozonotherapy in complex treatment of inner female genital inflammatory diseases". In: INTERNATIONAL OZONE ASSOCIATION (Eds.), Proceedings Ozone in Medicine, 12th World Congress of the International Ozone Association, 15th to 18th May 1995, Lille France (Tours: Instaprint S.A., 1995), pp.145-155.

61. V.I.Bulinin, N. V. Solod, and I. P. Moshurov, "The first experience of chronic abscesses and pleura emphyemas treatment by the method of ozonization". In: The ozone in biology and medicine. 2nd all Russian scientific-practical conference, September 6-8, 1995. Russian association of ozone therapy, Reshetnikovskaya street 2, Nizhni Novgorod, 603006 Russia1995), pp. 20.

62. B.P.Kudravcev, S. J. Miroshin, and S. V. Semyonov, "The use of ozonized solutions in complex treatment of peritonitis". In: The ozone in biology and medicine. 2nd all Russian Scientific-Practical Conference, September 6-8, 1995, Russian Association of Ozone therapy, Reshetnikovskaya stret, 2 - Nizhni Novgorod, 603006 Russia (Nizhni Novgorod1995), pp. 20.

63. S.A.Kasumjan, A. D. Lelyanov, E. D. Guseva, and B. P. Alexeev, "The ozone therapy of the acute suppurative infection". In: The ozone in biology and medicine. 2nd all Russian scientific-practical conference, September 6-8, 1995. Russian association of ozone therapy, Reshetnikovskaya street 2, Nizhni Novgorod, 603006 Russia1995), pp. 16.

64. V.Bocci, "A reasonable approach for the treatment of HIV infection in the early phase with ozone therap (autohemotherapy). How inflammatory cytokii may have a therapeutic role", Mediat. Inflamm. 3: 315-321 (1994).

65. V.Bocci, "Ozone therapy as a possible biological response modifier in cancer", Forsch. Komplementärmed. 5: 54-60 (1998).

66. G.E.Garber, D.W.Cameron, N.Hawley-Foss, D.Greenway, M.E.Shannon, "The use of ozone-treated blood in the therapy of HIV infection and immune disease: a pilot study of safety and efficacy.", AIDS 5: 981-984 (1991).

67. G.Valacchi, V.Bocci, "Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets", Mediat. Inflamm. 8: 205-209 (1999).

68. H.Werkmeister, "Dekubitalgeschwüre und die Behandlung mit der Ozon-Unterdruckbegasung". In BECK and VIEBAHN-HÄNSLER (Eds.), Ozon-Handbuck. Grundlagen. Prävention. Therapie (Landsberg/Lech: Ecomed, 1995), pp. V-7.1 1-V-7.1 22.

69. R.Wong, S. Menendez, J. Castañer, P. Pérez, and Abreu, "Ozone therapy in ischemic cardiopathy". International Ozone Association (Eds.), Proceedings Ozone in Medicine 12th World Congress of the International Ozone Association, 15th to 18th May 1995, Lille France (Tours: Instaprint, 1995), pp. 73-77.

70. G.H.Wasser, "Zerebrale Durchblutungsstörungen" In: Beck and Viebahn-Hänsler (Eds.), Ozon-Handbuch. Grundlagen. Prävention. Therapie (Landsberg/Lech: Ecomed, 1995), pp. V-6.3 1-V-6.3 12.

71. K.Kraft, E.Stenkamp, A.Sachinidis, S.Seewald, H.Vetter, "Effect of autohemotherapy with ozone on cardiovascular risk factors in patients with mild hypertension", Perfusion 11: 216-219 (1998).

72. F.Hernàndez, S.Menéndez, R.Wong, "Decrease of blood cholesterol and stimulation of antioxidant response in cardiopathy patients treated with endovenous ozone therapy", Free Radical Biol. Med. 19: 115-119 (1995).

73. M.A.Gomez Moraleda, "Ozone therapy in the functional recovery from diseases involving damage to central nervous system cells". In: International Ozone Association (Eds.), Proceedings Ozone Medicine 12th World Congress of th International

Ozone Association, 15th to 18th May 1995, Lille, France. (Tours: Instaprint S.A., 1995), pp.111-123.

74. C.-H.Siemsen, "Ozon-Anwendung bei akuten und chronischen Gelenkerkrankungen". In: Beck and Viebahn-Hänsler (Eds.), Ozon-Handbuch. Grundlagen. Prävention. Therapie (Landsberg/Lech: Ecomed, 1995), pp. V-9.2 1-V-9.2 14.

75. A.Alexandre and G. Fumo, "Discolisi percutanea mediante O_2O_3 nell'ernia discale lombare". In: F.Ceccherelli and A. Ricciardi (Eds.), Lombalgie e lombosciatalgie. Criteri di diagnosi e cura (Torino: Edizioni Libreria Cortina, 1998), pp.367-377.

76. V.Bocci, "Ipotetici meccanismi di azione dell'ozono nel trattamento del conflitto discoradicolare". In: F.Ceccherelli and A. Ricciardi (Eds.), Lombalgie e lombosciatalgie. Criteri di diagnosi e cura (Torino: Edizioni Libreria Cortina, 1998), pp.331-340.

77. M.Brayda-Bruno and P. Cinnella, "Il trattamento dell'ernia discale con infiltrazioni di ossigeno-ozono in paravertebrale". In: F.Ceccherelli and A. Ricciardi (Eds.), Lombalgie e lombosciatalgie. Criteri di diagnosi e cura (Torino: Edizioni Libreria Cortina, 1998), pp.361-365.

78. V.Bocci, "Determinants of erythrocyte ageing: a reappraisal.", Brit. J. Haematol. 48: 515-522 (1981).

79. E.Marshall, "Gene therapy on trial", Science 288: 951-957 (2000).

Budavari S. 1996 The Merck Index 12th Ed. Whitehouse Station NJ Merck and Co.

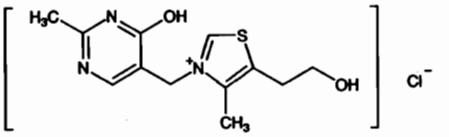
Disodium salt dihydrate, $C_{22}H_{22}N_2Na_2O_9.2H_2O$, yellow crystals; darkens on standing. Soly in abs alc: 8,000 γ/ml, in methanol: 1500 γ/ml.

USE: To treat lethal yellowing in palm trees.

THERAP CAT: Antibacterial.

THERAP CAT (VET): Antibacterial.

7112. Oxythiamine. *3-[(1,4-Dihydro-2-methyl-4-oxo-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride; 5-(2-hydroxyethyl)-3-[(4-hydroxy-2-methyl-5-pyrimidinyl)methyl]-4-methylthiazolium chloride.* $C_{12}H_{16}ClN_3O_2S$; mol wt 301.80. C 47.76%, H 5.34%, Cl 11.75%, N 13.92%, O 10.60%, S 10.63%. Prepn: F. Bergel, A. R. Todd, *J. Chem. Soc.* **1937,** 1504; M. Soodak, L. R. Cerecedo, *J. Am. Chem. Soc.* **66,** 1988 (1944). Improved prepn: H. N. Rydon, *Biochem. J.* **48,** 383 (1951). Thiamine antagonist activity: A. J. Eusebi, L. R. Cerecedo, *Science* **110,** 162 (1949); L. J. Daniel, L. C. Norris, *Proc. Soc. Exp. Biol. Med.* **72,** 165 (1949); and distribution in tissues: C. J. Gubler, D. S. Murdock, *J. Nutr. Sci. Vitaminol.* **28,** 217 (1982). Proposed mechanism of action: S. A. Strumilo *et al., Biomed. Biochim. Acta* **43,** 159 (1984). Determn by HPLC: B. C. Hemming, C. J. Gubler, *J. Liq. Chromatog.* **3,** 1697 (1980).



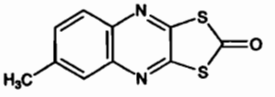
Hydrochloride, $C_{12}H_{16}ClN_3O_2S.HCl$, flat needles grouped in rosettes, dec 195°. uv max (acid soln): 265, 258, 228, 223 nm; (alkaline soln): 268, 260, 228, 221 nm. Does not give the thiochrome reaction.

Diphosphate, crystals, mp 127-129°.

Monophosphoric acid ester, hygroscopic crystals, dec 185-186°.

Triphosphoric acid ester, minute crystals, dec 245-255°.

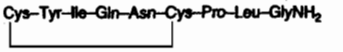
7113. Oxythioquinox. *6-Methyl-1,3-dithiolo[4,5-b]-quinoxalin-2-one; dithiocarbonic acid cyclic S,S-(6-methyl-2,3-quinoxalinediyl) ester;* 6-methyl-2,3-quinoxalinedithiol cyclic *S,S*-dithiocarbonate; 6-methyl-2-oxo-1,3-dithio[4,5-*b*]-quinoxaline; chinomethionat(e); quinomethionate; Bayer 36205; Forstan; Morestan. $C_{10}H_6N_2OS_2$; mol wt 234.30. C 51.26%, H 2.58%, N 11.96%, O 6.83%, S 27.37%. Prepn and review (in English) of chemical and fungicidal properties and toxicology: F. Grewe, H. Kaspers, *Pflanzenschutz Nachr. Bayer* **18** (1), 1-23 (1965), *C.A.* **64,** 5689h (1966). Toxicity study: T. B. Gaines, *Toxicol. Appl. Pharmacol.* **14,** 515 (1969).



Yellow crystals from benzene, mp 172°. Practically insol in water. Freely sol in DMF. Sol in hot benzene, toluene, dioxane. Slightly sol in methanol, ethanol, acetone. LD_{50} in male, female rats (mg/kg): 1800, 1100 orally (Gaines).

USE: Acaricide; agricultural fungicide.

7114. Oxytocin. Alpha-hypophamine; ocytocin; Intertocine-S; Perlacton; Pitocin; Syntocinon; Orasthin; Oxystin; Partocon; Synpitan; Uteracon. $C_{43}H_{66}N_{12}O_{12}S_2$; mol wt 1007.20. C 51.28%, H 6.60%, N 16.69%, O 19.06%, S 6.37%. The principal uterus-contracting and lactation-stimulating hormone of the posterior pituitary gland. Isoln: Pierce *et al., J. Biol. Chem.* **199,** 929 (1952). Structure and synthesis: Tuppy, Michl, *Monatsh.* **84,** 1011 (1953); Tuppy, *Biochim. Biophys. Acta* **11,** 449 (1953); du Vigneaud *et al., J. Am. Chem. Soc.* **75,** 4879 (1953); **76,** 3115 (1954); Bodanszky, du Vigneaud, *ibid.* **81,** 2504 (1959); Cash *et al., J. Med. Pharm. Chem.* **5,** 413 (1962); Sakakibara *et al., Bull. Chem. Soc. Japan* **38,** 120 (1965). Solid phase synthesis: Bayer, Hagenmaier, *Tetrahedron Letters* **1968,** 2037; Ives, *Can. J. Chem.* **46,** 2318 (1968). Synthesis of D-oxytocin: Flouret, du Vigneaud, *J. Am. Chem. Soc.* **87,** 3775 (1965). Description of commercial process: Velluz *et al.,* U.S. pats. **2,938,891** and **3,076,797** (1960, 1963, both to Roussel-UCLAF). Radioimmunoassay: T. Chard, *Clin. Biochem. Anal.* **5,** 209 (1977). *Review:* du Vigneaud, *Experientia Suppl.* **II** (14th Intl. Congr. Pure and Appl. Chem.), 9-26 (1955); R. Caldeyro-Barcia, H. Heller, *Proc. of an Intl. Symp. on Oxytocin* (Montevideo 1959) 443 pp; several authors, *Advan. Exp. Med. Biol.* **2,** 53-104 (1968); C. R. W. Edwards in *Hormones in Blood* **vol. 2,** C. H. Gray, V. James, Ed. (Academic Press, New York, 3rd ed., 1979) pp 401-421. Review of role in parturition: A.-R. Fuchs, F. Fuchs, *Advan. Exp. Med.* **1980,** 403-428. Comprehensive description: F. Nachtmann *et al., Anal. Profiles Drug Subs.* **10,** 563-600 (1981).



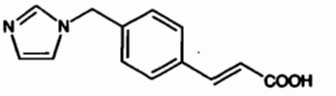
White powder. $[\alpha]_D^{22}$ −26.2° (c = 0.53). Sol in water, 1-butanol, 2-butanol.

Citrate, *Pitocin-Buccal.*

THERAP CAT: Oxytocic.

THERAP CAT (VET): Stimulates milk let-down, uterine contraction.

7115. Ozagrel. *(E)-3-[4-(1H-Imidazol-1-ylmethyl)phenyl]-2-propenoic acid; (E)*-4-(imidazol-1-ylmethyl)cinnamic acid; OKY-046. $C_{13}H_{12}N_2O_2$; mol wt 228.25. C 68.41%, H 5.30%, N 12.27%, O 14.02%. Prepn: K. Iizuka *et al.,* Ger. pat. **2,923,815;** *eidem,* U.S. pat. **4,226,878** (both 1980 to Ono; Kissei). Synthesis and thromboxane synthetase inhibitory activity: K. Iizuka *et al., J. Med. Chem.* **24,** 1139 (1981). Pharmacology: S. Hiraku *et al., Japan. J. Pharmacol.* **41,** 393 (1986). Pulmonary vascular effects: R. Garcia-Szabo *et al., Prostaglandins* **28,** 851 (1984). Metabolism in animals: M. Shimizu *et al., Iyakuhin Kenkyu* **17,** 289 (1986), *C.A.* **105,** 72012q (1986). HPLC determn in biological fluids: *eidem, ibid.* 298, *C.A.* **105,** 72013r (1986). Clinical pharmacology and evaluation in myocardial infarction: T. Ito *et al., Biomed. Biochim. Acta* **43,** S125 (1984). Clinical evaluation in prevention of cerebral vasospasm: S. Suzuki *et al., Acta Neurochir.* **77,** 133 (1985); in coronary artery disease: M. Shikano *et al., Japan. Heart J.* **28,** 663 (1987). Toxicity data: T. Nishigake *et al., Clin. Rep.* **20,** 2671 (1986). Series of articles on pharmacology: *Pharmacometrics* **31,** 527-565 (1986), *C.A.* **105,** 35349-35352 (1986).



Prisms from ethanol-ether, mp 223-224°.

Hydrochloride, $C_{13}H_{12}N_2O_2.HCl$, crystals from ethanol-ether, mp 214-217°.

Sodium salt, $C_{13}H_{11}N_2NaO_2$, *Cataclot, Xanbon.* LD_{50} in male, female mice, male, female rats (mg/kg): 1940, 1580, 1150, 1300 i.v.; 3800, 3600, 5900, 5700 orally; 2450, 2100, 2300, 2250 s.c. (Nishigake).

THERAP CAT: Antithrombotic; antianginal.

7116. Ozone. Triatomic oxygen. O_3; mol wt 48.00. Found in the atm in varying proportions (about 0.05 ppm at sea level), since it is produced continuously in the outer layers of the atm by the action of solar uv radiation on the oxygen of the air. So-called sterilizing lamps operate on the same principle. In the laboratory ozone is prepd by passing dry air between two plate electrodes connected to an alternating current source of several thousand volts. The reaction is reversible, and after a little ozone has been produced it is dec at the same rate as it is generated. Obtained in pure form by cooling ozonized air to −180° when it separates as a dark blue liquid. *See also* C. E. Thorp, *Bibliography of Ozone Technology* (Armour Res. Found., Chicago). Lab prepn: *Org. Syn.* **coll. vol. III,** 673 (1955). Monograph: *Ozone Chemistry and Technology,* Advances in Chemistry Series no. **21** (A.C.S., Washington D.C., 1959) 465 pp. *Review:* C. Nebel in Kirk-Othmer *Encyclopedia of Chemical Technology* **vol. 16** (Wiley-Interscience, New York, 3rd ed., 1981) pp 683-713.

Bluish, explosiv
teristic odor in c
injurious in high
(gas): 2.144 g/l;
−111.9°. Critica
Heat of formatio
tion band beginni
contg ozone expl
liq oxygen: Cool
bide). Although
as alkalinity rises

Bluish, explosive gas or blue liquid. Pleasant, characteristic odor in concns of less than 2 ppm. Irritating and injurious in higher concns. Powerful oxidizing agent. d^0 (gas): 2.144 g/l; $d^{-195.4}$ (liq) 1.614 g/ml. mp −193°. bp −111.9°. Critical temp −12.1°. Critical press. 53.8 atm. Heat of formation 34.4 kcal/mole at 25°. Intense absorption band beginning at about 290 nm. Unstable. Solutions contg ozone explode on warming. Prepn of ozone solns in liq oxygen: Cook, U.S. pat. 3,008,902 (1961 to Union Carbide). Although the stability of ozone in aq solns decreases as alkalinity rises, this effect is reversed at high concns. For example, the half life of ozone is 2 min in 1*N* NaOH; it is increased to 83 hrs in 20*N* NaOH: Heidt, Landi, *J. Chem. Phys.* 41, 176 (1964).

Caution: Potential symptoms of overexposure are irritation of eyes and mucous membranes; pulmonary edema; chronic respiratory disease. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 90-117, 1990) p 172.

USE: As disinfectant for air and water by virtue of its oxidizing power. For bleaching waxes, textiles, oils. In organic syntheses. Forms ozonides which are sometimes useful oxidizing compds.

HortScience 34(6):1085–1089. 1999.

Heat-retentive Films for Increasing Soil Temperatures during Solarization in a Humid, Cloudy Environment

Carlene A. Chase and Thomas R. Sinclair
U.S. Department of Agriculture, Agricultural Research Service, Agronomy Physiology and Genetics Laboratory, P.O. Box 110965, University of Florida, Gainesville, FL 32611

Daniel O. Chellemi[1] and Stephen M. Olson
North Florida Research and Education Center, University of Florida, Quincy, FL 32351

James P. Gilreath
Gulf Coast Research and Education Center, University of Florida, Bradenton, FL 34203

Salvadore J. Locascio
Horticultural Sciences Department, University of Florida, Gainesville, FL 32611

Additional index words. methyl bromide alternatives, soil solarization, polyethylene mulch

Abstract. **Although summer soil solarization is a viable technique for the control of soilborne pests, periods of cloudy skies and high rainfall typical of the southeastern United States limit the heating of soils. This study was devised to evaluate whether polyethylene films designed for improved heat conservation could increase soil solarization temperatures under humid environmental conditions. Soil solarization was conducted in Summer 1996 at Quincy, Gainesville, and Bradenton: located in north, north central, and west central Florida, respectively. Temperatures at soil depths of 5, 10, and 25 cm were higher under clear solarization films than under black polyethylene. A clear, thermal-infrared absorbing film (TIR) was consistently more effective in increasing soil temperature than was a double-layered, clear bubble film or a 30 μm clear, low-density polyethylene film. Soil temperatures under all film types were reduced by rainfall, but remained highest under TIR film. On cloudy days with light rainfall, temperatures under TIR film exceeded 45 °C at 5 cm depth. Soil temperatures rose rapidly when rainy weather was followed by a clear day. Cumulative exposure to temperatures ≥45 and 50 °C was greater with the TIR film than with the other films, indicating that it has the greatest potential for soil solarization in humid climates.**

With the impending ban on the production and importation of methyl bromide in the year 2005, soil solarization has been identified as an attractive nonchemical approach to soil disinfestation (Chellemi et al., 1997). Although the benefits of soil solarization have been demonstrated in arid climates (Katan et al., 1976; Lodha et al., 1997; Stapleton and DeVay, 1983), the feasibility of soil solarization in humid, cloudy environments has been questioned (Chellemi et al., 1993; McSorley and Parrado, 1986; Overman and Jones, 1986). In Florida, frequent cloud cover and rainfall during the summer, and high water tables, can prevent the development of sufficiently high temperatures under the solarization film. Despite this, the use of clear low-density polyethylene and green photoselective films has been effective in several studies in Florida. Overman and Jones (1986) found that summer soil solarization was potentially an effective alternative to chemical fumigation for the control of soilborne pests of tomato (*Lycopersicon esculentum* Mill.) in Florida. With rainfall occurring on 60% of the 63 d of solarization, soil solarization decreased nutsedge (*Cyperus* spp.) and nematode densities such that tomato yields were not significantly improved with a treatment in which methyl bromide was applied after soil solarization (McSorley and Parrado, 1986). Soil solarization reduced the incidence of phytoparasitic nematodes and soilborne pathogens of tomato (Chellemi et al., 1993, 1994, 1997; Overman, 1985), and was as effective as methyl bromide in controlling the sting nematode in strawberry (*Fragaria ×ananassa* Duchesne) (Overman et al., 1987).

Lamberti and Basile (1991) have discussed the characteristics of plastic films that can improve soil heating. Solarization film should allow maximum transmission of visible solar radiation and shortwave infrared (IR) radiation and minimize energy losses from the soil. The energy losses occur by means of thermic conduction, thermic convection, and irradiation losses through medium-longwave IR radiation. In most previous studies of soil temperature under solarization, clear, low-density polyethylene films were generally used (Mahrer, 1979; Mahrer et al., 1984). Such films are poor barriers to medium-long wave IR radiation. While other alternative films have been evaluated (Chellemi et al., 1993, 1994, 1997; Ham et al., 1993), the formulation and evaluation of heat-retentive films dedicated to soil solarization have not.

There are at least two possibilities for increasing the heat retention of clear, low-density polyethylene films. One option is to reduce the transmittance of radiation through the film in the thermal IR wavebands. Thermal IR radiation is emitted from all surfaces as a function of their temperature, and is usually the major mechanism for heat loss. For a surface such as soil, with a temperature of 40 °C, the wavelength of peak radiation loss is 9.3 μm, and most of the reradiated energy is in the waveband of 6 to 20 μm (Sinclair and Gardner, 1998). Conventional polyethylene films readily transmit these thermal IR wavebands, so virtually all of the radiant energy is lost to the environment. Calculations have shown that decreasing the transmittance of films in the thermal IR wavebands would increase soil temperature (Ham and Kluitenberg, 1994; Wu et al., 1996). Additives such as inorganic salts can be incorporated into polyethylene films to increase the absorption in the thermal IR wavelengths (Lamberti and Basile, 1991; Stevens et al., 1991).

A second option for increasing heat retention during soil solarization is to reduce convective heat loss from the planting bed. Generally, polyethylene mulches are ≈25 μm thick, so that heat is readily conducted through the film. The temperature of the outer surface of such a film is the same as that of the air under the film. As a result, heat is readily convected into the atmosphere from the solarization system at the outer surface of the film. When using two layers of film, heat must first be transferred from the inner to the outer layer of film before it is convected to the atmosphere. In calculating the energy balance of polyethylene mulches, Ham and Kluitenberg (1994) suggested that increased resistance to convective heat losses increases soil temperatures under a clear mulch.

The temperatures achieved and the duration of high temperature are critical determinants of the effectiveness of soil solarization. Pullman et al. (1981) showed that there is a logarithmic relationship between time and temperature for pathogen mortality. For example, exposure to 50 °C for 10 min killed cultures of *Rhizoctonia solani* Kuehn, whereas 14 d were required at 39 °C. In Florida, Chellemi et al. (1993) conducted solarization for 32 d with a green photoselective film and obtained maximum soil temperatures of 49.5, 46.0, and 40.5

Received for publication 5 Nov. 1998. Accepted for publication 22 Mar. 1999. Florida Agricultural Experiment Station, Journal Series No. R-06870. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Current address: USDA–ARS, 2199 South Rock Road, Ft. Pierce, FL 34945.

°C at soil depths of 5, 15, and 25 cm, respectively. While densities of *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker and *F. o. lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. were suppressed only to a depth of 5 cm, there were significant reductions in the densities of *Pseudomonas* (=*Ralstonia*) *solanacearum* (Smith), phytoparasitic nematodes and *Phytophthora nicotianae* Breda de haan var *parasitica* (Dastur) to depths of 15, 20, and 25 cm, respectively. Horowitz et al. (1983) found that soil solarization effectively controlled weeds when maximum soil temperatures were ≥45 °C, and that control improved as the number of days with temperatures above that threshold increased. In laboratory studies, we confirmed the importance of the 45 °C threshold in killing nutsedge tubers (*C. esculentus* L. and *C. rotundus* L.). Exposure to daily oscillating soil temperatures with maxima exceeding 45 °C over a 2-week period resulted in complete tuber mortality (Chase et al., 1999). We have previously reported on improved nutsedge control with TIR film vs. conventional clear film (Chase et al., 1998). Foliar scorching was also an important mechanism for controlling nutsedge shoots that emerged from tubers that were not exposed to lethal temperatures.

The objectives of this research were to 1) verify experimentally the effect on soil temperature of the use of a high-transparency, thermal-IR absorbing film during solarization, and 2) determine whether decreasing convective heat loss by using a double-layered mulch formulated as a bubble-wrap film would further increase soil temperatures. Field experiments designed for evaluating alternatives to methyl bromide fumigation were used to monitor soil temperatures under several polyethylene films installed on planting beds prior to crop transplanting. Soil solarization was conducted during summer months at three geographic locations where cloudy, rainy conditions prevailed.

Materials and Methods

Solarization environments. Soil solarization was conducted in Summer 1996 at three locations within the state of Florida (Quincy, Gainesville, and Bradenton) that provided a diversity of soil and weather conditions. At each location, planting beds were formed mechanically prior to application of the various films. Beds were 0.2 m high and 0.9 m wide, and were oriented in a north-south direction. Films were applied using machinery that laid the film over the beds and covered the edges of the film with soil next to the base of the bed.

The Quincy experimental site was located at the North Florida Research and Education Center, where the soil was a Ruston loamy sand (fine-loamy, siliceous, thermic, Typic Kandiudult). Overhead irrigation was employed prior to mulching, since soil moisture is required for efficient heat transfer during solarization and for activating dormant structures and propagules. Solarization was initiated on 6 June and soil temperatures were measured from 9 June to 10 July. At Gainesville, solarization films were installed on 10 July on a Millhopper fine sand (loamy, siliceous, hyperthermic, Grossarenic Paleudults). There had been heavy rainfall 3 d prior to mulching. Soil temperatures were measured from 14–23 July and 2–14 Aug. At the Gulf Coast Research and Education Center, Bradenton, soil solarization was conducted on an EauGallie fine sand (sandy, siliceous, hyperthermic Alfic Haplaquods). This site was subsurface irrigated throughout the solarization period to maintain a high water table for irrigation of the subsequent tomato crop. At the other two locations, moisture was replenished only by rainfall. Solarization was initiated on 17 July and soil temperatures were measured from 19 July to 18 Aug.

Films. At all three locations a 30 μm clear low-density polyethylene film (CLR) (AEP Industries, Hackensack, N.J.) was compared with a 100 μm thermal-IR absorbing (TIR) film, manufactured by AT Plastics (Edmonton, Alberta, Canada) specifically for this test. The film was clear and transmitted >95% in the solar radiation wavebands. Importantly, in the wavebands near the peak of thermal IR reradiation for a soil, the transmittance of the film was only ≈25%, i.e., it absorbed ≈75% (Fig. 1). Its low transmittance in the thermal IR wavebands is in marked contrast to the CLR film (Fig. 1). Bubble film, stabilized against ultraviolet radiation, was manufactured (Tenneco Packaging, Glens Falls, N.Y.) from two layers of 75 μm-thick polyethylene film, using the configuration of the commercial wrapping material with a bubble height of 3.2 mm and a diameter of 10 mm. The bubble side of the film was placed next to the soil so that the smooth surface was exposed to the atmosphere. The film was highly transmissive of visible radiation but the bubble configuration resulted in considerable backscattering of solar radiation. This film was used at Quincy and Bradenton but not at Gainesville. At all three sites a black film was also included in the study. At Gainesville and Bradenton, 38 μm conventional, embossed, black film (BLK) (Edison Plastics, Williamsburg, Va., at Gainesville, and Consolidated Thermoplastics, Harrington, Del., at Bradenton) was used; while at Quincy, the black film was a 30-μm gas-impermeable black film (Bromotec film; Lawson Mardon Packaging, Workington, Cumbria, U.K.).

Temperature measurements. Soil temperatures were measured under each film using dataloggers (model CR10 or CR10X; Campbell Scientific, Logan, Utah) equipped with copper-constantan thermocouples positioned at 0-, 5-, 10-, and 25-cm depths equidistant from the edges of the bed and at least 1 m from the end of the plot. At Bradenton and Gainesville soil temperatures were monitored at 30-min intervals at all depths. At Quincy, because of limitations in data storage capacity, temperatures at the surface and at 5-cm depth were logged at half-hour intervals and temperatures at 10- and 25-cm depths were measured hourly. Soil temperature was measured for one replication of each film at each location and data for the solarization period were summarized to obtain the daily maximum, mean daily maximum, and the overall mean temperature for each location. The cumulative hours of exposure to temperatures ≥45 and 50 °C were also calculated for 30 consecutive days of soil solarization at Quincy and at Bradenton.

Results and Discussion

Diurnal variation in soil temperature. The daily cycle of soil temperature under the films was characteristic of a bare soil (Wu et al., 1996), with the greatest variation through the daily cycle occurring at the soil surface. Diurnal fluctuation in temperature at the soil surface on a nearly clear day was typically >30 °C (Fig. 2). The daily variation in temperature decreased as soil depth increased, such that it was only ≈5 °C at a depth of 25 cm (Fig. 2). Also, the times at which the maximum and minimum temperature occurred were delayed as soil depth increased. Maximum and minimum temperatures at the surface occurred at

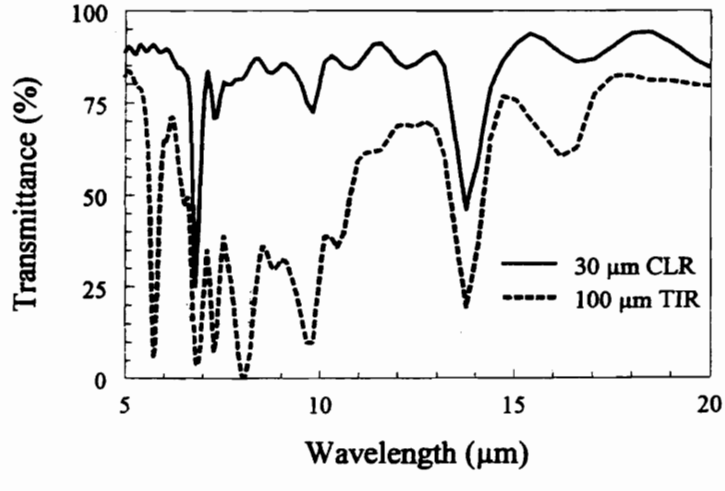


Fig. 1. Comparative transmittance of infrared radiation of a clear, low-density polyethylene film (CLR) vs. a heat-conserving thermal-infrared absorbing film (TIR).

≈1300 and 0600 HR, respectively, while at the 25-cm depth they occurred at 1900 and 0900 HR, respectively.

Soil temperatures at midday were higher under the three clear solarization films than under conventional black film (Fig. 2). Temperature differences with the various films were greater at the surface and diminished with depth. Among the three solarization films used at the Bradenton location, the TIR film resulted in the highest soil temperatures at all soil depths. There was little difference in soil temperatures at night among the four films except for the slightly higher temperatures at the soil surface under the bubble film.

Environmental sensitivity. A concern in undertaking soil solarization in a cloudy, humid environment is the influence of reduced solar radiation. Rainfall, in particular, presents a problem because both the cloudy conditions and the temperature of the rain can readily depress soil temperatures. However, temperatures at and near the soil surface recovered rapidly following rainfall events. Temperatures measured at the 5-cm depth for a sequence of 4 d associated with rainfall at Quincy (Fig. 3) illustrated the rapid temperature recovery. The first day was overcast with 10 mm of rainfall. The second and third days were less overcast but light showers did occur. No rain occurred on the fourth day. The soil temperature on each day appeared to be nearly independent of that on the previous day, with rapid recovery from the effects of rainy conditions. In this example, temperatures under all films were very low on the first day of rainfall but reached reasonably high temperatures on the following 3 d.

As observed previously, all three solarization films raised soil temperatures substantially more than did black film (Fig. 3), with TIR film being the most effective. The 3 to 4 °C higher temperature with the TIR film resulted in temperatures >45 °C under this film, even on the days of light rainfall. The relative temperatures among the various films during the night at Quincy (Fig. 3) differed from those at Bradenton (Fig. 2). At Bradenton the soil temperatures with all films were within 0.4 °C at 5-cm depth; at Quincy, minimum soil temperatures under TIR film were ≈2 °C higher than under the bubble film, which had the next highest night temperatures. This result might be related to the fact that the soil at Bradenton was kept in a very moist state by continuous subsurface irrigation so that heat conduction within the soil was high. At Quincy, the soil was not irrigated during solarization so that conduction was lessened and the temperature advantage gained by the TIR absorbing film during the day was preserved through the night.

Soil surface temperatures. The highest soil surface temperatures occurred at Quincy (Table 1), where the maximum temperature of the solarization period with all films exceeded 67 °C and the mean maximum daily temperature exceeded 58 °C. The next highest soil surface temperatures occurred at Gainesville with a maximum >63 °C and a mean daily maximum of 52 °C or greater. The soil at these two locations was not rewetted during the solarization period except by rainfall, so that the loss of moisture at the soil surface may have contributed to these high temperatures. At Bradenton, where the soil was subsurface irrigated, the maximum soil surface temperature was lower than at the other two locations, except under the TIR film. The maximum temperature under the TIR film exceeded 65 °C while temperatures were <60 °C under the other three films. Similar differences among the films were observed at Bradenton in mean daily maximum temperatures.

At Gainesville and Bradenton, higher soil surface temperatures were obtained with the solarization films than with the black film. At both of these locations, the TIR film provided the highest mean daily maximum temperature and the black film resulted in the lowest. There was little difference among the four films at Quincy, but this may have been due to the drying of the soil surface at this location. Even under the black film, soil surface temperatures were high at Quincy. The difference in soil heating characteristics is illustrated by the mean daily maximum temperatures at the soil surface under the black film, which were 61.5, 51.9, and 48.8 °C at Quincy, Gainesville, and Bradenton, respectively.

Soil temperature at various depths. Solarization films consistently produced higher soil temperatures than black films at all three locations (Table 1). At the 5-cm depth, the mean daily maximum temperature under the solarization films exceeded 45 °C, and was ≈5 °C lower under the black film. The highest mean daily maximum temperatures were recorded with TIR film at each of the three locations. Similarly, higher soil temperatures were ob-

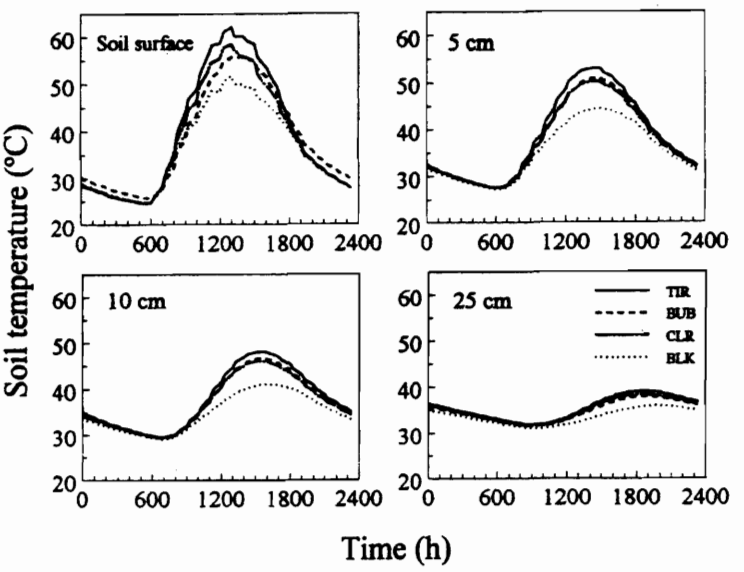


Fig. 2. Diurnal temperature changes measured at four soil depths for four film types at Bradenton, Fla., on a rain-free day (27 July 1996). Polyethylene mulches were: 100 µm thermal-infrared absorbing film (TIR), UV-stabilized bubble film manufactured with two layers of 75 µm-thick polyethylene film (BUB), 30 µm clear low-density polyethylene film (CLR), and 38 µm conventional, embossed black film.

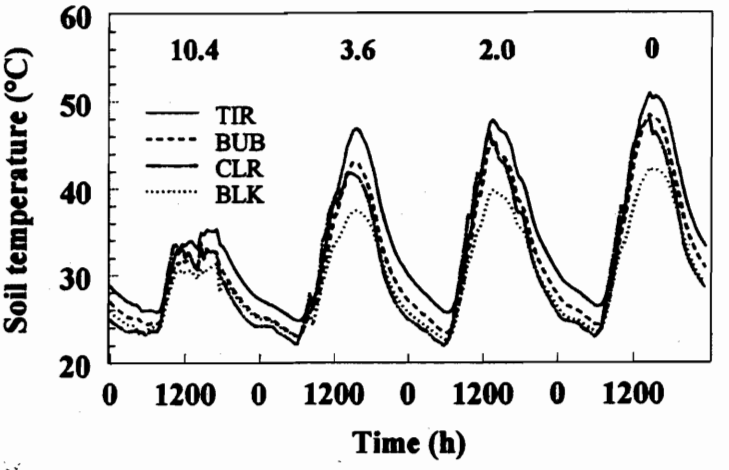


Fig. 3. Effect of rainfall on soil temperatures under four polyethylene mulches at a 5-cm depth at Quincy, Fla., 8 to 11 June 1996. Polyethylene mulches were: 100 µm thermal- infrared absorbing film (TIR), UV-stabilized bubble film manufactured with two layers of 75 µm-thick polyethylene film (BUB), 30 µm clear low-density polyethylene film (CLR), and 30 µm gas-impermeable black film. Numbers above curves indicate rainfall (mm) each day.

Table 1. Influence of polyethylene mulch films[z] and soil depth on soil temperatures at Quincy, Gainesville, and Bradenton, Fla., in 1996.

	Quincy[y]				Gainesville[x]			Bradenton[w]			
Temp variable	BLK1	CLR	BUB	TIR	BLK2	CLR	TIR	BLK2	CLR	BUB	TIR
					Soil depth: 0 cm						
Mean	34.8	34.3	35.9	34.6	33.7	34.4	35.5	34.5	36.1	36.3	36.7
Mean daily maximum	61.5	58.3	59.3	60.5	51.9	55.2	57.3	48.8	55.2	52.9	58.4
Maximum	69.5	68.0	67.9	67.8	65.3	63.6	64.7	59.0	59.6	57.6	65.1
					Soil depth: 5 cm						
Mean	32.6	34.0	34.5	36.2	33.0	34.8	35.7	33.8	35.4	35.7	36.3
Mean daily maximum	41.1	46.9	45.6	47.7	40.5	46.4	46.5	42.3	47.0	47.7	49.3
Maximum	46.4	54.5	52.0	53.7	45.6	51.5	52.0	46.2	50.9	52.1	53.9
					Soil depth: 10 cm						
Mean	32.4	34.1	34.2	35.8	33.1	35.0	35.8	33.4	35.0	35.1	35.6
Mean daily maximum	38.7	40.3	41.2	43.2	38.3	43.3	43.8	39.0	43.1	43.6	44.5
Maximum	43.5	46.1	46.8	48.4	42.4	48.0	49.4	42.5	46.5	47.5	48.4
					Soil depth: 25 cm						
Mean	31.4	32.6	32.6	33.4	32.6	34.0	34.9	32.2	33.4	33.3	33.7
Mean daily maximum	34.0	35.0	34.9	35.8	35.0	37.3	38.6	34.3	36.3	36.0	36.7
Maximum	38.0	39.6	39.2	39.7	38.4	40.6	42.0	36.5	38.6	38.1	39.2

[z]BLK1 = 30-µm gas-impermeable black film; BLK2 = 38-µm conventional embossed black film; CLR = 30-µm clear low-density polyethylene film; BUB = UV-stabilized bubble film manufactured with two layers of 75-µm-thick polyethylene film; and TIR = 100-µm thermal-infrared absorbing film.
[y]9 June–10 July.
[x]14–23 July and 2–14 Aug.
[w]19 July–18 Aug.

tained with the solarization films than with the black films at 10-cm depth. At this depth, the differences among the films were not as great as at the shallower soil depth, and, although maximum temperatures achieved under the solarization films exceeded 45 °C, the mean maximum temperature did not. Temperatures were highest under the TIR film. The difference in soil temperatures under black film vs. solarization films was much less at the 25-cm depth than at shallower depths, but temperatures among the solarization films were very similar.

Cumulative time of exposure to high temperature. The cumulative exposure times during which temperatures were ≥45 and 50 °C were consistently higher with solarization films than with black films (Table 2) at Quincy and Bradenton. (Data were not tabulated for Gainesville because the temperature record was not continuous). The black films were the only films under which soil temperatures at 10 cm did not rise to 45 °C. The TIR film provided the highest cumulative exposure at all sites. At the 10-cm depth at Quincy, cumulative exposure was 38 and 48 h greater with TIR film than with bubble and CLR films, respectively. Similarly, for the 10-cm depth at Bradenton, cumulative exposure was 18 and 30 h greater with TIR film than with bubble and CLR films, respectively. There was no consistent difference between the bubble and CLR films across the two locations.

Conclusions. Our current studies demonstrate that the use of heat-retentive TIR film may have potential for enhancing the utility of soil solarization under cloudy, humid environments. While the temperature advantage varied among locations and soil depths, the TIR film was the most effective of the solarization films in increasing soil temperatures. The mean daily maximum temperatures at each of the three locations was at least 57, 46, and 43 °C for the surface, 5-cm, and 10-cm depths, respectively. The temperatures in the upper 10 cm of soil were sufficiently high to be potentially lethal to mesophilic pathogens and weed propagules, and also the cumulative time of exposure to temperatures exceeding the threshold were highest with TIR films. More study is warranted with oscillating temperatures to determine lethal temperatures and durations required for specific soilborne pests and pathogens. The bubble film, which was tested as a means of reducing convective heat loss from the solarized beds, failed to increase soil temperatures above that achieved with a single layer of clear polyethylene film in virtually all comparisons. The backscattering of solar radiation as a result of the bubble configuration may have decreased its transmission to the soil. Further, the bulkiness of the bubble film makes it unattractive for commercial use.

Overall, these results indicate that TIR film can promote more effective soil heating than can CLR film. The enhanced capacity for soil heating with TIR film promotes conditions that are detrimental to soilborne pests, even when the background environment may be somewhat adverse for other films. Therefore, the use of TIR film in soil solarization appears to be a viable option for enhancing the control of soilborne pests in humid, subtropical environments. The efficacy of control has been documented for nutsedge (Chase et al., 1998, 1999) and additional information on pathogen control and tomato yield are subjects of a subsequent paper. The evaluation of TIR film for solarization in late summer and early fall for production of strawberry is in progress.

Table 2. Influence of polyethylene mulch and soil depth on cumulative hours of exposure to temperatures ≥45 °C and 50 °C at Quincy, Fla., and Bradenton, Fla., over 30 consecutive days of soil solarization.

	Soil depth	Polyethylene film[z]			
Location	(cm)	Black	Clear	Bubble	TIR
			Cumulative hours ≥45 °C		
Quincy	0	173	175	181	181
	5	12	107	91	129
	10	0	14	24	62
Bradenton	0	92	171	166	195
	5	15	100	111	133
	10	0	26	56	38
			Cumulative hours ≥50 °C		
Quincy	0	134	133	140	146
	5	0	28	16	44
Bradenton	0	27	100	90	130
	5	0	7	22	46

[z]30-µm gas-impermeable black film (38-µm conventional embossed black film at Bradenton), 30-µm clear low-density polyethylene film, UV-stabilized bubble film manufactured with two layers of 75-µm-thick polyethylene film, and 100-µm thermal-infrared absorbing (TIR) film, respectively.

Literature Cited

Chase, C.A., T.R. Sinclair, and S.J. Locascio. 1999. Effects of soil temperature and tuber depth on *Cyperus* spp. control. Weed Sci. 47:467–472.

Chase, C.A., T.R. Sinclair, D.G. Shilling, J.P. Gilreath, and S.J. Locascio. 1998. Light effects on rhizome morphogenesis in nutsedges (*Cyperus* spp.): Implications for control by soil solarization. Weed Sci. 46:575–580.

Chellemi, D.O., S.M. Olson, and D.J. Mitchell. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. Plant Dis. 78:1167–1172.

Chellemi, D.O., S.M. Olson, D.J. Mitchell, I. Secker,

and R. McSorley. 1997. Adaptation of soil solarization to the integrated management of soilborne pests of tomato under humid conditions. Phytopathology 87:250–258.

Chellemi, D.O., S.M. Olson, J.W. Scott, D.J. Mitchell, and R. McSorley. 1993. Reduction of phytoparasitic nematodes on tomato by soil solarization and genotype. J. Nematol. 25 (suppl.):800–805.

Davis, C.H. and R.S. Hawkins. 1943. Eradication and control of nut grass. Arizona Agr. Expt. Sta. Bul. 189.

Friesen, G.H. and A.S. Hamill. 1977. Sprouting and development of yellow nutsedge tubers. Can. J. Plant Sci. 57:509–514.

Ham, J.M. and G.J. Kluitenberg. 1994. Modeling the effect of mulch optical properties and mulch-soil contact resistance on soil heating under plastic mulch culture. Agr. For. Meteorol. 71:403–424.

Ham, J.M., G.J. Kluitenberg, and W.J. Lamont. 1993. Optical properties of plastic mulches affect the field temperature regime. J. Amer. Soc. Hort. Sci. 118:188–193.

Horowitz, M., Y. Regev, and G. Herzlinger. 1983. Solarization for weed control. Weed Sci. 31:170–179.

Katan, J., A. Greenberger, H. Alon, and A. Grinstein. 1976. Solar heating by polyethylene mulching for the control of diseases caused by soil-borne pathogens. Phytopathology 66:683–688.

Lamberti, F. and M. Basile. 1991. Improvement in plastic technology for soil heating, p. 309–330. In: J.E. DeVay, J.J. Stapleton, and C.L. Elmore (eds.). Soil solarization. FAO Plant Production and Protection Paper 109. Rome.

Lodha, S., S.K. Sharma, and R.K. Aggarwal. 1997. Solarization and natural heating of irrigated soil amended with cruciferous residues for improved control of *Macrophomina phaseolina*. Plant Pathol. 46:186–190.

Loustalot, A.J., T.J. Muzik, and H.J. Cruzado. 1954. Studies on nutgrass (*Cyperus rotundus* L.) and its control. Puerto Rico Federal Expt. Sta. Bul. 52.

Mahrer, Y. 1979. Prediction of soil temperature of a soil mulched with transparent polyethylene. J. Appl. Meteorol. 18:1263–1267.

Mahrer, Y., O. Naot, E. Rawitz, and J. Katan. 1984. Temperature and moisture regimes in soils mulched with transparent polyethylene. Soil Sci. Soc. Amer. J. 48:362–367.

McSorley, R. and J.L. Parrado. 1986. Application of soil solarization to Rockdale soils in a subtropical environment. Nematropica 16:125–140.

Overman, A.J. 1985. Off-season land management, soil solarization and fumigation for tomato. Proc. Soil and Crop Sci. Soc. Florida. 44:35–39.

Overman, A.J., C.M. Howard, and E.E. Albregts. 1987. Soil solarization for strawberries. Proc. Florida. State Hort. Soc. 100:236–239.

Overman, A.J. and J.P. Jones. 1986. Soil solarization, reaction, and fumigation effects on double-cropped tomato under full-bed mulch. Proc. Florida State Hort. Soc. 99:315–318.

Pullman, G.S., J.E. DeVay, and R.H. Garber. 1981. Soil solarization and thermal death: A logarithmic relationship between time and temperature for four soilborne plant pathogens. Phytopathology 71:959–964.

Sinclair, T.R. and F.P. Gardner. 1998. Principles of ecology in plant production. CAB Intl., Wallingford, U.K.

Stapleton, J.J. and J.E. DeVay. 1983. Response of phytoparasitic and free-living nematodes to soil solarization and 1,3-dichloropropene in California. Phytopathology 73:1429–1436.

Stevens, C., V.A. Khan, J.E. Brown, G.J. Hochmuth, W.E. Splittstoesser, and D.M. Granberry. 1991. Plastic chemistry and technology as related to plasticulture and solar heating of soil, p. 141–158. In: J. Katan and J.E. DeVay (eds.). Soil solarization. CRC Press, Boca Raton, Fla.

Wu, Y., K.B. Perry, and J.B. Ristaino. 1996. Estimating temperature of mulched and bare soil from meteorological data. Agr. For. Meteorol. 81:299–323.

Weed Science, 47:467–472. 1999

Effects of soil temperature and tuber depth on *Cyperus* spp. control

Carlene A. Chase
Corresponding author. U.S. Department of Agriculture-Agricultural Research Service, Gainesville, FL 32611. Mailing address: Agronomy Physiology and Genetics Laboratory, P.O. Box 110965, University of Florida, Gainesville, FL 32611-0965; cach@gnv.ifas.ufl.edu

Thomas R. Sinclair
U.S. Department of Agriculture-Agricultural Research Service, Gainesville, FL 32611-0965

Salvadore J. Locascio
Horticultural Sciences Department, University of Florida, Gainesville, FL 32611-0690

Studies were conducted to determine lethal temperatures for *Cyperus esculentus* and *Cyperus rotundus* tubers using diurnal oscillations in soil temperature with maxima of 40, 45, 50, and 55 C and a minimum of 26 C. Growth of *Cyperus* spp. plants was faster at 40 C than at a constant temperature of 26 C. The 45 C treatment delayed *Cyperus* spp. emergence but was not lethal to tubers. Tuber mortality was 100% for both *Cyperus* spp. with the 50 and 55 C temperature regimes. Soil solarization with thermal-infrared-retentive (TIR) films resulted in higher soil temperatures than with a 30-μm low-density polyethylene (LDPE) clear film. With TIR films, greater proportions of emerged *C. rotundus* plants were killed by foliar scorching, and 6 wk of soil solarization was more effective at reducing *C. rotundus* density than with the LDPE film. Four weeks after film removal, the lowest level of control was obtained with the LDPE film. For *C. rotundus* tubers planted 5 and 10 cm deep, 62% control was obtained with the LDPE film, and it decreased to 32% with a 15-cm planting depth. The best residual control was 95 and 92% with the 75- and 100-μm TIR films, respectively. With the TIR films, there was no significant change in *C. rotundus* control with planting depth.

Nomenclature: Methyl bromide; *Cyperus rotundus* L. CYPRO, purple nutsedge; *Cyperus esculentus* L. CYPES, yellow nutsedge.

Key words: Methyl bromide alternatives, lethal temperature, CYPES, CYPRO.

Black polyethylene or white-on-black polyethylene mulches are used commonly in association with methyl-bromide : chloropicrin soil fumigation in Florida for the production of high-value horticultural crops such as *Lycopersicon esculentum* Mill. (tomato), *Capsicum annuum* L. (bell pepper), and *Fragaria xananassa* Duchesne (strawberry). The ban on the production and importation of methyl bromide after January 1, 2005, has stimulated a concerted effort to find suitable alternatives. In evaluations of chemical alternatives to methyl bromide, Gilreath et al. (1994) found that in most of their tests, *C. rotundus* and *C. esculentus* were the most damaging pests and the most difficult to control with fumigants alone. Fumigation with 1,3-dichloropropene : chloropicrin plus a preplant-incorporated application of pebulate provided some control of *Cyperus* spp. (Locascio et al. 1997). Summer soil solarization for fall production is another alternative that has shown promise as an alternative to methyl-bromide fumigation. Chellemi et al. (1997) reported that *Cyperus* spp. (mixed populations) control by soil solarization was equivalent to that of methyl bromide at a site with a low *Cyperus* spp. population (12 plants 0.27 m^{-2} with white film) and was more effective than methyl bromide at a site with a high *Cyperus* spp. population (33.4 plants 0.27 m^{-2}). In soil solarization, radiant energy from the sun is transmitted through clear polyethylene film so that soil can be heated to biologically lethal temperatures. The solarization of soil has been successfully utilized in arid climates for weed and soilborne disease control (Katan et al. 1976).

Soil solarization has been shown to provide excellent control of annual weeds but is less effective for control of perennial weeds. Rubin and Benjamin (1983) found that control of *C. rotundus, Sorghum halepense* (L.) Pers. (johnsongrass), and *Cynodon dactylon* (L.) Pers. (bermudagrass) was significantly improved by an extended solarization period of 8 to 10 wk. Egley (1983) found that 3 to 4 wk of solarization did not decrease *C. rotundus* emergence and that, in some cases, emergence increased. Fluctuating temperatures were more effective than constant temperatures in promoting rapid and complete sprouting of *C. rotundus* tubers (Miles et al. 1996), suggesting that stimulation of *Cyperus* spp. emergence in some studies was probably due to the more pronounced diurnal temperature variation that is a characteristic of solarization. A single temperature pulse from 20 to 35 C has been shown to release dormancy in *C. rotundus* tubers (Sun and Nishimoto 1997).

Soil solarization controls weeds directly by killing weed propagules and indirectly by the foliar scorching of plants that emerge under the polyethylene mulch (Horowitz et al. 1983). Rubin and Benjamin (1984) proposed that only weed propagules in the top 10 cm were being killed by soil solarization and that those located at greater depths were escaping. In the studies of Standifer et al. (1984), seeds of *Cyperus* spp. (annual sedges) and *Echinochloa crus-galli* (L.) Beauv. (barnyardgrass) were killed only in the upper 3 to 4 cm of soil, whereas seeds of *Eleusine indica* (L.) Gaertn. (goosegrass) and *Commelina communis* L. (Asiatic dayflower) were killed within the upper 5 and 11 cm, respectively. It is likely that *Cyperus* spp. tubers are directly killed by soil solarization in the shallower soil depths and stimulated to sprout at depths that do not heat to lethal temperature (Rubin and Benjamin 1984). A light-dependent morphogenic change from rhizome elongation to leaf expansion was proposed to explain differential penetration of opaque and clear mulch films (Chase et al. 1998). Expanding leaves are

trapped under the clear mulch, and the shoots succumb to foliar scorching.

Lethal temperatures for *C. esculentus* and *C. rotundus* tubers have been previously investigated. Holt and Orcutt (1996) found that when incubated for 2 wk at constant temperature, the lethal temperatures were 43 and 44 C, respectively. Rubin and Benjamin (1984) found that when *C. rotundus* tubers were subjected to a single 30-min exposure, temperatures > 60 C were required to reduce tuber viability significantly. Because solarization produces a pronounced diurnal variation in soil temperature, the objectives of these studies were (1) to determine the cumulative effect of diurnal temperature fluctuation on *Cyperus* spp. tuber viability, with a maximum temperature range of 40 to 55 C; and (2) to compare the effects of TIR films of varying thickness with a conventional LDPE clear film on soil temperature and *C. rotundus* control.

Materials and Methods

Laboratory Experiments

Laboratory experiments were conducted in pots to determine the lethal temperature for *C. esculentus* and *C. rotundus* tubers under temperature regimes that approximated those of solarization in the field. *Cyperus* spp. tubers[1,2] were presprouted to ensure viability. *Cyperus rotundus* tubers were stored at 25 C wrapped in moist newspaper. Sprouted tubers were selected for use in experiments. *Cyperus esculentus* tubers were placed in moist soil (Scotts Terralite Agricultural Mix)[3] and exposed to 30 C for 6 h followed by 25 C for 18 h over a 3-d incubation period. The buds and roots were removed by trimming, and four tubers were planted 5 cm deep in each pot (11 cm in diam, 11 cm tall). The soil was a Millhopper fine sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult) obtained from the University of Florida Horticultural Research Unit, Gainesville. Pots were covered with LDPE film that was secured around the pots with rubber bands to limit convective heat loss from the soil surface. All but 2 cm of the pots were immersed in well-stirred water baths and heated, with maximum soil temperatures of 40, 45, 50, or 55 C. Pots without drain holes were used to prevent direct contact between the soil in the pots and the water in the baths. Four replicate pots were randomly allocated to temperature treatments. Water baths were controlled by timers to allow heating for just 6 h each day, beginning at 10 A.M., and allowed to cool to 26 C after the heating period to generate the diurnal oscillations of soil temperature. The control treatment was held at 26 C. Soil temperatures were monitored at 30-min intervals 5 cm deep at the center of each pot using a CR10 data logger.[4]

The temperature treatments were discontinued after 2 wk, and the number of shoots was recorded. Pots with unsprouted tubers were held at 30/25 C (12 h each daily) in a growth chamber for another week, after which the viability of unsprouted tubers was determined using 0.1% triphenyl tetrazolium chloride (TTC) (Miles et al. 1996). Unsprouted tubers and tubers from the control plants were washed and sectioned into halves. One-half of each tuber was immersed in 0.1% TTC and held at 30 C for 2 h. The other half was held in deionized water at room temperature.

Experiments were conducted twice with each *Cyperus* sp. There was no interaction between experiment and temperature, and data were pooled and analyzed using ANOVA. Fisher's Protected LSD test with a 5% level of significance was used for mean separation.

Field Experiment

A soil solarization experiment was conducted in summer 1997 on a Millhopper fine sand soil (loamy, siliceous, hyperthermic, Grossarenic Paleudult) at Gainesville, FL, to confirm the laboratory data, determine the soil depths at which lethal temperatures occurred, and evaluate the efficacy of *C. rotundus* control. The experimental design was a split plot, with four solarization films and a nonmulched (bare) treatment as the main plot treatments, and subplot treatments were three tuber planting depths (5, 10, and 15 cm). The main plots (3.6 m long and 0.9 m wide) were arranged in a 5 by 5 latin square. Subplots (0.9 by 0.9 m) were completely randomized within the main plots.

The 0.9-m-wide planting beds on 1.8-m centers were treated with methyl bromide at 44.8 g m^{-2} and covered with black polyethylene mulch to kill existing weed species. The fumigation mulch was removed after 1 wk, the field was irrigated, and 12 *C. rotundus* tubers were planted in each subplot in a rectangular grid with 0.2 m between planting positions. Solarization films were then installed. Three of the solarization films consisted of a TIR formulation that had been extruded in thicknesses of 50, 75, and 100 μm.[5] The fourth film was a conventional 30-μm clear LDPE film.[6]

Soil temperatures in three main plot replications were recorded at 30-min intervals using a CR10 data logger. A copper-constantan thermocouple was inserted in the middle of each subplot to the depth of planting. Soil surface temperatures were also measured in a single replication of the 100-μm TIR and the 30-μm LDPE treatments.

Soil solarization was conducted from July 23, 1997, to September 3, 1997. During the 6-wk solarization period, counts were made of live and dead (foliar scorched) *C. rotundus* plants trapped under the films and plants penetrating the films. *Cyperus rotundus* plants that emerged under and through the films were counted again at the end of the solarization period. The solarization films were removed, *C. rotundus* shoots were cut off at soil level, and shoot dry weights were determined. *Cyperus rotundus* shoot density and biomass were expressed on a per square meter basis. In addition, *C. rotundus* control was determined by expressing the plant density from each treatment as a percentage of the density of the nonsolarized treatment. The persistence of control was assessed 4 wk later on October 1, 1997, by counting the number of emerged *C. rotundus* plants and expressing the counts as a percentage of the nonsolarized treatment. ANOVA was performed using the MIXED procedure in SAS (1996). Film and depth were considered to be fixed effects, and column and row were considered random effects. Multiple comparisons of films and depths were performed by use of contrasts or by applying Fisher's Protected LSD test to the least-squares means.

Results and Discussion

Laboratory Experiments

The diurnal variation in temperature obtained using the water baths is illustrated in Figure 1. Soil temperatures in-

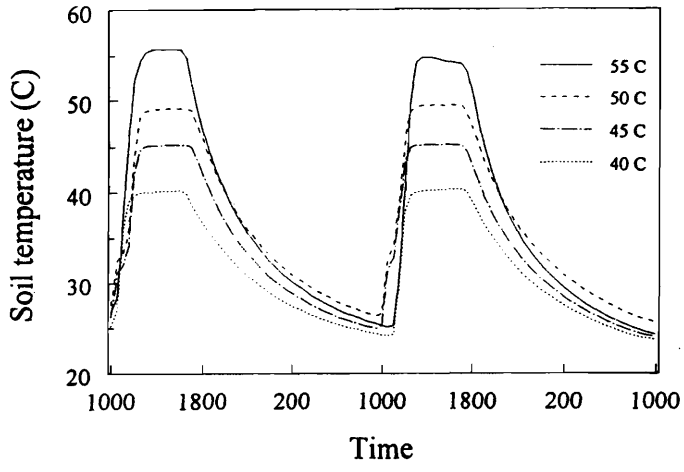


FIGURE 1. Diurnal soil temperature variation produced in laboratory study using water baths over a 48-h period.

creased rapidly when the heaters were turned on and reached the prescribed treatment temperature within 1 h. After the heaters were turned off, soil temperature decreased slowly, approaching 26 C before the heaters were again turned on. The daily cycle of temperature was similar to that observed in the field 5 cm deep, except that soil heating occurred at a faster rate and resulted in more of a plateau at maximum temperature (Figure 2).

Cyperus spp. sprouting and growth were faster at 40 C than at room temperature. Multiple sprouts arose from some tubers, so that there was more than one shoot per tuber in some pots. This resulted in a significantly greater number of *C. rotundus* shoots at the end of the 2-wk incubation (Table 1). Although shoot emergence was delayed (data not shown), the 45 C treatment was not lethal to tubers. Sprouting in both *Cyperus* spp. was completely inhibited by the 50 and 55 C treatments (Table 1).

The nonviability of tubers from the 50 and 55 C treatments was confirmed using the TTC test (data not shown). Tubers held at room temperature were ivory in cross-section and retained that color when held in deionized water. After a 2-h incubation in 0.1% TTC, these tubers developed an intense pink color around the pith. The tubers subjected to 50 C were a dark cream color with light brown streaks when first cut. Some of the 50 C-treated tubers developed a slightly pink color, but the intensity was not comparable to that of the control tubers, and they were considered nonviable.

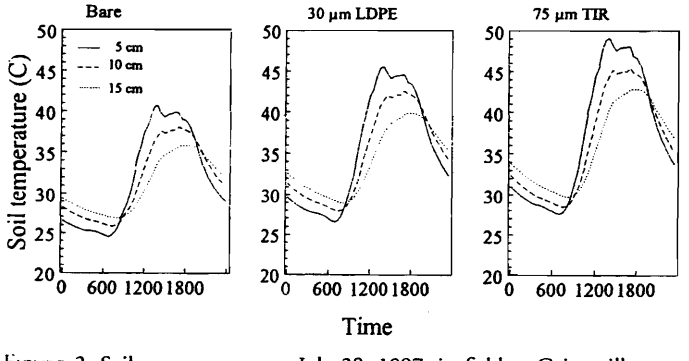


FIGURE 2. Soil temperatures on July 28, 1997, in field at Gainesville over a 24-h period.

TABLE 1. Effect of soil temperature on *Cyperus* spp. shoot emergence.

Soil temperature (C)	*Cyperus* spp. (plants pot^{-1})[a]	
	C. rotundus	*C. esculentus*
26	4	4
40	8	5
45	5	4
50	0	0
55	0	0
LSD (0.05)	2	1

[a] Values in each column are means of two experiments.

Tubers held at 55 C were light brown when sectioned and did not develop color after incubation.

Although it has been reported that a constant incubation temperature of 44 C was sufficient to kill *C. rotundus* and *C. esculentus* tubers (Holt and Orcutt 1996), diurnally fluctuating temperatures with a maximum temperature of 45 C in our experiments only slowed emergence. Miles et al. (1996) have pointed out the limitation of studying *C. rotundus* sprouting at constant temperatures. There is a characteristic diurnal variation in field soil temperature that is even more pronounced with soil solarization.

Field Experiment

The typical diurnal variation of temperature that occurred in soil on a sunny day and the increase in soil temperature under clear polyethylene films are illustrated in Figure 2. Soil under clear mulches heated more rapidly than nonmulched soil, resulting in higher daily maximum temperatures. Minimum soil temperatures of mulched soil were also not as low as those of nonmulched soil (Figure 2). All of the solarization films increased the mean daily maximum soil temperature by at least 10.3, 8.7, and 7.3 C over nonsolarized soil, 5, 10, and 15 cm deep, respectively (Table 2). Surface soil temperatures were higher under the 100-µm TIR film than under the 30-µm LDPE, so that temperature exceeded 60 C on 49% of the days of the solarization period under TIR and attained those temperatures only 8% of the days under the LDPE film. Although temperatures > 50 C occurred on at least 18% of the days under solarization mulches, 50 C was never attained at 10- or 15-cm depths. The TIR films promoted higher soil temperatures than the

TABLE 2. Soil temperatures[a] and the percentage of days on which threshold temperatures were exceeded at Gainesville from July 24 to September 2, 1997.

	Mean daily maximum temperature (C)				Percentage of days	
					> 60 C	> 50 C
	Soil depth (cm)				Soil depth (cm)	
Film	0	5	10	15	0	5
Bare soil	—	36.5	34.5	33.2	—	0
LDPE-30 µm	53.5	46.8	43.2	40.5	8.1	17.6
TIR-50 µm	—	47.6	44.2	41.1	—	26.5
TIR-75 µm	—	47.6	43.8	40.9	—	26.5
TIR-100 µm	58.8	47.9	43.7	40.8	48.6	29.4

[a] Temperatures measured in three replications of each main plot treatment for 34 d of the solarization period were used.

TABLE 3. Percentage of days during solarization when soil temperature exceeded 45 C (based on temperatures for 34 d of the solarization period).

Film	Soil depth (cm)		
	5	10	15
	%		
Bare soil	0	0	0
LDPE-30	77	32	3
TIR-50	80	44	3
TIR-75	82	38	3
TIR-100	80	44	3

LDPE film. This resulted in 9 to 12% more days on which maximum soil temperature exceeded 50 C and thus TIR film had greater potential for promoting *C. rotundus* tuber mortality.

Because laboratory soil temperatures > 45 C were required for tuber mortality, the field soil temperature data were summarized to determine the percentage of days during the solarization period on which the daily maximum soil temperature exceeded 45 C (Table 3). Bare soil did not heat to 45 C at any of the depths measured. At 5-cm depth, the three TIR films resulted in daily maximum temperatures > 45 C for at least 80% of the days, compared with 77% for the 30-μm LDPE film. With TIR film, at least 38% of the days resulted in > 45 C soil temperatures 10 cm deep but only 32% with the 30-μm LDPE film. For all of the solarization films, temperatures 15 cm deep exceeded 45 C for just 3% of the days. Therefore, only the upper 10 cm of soil consistently produced soil temperatures that would have been lethal to *Cyperus* spp. tubers. Horowitz et al. (1983) ascribed the resistance of perennial weeds to soil solarization to the occurrence of perennating organs at depths that do not heat to lethal temperature.

Cyperus rotundus Establishment in Nonsolarized Soil

There was 100% emergence from tubers planted in nonsolarized soil (data not shown). However, the rate of emergence and production of daughter plants differed with tuber planting depth, so that the density of emerged *C. rotundus* plants decreased as tuber planting depth increased (Table 4). Two weeks after the beginning of soil solarization, the *C. rotundus* densities for the 5-, 10-, and 15-cm tuber planting depths were 32, 23, and 14 plants m^{-2}, respectively. By the fourth week, *C. rotundus* densities had increased one order of magnitude to 203, 183, and 155 plants m^{-2} with 5-, 10–, and 15-cm depths, respectively. There were 315, 292, and 280 m^{-2} for the 5-, 10-, and 15-cm depths by week 6. The inverse linear relationship between *C. rotundus* density and tuber planting depth may have been due to stimulation of tuber sprouting and plant growth by the higher soil temperatures and wider diurnal temperature fluctuations near the soil surface of nonmulched plots. In a study in which the range of temperature fluctuation was varied from 0 to 6 C, there was a linear increase in *C. rotundus* tuber sprouting as the temperature difference increased (Miles et al. 1996). Alternatively, the energy requirements of emergence from greater depths may have decreased the rate of plant establishment and proliferation of daughter plants. Rubin and Benjamin (1984) found no differences in the emergence of *C. rotundus* planted 2 to 10 cm deep when assessed 60 d after planting. However, emergence decreased from 74 to 55% when planting depth was increased from 10 to 20 cm, and emergence was only 5% with a 30-cm planting depth.

TABLE 4. Effect of soil depth of tuber planting on *C. rotundus* density in nonsolarized soil.

Tuber depth (cm)	*C. rotundus* density (plants m^{-2})		
	Week 2	Week 4	Week 6
5	32	203	315
10	23	183	292
15	14	155	280
P (linear effect)	0.0001	0.0004	0.02

Cyperus rotundus Control in Solarized Soil

There was no interaction between solarization film and tuber planting depth; therefore, the main effects of film and planting depth were assessed. The effect of solarization film on *C. rotundus* density was significant (Table 5). Fewer *C. rotundus* plants emerged with the solarization treatments than with the bare soil. This appeared to result from a combination of factors associated with having a clear film over the soil. Temperatures were sufficiently high to cause thermal death of tubers in the upper 10 cm of soil. We have previously reported that the differential penetration of opaque and clear films by *Cyperus* spp. plants was due to a photomorphogenic change from rhizome to leaf growth under clear film, which does not occur under opaque film (Chase et al. 1998). As a result of this light-dependent morphological change to leaf growth, there was a reduction in *C. rotundus* density as plants were trapped under the films, and foliar scorching resulted in necrosis and death (Table 5).

A greater proportion of the emerged plants were killed by foliar scorching with the TIR treatments than with LDPE film (Table 6). After 4 wk of solarization, the LDPE film resulted in 21 live plants m^{-2}, whereas the plant densities with the TIR films were 3, 0, and 0 for TIR-50, TIR-75, and TIR-100, respectively. This probably was due to hotter soil surface temperatures under the TIR films than under the LDPE film. Mean daily soil temperature at the soil surface with TIR-100 film was 5.3 C higher than with LDPE film (Table 2). For 49% of the solarization period, soil surface temperatures under TIR-100 exceeded 60 C, compared with 8% with LDPE film.

At the end of the 6-wk soil solarization period, in the nonsolarized treatment, the initial 12 tubers resulted in a *C. rotundus* density of 296 plants m^{-2} (Table 5). The use of solarization considerably reduced *C. rotundus* density. TIR films were more effective than the LDPE film. While solarization with TIR films resulted in < 15 plants m^{-2}, the *C. rotundus* density with LDPE film was 65 plants m^{-2}. If the nonsolarized treatment is considered to be a weedy check, this density of 65 plants m^{-2} represents a control level of 78%. The least effective of the TIR films, the TIR-50, provided 95% control of *C. rotundus*. TIR-75 and TIR-100 resulted in nearly 100% control of *C. rotundus*. The effect of soil solarization on shoot biomass paralleled that of shoot density.

TABLE 5. Comparative effects of solarization film on *C. rotundus* control at the end of a 6-wk period of soil solarization.

Film	*C. rotundus* density (plants m^{-2})			Control[c] (%)	Shoot biomass (g)		
	Under[a]	Through[b]	Total		Under	Through	Total
Bare	—	—	296 a	—	—	—	125.2 a
LDPE-30	58 a[d]	7 a	65 b	78.1 a	16.1 a	6.7 a	22.8 b
TIR-50	12 b	2 b	14 c	95.2 b	2.5 b	1.3 b	3.8 c
TIR-75	1 b	0 b	1 d	99.5 b	0.1 b	0 b	0.1 d
TIR-100	1 b	0 b	1 d	99.6 b	0.1 b	0 b	0.1 d

[a] *C. rotundus* plants trapped under solarization film.
[b] *C. rotundus* plants penetrating solarization film.
[c] *C. rotundus* densities of solarized treatments were expressed as percentages of the nonsolarized treatment.
[d] Means followed by the same letter within columns are not significantly different ($\alpha = 0.05$) as determined by contrasts. Values are means of 15 replicates, averaged over depth.

The thinner films (LDPE-30 and TIR-50) were more susceptible to puncture by the sharp leaf tips of the *C. rotundus* plants (Table 5). Plants that emerged through the films generally survived soil solarization and resulted in a proliferation of daughter plants from their basal bulbs. This contributed to the greater number of plants present under the thinner films at the end of the solarization period.

The main effect of soil depth of tuber planting (averaged over film with the nonsolarized treatment included) on *C. rotundus* density was not significant (Table 7). This indicated that there was no change in *C. rotundus* density with increasing planting depths, a result that was probably strongly influenced by the densities obtained with the nonsolarized treatment (Table 4). Because nonsolarized soil was not expected to, and did not, result in lethal temperatures, the main effect of planting depth on *C. rotundus* density was reassessed after excluding the nonsolarized treatment. Although there was a trend for *C. rotundus* density to increase as depth of tuber planting increased, the effect of planting depth on *C. rotundus* density was not significant. The biological implication of this trend is that the control by soil solarization of *C. rotundus* tubers located at soil depths where they are not exposed to lethal temperatures is dependent on tuber inactivation or depletion as a result of foliar scorching of successive shoots that arise from the tubers. Another explanation could be that there is decreased *C. rotundus* emergence as tuber planting depth increases that may contribute to the reduced densities observed with increased depth. However, despite a previous report of significant reductions in *C. rotundus* emergence with tuber planting depths of ≥ 20 cm (Rubin and Benjamin 1984), the lack of difference in *C. rotundus* density with soil depth in this study is unlikely to be related to poor emergence from 15 cm deep. In the absence of solarization, *C. rotundus* density with a 15-cm planting depth was 280 plants m^{-2} compared with 25 plants m^{-2} with solarized soil. This indicates considerable *C. rotundus* suppression resulting from the use of solarization film.

Persistence of Control After Film Removal

When the persistence of *C. rotundus* control was assessed 4 wk after the films had been removed, the lowest level of control was obtained with the LDPE film (Figure 3). With the LDPE film, the 5- and 10-cm planting depths both resulted in 62% control. However, *C. rotundus* control decreased to 32% when planting depth increased to 15 cm. There was a trend for a decrease in control with planting depth with the 50-μm TIR film, which was not significant. *Cyperus rotundus* control with 75- and 100-μm TIR films was still quite high 4 wk after removing the solarization films. Control with these two films did not differ significantly and was equally effective for all planting depths.

In this study, lethal temperature for *Cyperus* spp. tubers under diurnally fluctuating temperatures was discovered to be > 45 C. While 45 C slowed the rate of emergence, it was not lethal to *Cyperus* spp. tubers in a diurnally fluctuating temperature regime. However, 50 and 55 C completely eliminated sprouting and resulted in 100% tuber mortality. In the field test, lethal temperatures were recorded 5 cm deep under all of the solarization films. TIR films resulted in lethal soil temperatures on a greater proportion of days and hotter temperatures at all soil depths than the non-TIR film. This is expected to contribute both to more extensive tuber mortality and to foliar scorching. Thinner so-

TABLE 6. Evaluation of *C. rotundus* density after 2 and 4 wk of soil solarization.

Film	*C. rotundus* (plants m^{-2})			
	Alive	Dead[a]	Alive	Dead
	Week 2		Week 4	
LDPE-30	12 a[b]	2 a	21 a	10
TIR-50	3 b	8 b	3 b	10
TIR-75	4 b	8 b	0 b	11
TIR-100	4 b	9 b	0 b	12

[a] Plants were killed by foliar scorching.
[b] Multiple comparisons performed by applying Fisher's LSD test to the least-squares means.

TABLE 7. Effect of soil depth of tuber planting on *C. rotundus* density after 6 wk of soil solarization.

Tuber depth	Bare and solarized[a]	Solarized[b]
cm	Plants m^{-2}	
5	76	17
10	73	19
15	76	25
$P > F_{0.05}$[c]	0.8	0.4

[a] ANOVA included all main plot treatments: bare or nonsolarized and solarized with 30-μm LDPE film and 50-, 75-, and 100-μm TIR.
[b] ANOVA included only *C. rotundus* densities obtained with the LDPE and the three TIR solarization films.
[c] Probability values > 0.05 are not significant.

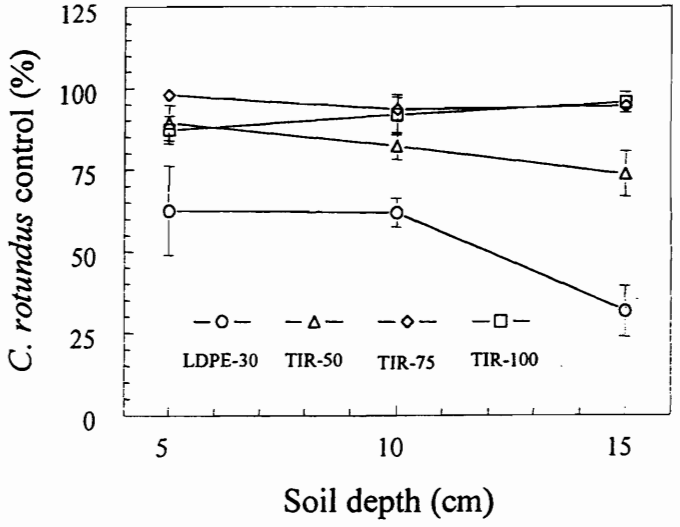


FIGURE 3. *Cyperus rotundus* control by soil solarization 4 wk after film removal and shoot biomass harvest.

larization films were more susceptible to puncture by *C. rotundus,* an occurrence that facilitated daughter plant production from basal bulbs and decreased the efficacy of soil solarization. Although temperatures > 45 C were rare 15 cm deep, the absence of a depth effect on the persistence of *C. rotundus* control with the TIR films indicates that foliar scorching of emerged *C. rotundus* shoots that are trapped under the film contributes significantly to *C. rotundus* control by soil solarization. The higher soil temperatures under TIR films contributed to more effective *C. rotundus* control by solarization than the LDPE film.

Sources of Materials

[1] *Cyperus esculentus* tubers, Valley Seed Service, P.O. Box 9335, Fresno, CA 93791.

[2] *Cyperus rotundus* tubers, clones from tubers collected at the University of Florida Horticultural Research Unit, Gainesville, FL 32611.

[3] Scotts Terralite Agricultural Mix, V. J. Growers Supply, Inc., 500 Orange Blossom Trail, Apopka, FL 32712-3498.

[4] CR10 data logger, Campbell Scientific, Inc, 815 West 1800 North, Logan, UT 84321-1784.

[5] TIR film, courtesy AT Plastics Inc., 4405 101st Avenue, Edmonton, Alberta, Canada T5J 2K1.

[6] LDPE film, courtesy AEP Industries, 125 Phillips Avenue, Hackensack, NJ 07606.

Acknowledgments

We thank Dr. D. O. Chellemi for his suggestions and advice and Dr. V. Chew and Jay Harrison for guidance with statistical analysis. Florida Agricultural Experiment Station, Journal Ser. R-06869. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval or the exclusion of other products that may also be suitable.

Literature Cited

Chase, C. A., T. R. Sinclair, D. G. Shilling, S. J. Locascio, and J. P. Gilreath. 1998. Light effects on nutsedge (*Cyperus* spp.) rhizome to shoot development: implications for control by soil solarization. Weed Sci. 46: 575–580.

Chellemi, D. O., S. M. Olson, D. J. Mitchell, I. Secker, and R. McSorley. 1997. Adaptation of soil solarization to the integrated management of soilborne of tomato under humid conditions. Phytopathology 87:250–258.

Egley, G. H. 1983. Weed seed and seedling reductions by soil solarization with transparent polyethylene sheets. Weed Sci. 31:404–409.

Gilreath, J. P., J. P. Jones, A. J. Overman, et al. 1994. Nutsedge and soilborne pathogen control with alternatives to methyl bromide. Pages 7–16 *in* Proceedings of the Florida Tomato Institute. Gainesville, FL: University of Florida.

Holt, J. S. and D. R. Orcutt. 1996. Temperature thresholds for bud sprouting in perennial weeds and seed germination in cotton. Weed Sci. 44: 523–533.

Horowitz, M., Y. Regev, and G. Herzlinger. 1983. Solarization for weed control. Weed Sci. 31:170–179.

Katan, J., A. Greenberger, H. Alon, and A. Grinstein. 1976. Solar heating by polyethylene mulching for the control of diseases caused by soilborne pathogens. Phytopathology 66:683–688.

Locascio, S. J., J. P. Gilreath, D. W. Dickson, T. A. Kucharek, J. P. Jones, and J. W. Noling. 1997. Fumigant alternatives to methyl bromide for polyethylene-mulched tomato. HortScience 32:1208–1211.

Miles, J. E., R. K. Nishimoto, and O. Kawabata. 1996. Diurnally alternating temperatures stimulate sprouting of purple nutsedge (*Cyperus rotundus*) tubers. Weed Sci. 44:122–125.

Rubin, B. and A. Benjamin. 1983. Solar heating of the soil: effect on weed control and on soil-incorporated herbicides. Weed Sci. 31:819–825.

Rubin, B. and A. Benjamin. 1984. Solar heating of the soil: involvement of environmental factors in the weed control process. Weed Sci. 32: 138–142.

[SAS] Statistical Analysis Systems. 1996. SAS/STAT Software: Changes and Enhancements Through Release 6.11. Cary, NC: Statistical Analysis Systems Institute.

Standifer, L. C., P. W. Wilson, and R. Porche-Sorbet. 1984. Effects of solarization on soil weed seed populations. Weed Sci. 32:569–573.

Sun, W. H. and R. K. Nishimoto. 1997. Dormancy release of purple nutsedge tuber buds by a single thermal pulse. J. Am. Soc. Hortic. Sci. 122:306–309.

Received June 5, 1998, and approved June 3, 1999.

Adaptation of Soil Solarization to the Integrated Management of Soilborne Pests of Tomato Under Humid Conditions

D. O. Chellemi, S. M. Olson, D. J. Mitchell, I. Secker, and R. McSorley

First and second authors: University of Florida, North Florida Research and Education Center, Route 3, Box 4370, Quincy 32351; third author: University of Florida, Department of Plant Pathology, Gainesville 32611; fourth author: Polyon Barkai, Kibbutz Barkai, Israel; and fifth author: University of Florida, Department of Entomology and Nematology, Gainesville 32611.

Accepted for publication 22 November 1996.

ABSTRACT

Chellemi, D. O., Olson, S. M., Mitchell, D. J., Secker, I., and McSorley, R. 1997. Adaptation of soil solarization to the integrated management of soilborne pests of tomato under humid conditions. Phytopathology 87:250-258.

Soil solarization was shown to be cost effective, compatible with other pest management tactics, readily integrated into standard production systems, and a valid alternative to preplant fumigation with methyl bromide under the tested conditions. Solarization using clear, photoselective, or gas-impermeable plastic was evaluated in combination with metham sodium, 1,3-dichloropropene + chloropicrin, methyl bromide + chloropicrin, pebulate, or cabbage residue. Strip solarization, applied to 20-cm-high, 0.9-m-wide beds, was conducted to achieve compatibility with standard production practices and resulted in soil temperatures 2 to 4°C above those temperatures resulting when using conventional flatbed solarization. Soil temperatures were 1 to 2°C higher at the edges of the raised beds, eliminating any border effects associated with solarization. Following a 40- to 55-day solarization period, the plastic was painted white and used as a production mulch for a subsequent tomato crop. The incidence of Southern blight and the density of *Paratrichodorus minor* and *Criconemella* spp. were lower ($P < 0.05$) in solarized plots. No differences ($P < 0.05$) in the incidence of Fusarium wilt and the density of nutsedge and *Helicotylenchus* spp. were observed between plots receiving solarization and plots fumigated with a mixture of methyl bromide + chloropicrin. The severity of root galling was lower ($P < 0.05$) when soil solarization was combined with 1,3-dichloropropene + chloropicrin (16 + 3.4 g/m^2) and a gas-impermeable film. The incidence of bacterial wilt was not affected by soil treatments. Marketable yields in plots using various combinations of soil solarization and other tactics were similar ($P < 0.05$) to yields obtained in plots fumigated with methyl bromide + chloropicrin. The results were validated in several large scale field experiments conducted by commercial growers.

Additional keywords: *Cyperus esculentus*, *C. rotundus*, *Fusarium oxysporum* f. sp. *lycopersici*, *Lycopersicon esculentum*, *Meloidogyne* spp., *Pseudomonas solanacearum*, *Rotylenchulus reniformis*, *Sclerotium rolfsii*.

In Florida and other states, field production of fresh market solanaceous crops is based upon high input, intensively managed production systems requiring the use of broad spectrum fumigants to provide economic control of soilborne pests (5). Since the 1970s, methyl bromide has been used almost exclusively, because of its consistent and effective control of soilborne pests under a range of soil moisture and temperature regimes, relatively low cost, and ease of handling (4,54,56). It has been estimated that a ban on the use of methyl bromide would cost producers and consumers in the United States over $1 billion annually (2) and would reduce production of tomato, pepper, and eggplant in Florida by 69, 63, and 100%, respectively (46). Concerns about the contribution of methyl bromide to the stratospheric depletion of ozone have led to a ban on its production and importation in the United States beginning January 2001 (10,13).

While several broad spectrum fumigants have been identified as alternatives to methyl bromide (11,21,35,38), use of a single tactic to control soilborne pests still leaves producers vulnerable to future regulatory and marketing policies. For example, half of the soil fumigants used in Florida before 1978 are no longer available, and the remainder have environmental or toxicity problems (4).

Integrated pest management (IPM), which consists of the coordinated use of multiple tactics to maintain pest damage below an economic threshold, offers several advantages over a single tactic approach. Selection of pest management tactics that address the specific needs and constraints of individual cropping systems eliminates unnecessary pesticide applications and optimizes the performance of existing tactics. IPM fosters the development of selective, biologically based tactics, because the emphasis is on the control of identified pest complexes, not perceived pest threats.

Historically, IPM has not been utilized to control soilborne pests during field production of fresh market solanaceous crops. Preharvest production costs often exceed $15,000 per hectare (45), leading producers to take measures that reduce the perceived risk of economic loss from soilborne pests. Assessment of soilborne pest complexes in the field is not routinely practiced, creating uncertainty in the selection of tactics. IPM programs are knowledge intensive; they require additional expertise and supplemental management decisions. The development of cultural pest management tactics that are easy to employ, cost effective, and can enhance the efficacy of chemical, cultural, and biological treatments has been limited (31).

Soil solarization is a pest management tactic that has potential application to IPM systems. It is compatible with and often enhances the performance of various chemical, biological, and cultural treatments (14,16,17,34,41–44,53). Soil solarization has been evaluated in several geographic regions where high value crops are cultivated (6,20,25,33,37,39). Its general contribution to IPM systems has been discussed, but it has not been examined within the constraints of specific crop production systems (27,49).

The objective of this study was to evaluate soil solarization as a component in an IPM program for soilborne pests of tomato. Soil solarization was examined for its adaptability to the production system used for this and other similar crops cultivated in humid

Corresponding author: D. Chellemi; E-mail address: doc@gnv.ifas.ufl.edu

Publication no. P-1997-0122-01R

regions, compatibility with other pest management tactics, and effect on soilborne pests and marketable yield.

MATERIALS AND METHODS

Characterization of the cropping system. Fall production of fresh market tomato (*Lycopersicon esculentum* Mill.) in north Florida and south Georgia was used as the model cropping system. Tomatoes are produced on 20-cm-high raised beds covered by white or white on black, low density polyethylene (LDPE) plastic mulch. Beds are prepared and fumigated in June. Tomatoes were transplanted in July and harvested in October. Key soilborne diseases are Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. (races 1, 2, and 3); bacterial wilt, caused by *Ralstonia solanacearum*; and Southern blight, caused by *Sclerotium rolfsii* Sacc. Key weed pests are yellow nutsedge (*Cyperus esculentus* L.) and purple nutsedge (*C. rotundus* L.). Important phytoparasitic nematodes include the root-knot nematode species *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, reniform nematode *Rotylenchulus reniformis* Linford & Oliveira, and stubby-root nematode *Paratrichodorus minor* (Colbran) Siddiqi.

In 1994, field experiments were conducted on tomato production farms located in Decatur County, GA (Georgia-94), and Gadsden County, FL (Florida-94). In 1995, an experiment was conducted at the North Florida Research and Education Center (Florida-95), Gadsden County. In 1995, large scale field validation studies were implemented in three commercial tomato production fields (validation sites I, II, and III). Soil characteristics are presented in Table 1. All locations had been cropped in tomato for five or more consecutive years except the Florida-95 site, which had been left as weed fallow following previous crops of peanut and tobacco.

Georgia-94 and Florida-94 experiments. Eighteen treatments were arranged in a randomized complete block with four replications per treatment. An incomplete factorial design was used in which 16 treatments consisted of all possible combinations of four soil treatments applied under four types of plastic film. Soil treatments were a mixture of methyl bromide + chloropicrin (13.1 + 6.5 g/m^2); metham sodium (5.8 g/m^2); and cabbage residue (*Brassica oleracea* cv. Constanza) that were dried, shredded, and incorporated into the soil by disking at a rate of 8.0 kg/m^2; and an untreated control. Plastic films included a 30-µm-thick, coextruded, white on black LDPE (Edison Plastics Co., Lee Hall, VA); a 30-µm-thick, clear LDPE used for solarization (Polydak film; Polyon Barkai Ltd., Kibbutz, Barkai, Israel); a 25-µm-thick, photoselective LDPE used for solarization (IRT film; AEP Industries, Hackensack, NJ); and a 30-µm-thick, clear, gas-impermeable film used for solarization (Bromotec film; Lawson Mardon Packaging, Workington, Cumbria, United Kingdom). Two additional treatments consisting of metham sodium (11.6 g/m^2) and methyl bromide + chloropicrin (26.1 + 13.0 g/m^2) were applied under white on black LDPE.

Each replicate plot was 20 m in length and consisted of a single raised bed 20 cm high and 0.9 m wide. Row orientation was north/south. Beds were spaced 1.8 m apart. Fertilizer was incorporated into the beds at 212 kg of N, 65 kg of P, and 212 kg of K per hectare, and drip irrigation tubing was placed 5 cm beneath the soil, 25 cm from the edge of the bed.

Cabbage was grown and harvested in the plots, and the residue incorporated on 23 and 24 May. Beds were prepared and fumigants applied on 3 and 15 June at the Georgia-94 and Florida-94 sites, respectively. The solarization period was terminated by painting the films with a white, latex-based paint (Kool Grow, Gainesville, FL) mixed at a 6:1 dilution with water and applied through a CO_2 backpack sprayer. The painted plastic functioned as a horticultural mulch. Tomato seedlings were transplanted into the plots on 27 July (cv. BHN 189) and 3 August (cv. Solar Set) at the Georgia-94 and Florida-94 sites, respectively.

Precipitation was recorded daily at both sites. Daily ambient temperature readings were obtained from a National Weather Service Reporting Station located in Gadsden County. Hourly soil temperatures were monitored at the Florida-94 site at depths of 10 and 25 cm using thermocouple sensors. Sensors were placed in the center and 10 cm from the edge of the beds. An electronic data logger automatically processed and recorded analog signals from the sensors (Omnidata International, Logan, UT). Two tropical storms severely damaged the Florida-94 site, preventing the collection of additional data from that site. Twelve contiguous plants in the center of each replicate plot were harvested from the Georgia-94 site on 7, 18, and 27 October. Fruit were sorted as marketable and unmarketable based upon size and cosmetic appearance.

Florida-95 experiment. Fifteen treatments were arranged in a randomized complete block with four replications per treatment. A factorial design that consisted of five soil treatments × three plastic films was used. Soil treatments were cabbage residue (cv. Constanza applied at 6.8 kg/m^2), pebulate (an herbicide applied at 0.1 g/m^2), a mixture of 1,3-dichloropropene + chloropicrin (16.2 + 3.4 g/m^2), a mixture of methyl bromide + chloropicrin (13.1 + 6.5 g/m^2), and an untreated control. Plastic films were the same as in 1994 except that the photoselective film was omitted.

Plot dimensions and row orientation were the same as in 1994. Cabbage was grown in another location, harvested, and incorporated into plots on 19 May using the same procedures as in 1994. Pebulate was applied and immediately disked into the soil on 25 May. The remaining soil treatments were applied on 26 May. Fertilizer rates were 196 kg of N, 26 kg of P, and 163 kg of K per hectare. The solarization period was terminated when the plastic was painted. Tomatoes (cv. Solar Set) were transplanted on 31 July. Yield data was collected from twelve contiguous plants in the center of each plot on 11, 18, and 26 October.

Daily rainfall and ambient temperature data were recorded. Hourly temperature changes in bare and solarized soil were monitored in beds located in another location approximately 0.5 km from the site. Temperatures were recorded at the soil surface and at depths of 5, 10, 15, 20, and 25 cm using thermocouple sensors connected to an electronic data logger (Campbell Scientific Inc., Logan, UT).

Validation site I. Five treatments were arranged in a randomized complete block with four replications per treatment. Treatments were soil solarization, soil solarization combined with 1,3-dichloropropene + chloropicrin (16.2 + 3.4 g/m^2), soil solarization combined with methyl bromide (22.4 g/m^2), 1,3-dichloropropene + chloropicrin (32.4 + 6.9 g/m^2) applied under white plastic, and methyl bromide (45.0 g/m^2) applied under white plastic. Soil solarization treatments were performed using clear, 25-µm-thick LDPE plastic (Polydak film). Each replicate plot consisted of three rows 182 m in length. Row-spacing orientation, bed dimensions, and plant spacings were the same as in previous experiments.

TABLE 1. Characteristics of field soils at the time of solarization and soil treatments

Site	pH	Soil water content (%)[y]	Organic matter (%)	Percent sand-silt-clay (%)	Soil class
Georgia-94	6.6	9.9	1.1	84.0 - 6.0 - 10.0	Kandiudult[z]
Florida-94	5.1	6.1	0.5	88.5 - 4.5 - 7.0	Kandiudult
Validation site I	6.3	9.8	0.4	86.0 - 5.5 - 8.5	Kandiudult
Florida-95	4.7	7.7	1.5	88.5 - 4.0 - 7.5	Kandiudult
Validation site II	6.2	10.3	0.8	87.0 - 6.5 - 6.5	Kandiudult
Validation site III	6.1	9.1	1.0	80.5 - 9.5 - 10.0	Kandiudult

[y] Water content expressed as a percentage of the mass of dry soil (gravimetric method).

[z] Ultisol with profiles similar to paleudults, but containing higher amounts of kaolinitic clay.

Treatments were applied on 9 June. The solarization period was terminated by painting the plastic, and tomatoes (cv. BHN 189) were transplanted on 19 July. Yield data were obtained by harvesting 12 contiguous plants from the middle row of each replicate plot on 28 September; 10, 19, and 25 October; and 1 November. Daily precipitation and ambient temperature data were obtained from a National Weather Service Reporting Station located 9 km from the study site.

Validation sites II and III. Unreplicated field plots were established on two additional commercial tomato production farms in 1995. Row orientation was north/south in site II and east/west in site III. Soil solarization was performed on 12 rows 210 m in length (0.48 ha) in site II using 30-µm-thick LDPE (Polydak film) and on seven rows 324 m in length (0.42 ha) in site III using 25-µm-thick LDPE (Polydak film). Rows adjacent to the solarization treatment were fumigated with methyl bromide (45 g/m^2) and covered with a white over black, coextruded LDPE (Edison Plastics Co.). Soil disinfestation treatments were applied on 9 June in site II and 13 June in site III. The solarization period was terminated by painting the plastic white. Tomato seedlings (cv. Solar Set) were transplanted into all treatments. Yield data were obtained by harvesting 12 contiguous plants from four different locations each within both the solarized and methyl bromide-treated areas.

Assessment of pest control. In locations where *Cyperus* spp. were present, assessment of control was made by counting the number of plants emerging through the plastic over a 12-m-long section and computed as an average per 0.27 m^2 of bed. Ratings were taken at the end of the solarization period, prior to transplanting. In locations where symptoms of soilborne diseases were present, disease incidence was assessed by counting the number of plants with symptoms in each replicate plot and dividing by the total number of plants present in each replicate plot. Soil samples for nematode analysis were collected by removing and combining soil cores 2.5 cm wide and 20 cm deep from the root zone of each of six plants per plot. Nematodes were extracted from 100-cm^3 soil subsamples with a modified sieving and centrifugation procedure (28), and then identified and counted. In locations where root galling from *Meloidogyne* spp. was present, root systems from five plants in each replicate plot were removed and rated for root galling on a 0 to 5 scale (52) in which 0 = no galls per root system, 1 = one to two galls per root system, 2 = three to 10 galls per root system, 3 = 11 to 30 galls per root system, 4 = 31 to 100 galls per root system, and 5 = more than 100 galls per root system.

Evaluation of soil heating under raised beds. Soil temperatures under a flat surface and 20-cm raised beds were recorded to obtain information on the efficiency of strip solarization performed on raised beds. The comparison was made between 30 August and 23 September 1995 in a location near the Florida-95

TABLE 2. Environmental conditions during solarization periods

Site	Total precipitation (cm)	Number of days: Solarization period	Number of days: Precipitation occurred	Number of days: Maximum ambient temperature ≥35°C
Georgia-94	46.0	49	30	0
Florida-94	38.4	48	31	0
Florida-95	30.1	55	17	6
Validation site I	17.4	40	12	6
Validation site II	NA[z]	40	NA	6
Validation site III	NA	36	NA	5

[z] Not available.

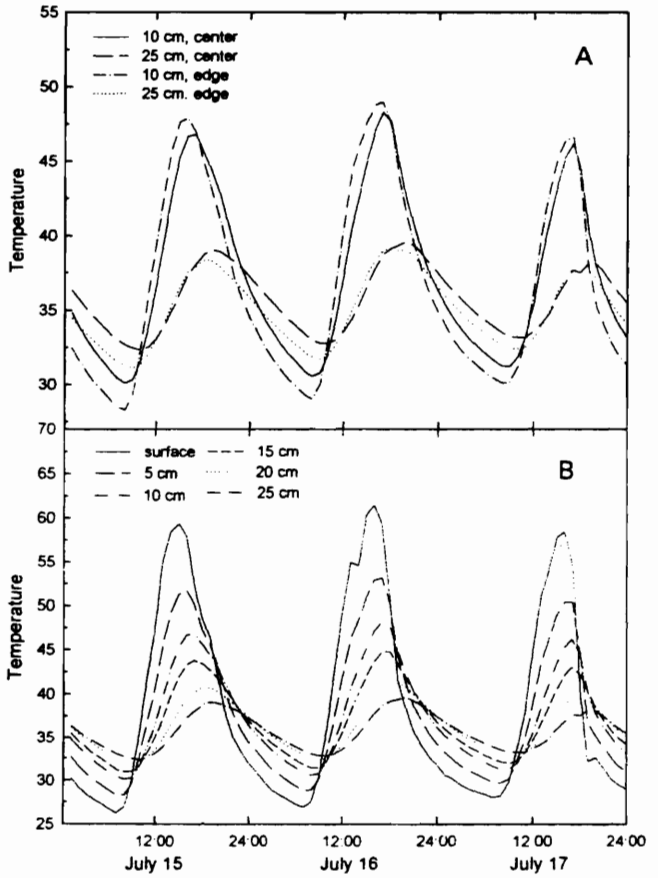


Fig. 1. Hourly fluctuations in soil temperatures over a 3-day period at the Florida-95 site. A, Comparison among temperatures at 10 and 25 cm depths in the center and 10 cm from the edge of a 0.9-m-wide, 20-cm-high bed covered by clear, low density polyethylene plastic. B, Temperature profile at various depths within the center of the raised bed.

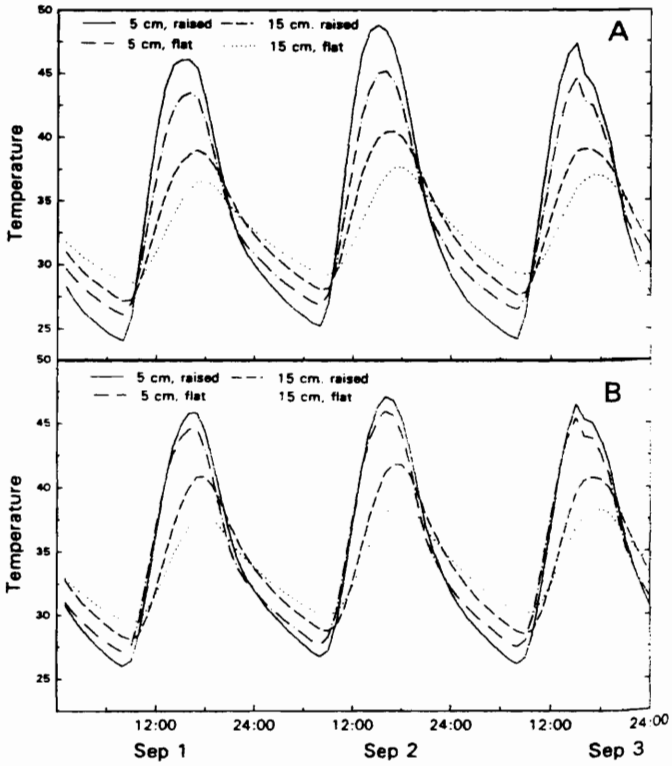


Fig. 2. Hourly fluctuations in soil temperatures at 5 and 15 cm depths over a 3-day period under a 0.9-m-wide sheet of clear, low density polyethylene plastic stretched over a flat surface or a 20-cm-high bed. A, Soil temperatures at 10 cm from the east edge of the plastic strip. B, Soil temperatures from the center of the plastic strip.

site. A 0.9 × 10.0-m sheet of clear, 25-µm-thick, LDPE (Polydak film) was stretched over a flat soil surface and a over raised bed 20 cm high and 0.9 m wide. Soil moisture at initiation of the comparison was 15.6%. Daily precipitation and ambient temperature data were recorded at the site. Hourly changes in soil temperatures were monitored in the raised and flat beds at 5 and 15 cm depths using thermocouple sensors connected to an electronic data logger (Campbell Scientific Inc.). Row orientation was north/south. Soil temperatures were monitored 10 cm from the edge on the west and east side of the bed and in the center of the bed.

Statistical analysis. Nematode counts were log-transformed ($\log_{10}[x + 1]$) and disease incidence data were transformed using the arc sine transformation ($\sin^{-1}$ square root x) prior to analysis. Analysis of variance was performed with PROC GLM of SAS (release 6.04 for personal computers; SAS Institute, Cary, NC) for all data except nematode density counts, which were analyzed using MSTAT (Michigan State University, East Lansing). The Waller-Duncan k-ratio t test was used to compare all treatment means except for nematode density counts, which were compared using Duncan's multiple range test.

RESULTS

Site conditions. Soil water content, expressed as a percentage of the mass of dry soil, ranged from 6.1 to 10.3% at the onset of solarization and fumigation treatments (Table 1). The solarization periods ranged from 36 to 55 days (Table 2). In 1994, precipitation was recorded on at least 30 different days in the solarization period at both sites (Table 2). In 1995, precipitation occurred on 12 and 17 different days in the solarization period at the Florida-95 site and validation site I, respectively.

Soil temperatures. At the Florida-94 site, the maximum temperature at a 10 cm depth was 41.5°C under bare soil and 41.8, 45.7, and 49.2°C under white, photoselective, and clear plastic, respectively. At a 25 cm depth, the maximum soil temperature was 34.6°C under bare soil and 34.7, 37.3, and 38.0°C under white, photoselective, and clear plastic, respectively. Addition of cabbage residue did not increase soil temperature.

At the Florida-95 site, the maximum soil temperature at a 10 cm depth was 42.4°C under bare soil and 48.7°C under clear LDPE. At a 25 cm depth, the maximum soil temperature was 35.1°C under bare soil and 40.3°C under clear LDPE. Soil temperatures near the edge of the plastic were 1 to 2°C higher than in the center (Fig. 1A). Under clear LDPE, the maximum soil temperature declined rapidly from 61.3°C at the surface to 53.2°C at a 5 cm depth (Fig. 1B). At depths of 5 and 15 cm, higher soil temperatures were achieved when solarization was performed on a raised bed as opposed to on a flat surface (Fig. 2A and B). The differences were more pronounced at the edges of the bed than in the center. On a raised bed, temperatures at the edges often exceeded those in the center (Fig. 2).

TABLE 3. Effects[w] of soil treatments and solarization with various plastic films on soilborne pests and yield of fresh market tomato at the Georgia-94 site

		Plastic film[x,y]				
Soil treatment	Rate per m²	Clear, GI	White	Clear	IRT	Mean[y]
Fusarium wilt						
None	...	0.0 d	7.2 a	0.0 d	1.0 cd	2.0
Cabbage	8.0 kg	0.9 cd	5.6 ab	0.0 d	2.0 cd	2.1
Metham-Na	5.8 g	0.0 d	3.8 bc	0.9 cd	0.9 cd	1.4
MBC[z]	13.1 + 6.5 g	0.0 d	0.0 d	0.0 d	0.0 d	0.0
Mean		0.2	4.2	0.2	1.0	
Bacterial wilt						
None	...	4.4	15.6	20.7	4.9	11.4
Cabbage	8.0 kg	10.0	20.5	2.9	23.6	14.2
Metham-Na	5.8 g	9.2	18.5	33.4	19.4	20.1
MBC	13.1 + 6.5 g	19.2	5.0	9.6	10.1	11.0
Mean		10.7	14.9	16.6	14.5	
Paratrichodorus minor						
None	...	13.8	9.5	18.5	21.2	15.8 C
Cabbage	8.0 kg	21.8	29.0	17.8	16.8	21.3 BC
Metham-Na	5.8 g	14.5	50.0	23.8	37.8	31.5 AB
MBC	13.1 + 6.5 g	21.8	62.2	38.8	21.0	35.9 A
Mean		17.9 B	37.7 A	24.7 B	24.2 B	
Cyperus species						
None	...	0.1 b	12.0 a	0.1 b	<0.1 b	3.1
Cabbage	8.0 kg	0.2 b	3.1 b	<0.1 b	0.5 b	1.0
Metham-Na	5.8 g	0.1 b	3.0 b	<0.1 b	0.2 b	0.8
MBC	13.1 + 6.5 g	<0.1 b	0.5 b	<0.1 b	0.2 b	0.2
Mean		0.1	4.6	0.1	0.2	
Marketable yield						
None	...	45.4 a	23.2 c	48.5 a	42.6 ab	39.9
Cabbage	8.0 kg	51.7 a	28.7 c	50.9 a	32.2 bc	40.9
Metham-Na	5.8 g	53.7 a	46.2 a	42.0 ab	45.7 a	46.9
MBC	13.1 + 6.5 g	46.7 a	49.3 a	47.0 a	46.0 a	47.2
Mean		49.4	36.9	47.1	41.6	

[w] Effects are stated for Fusarium wilt and bacterial wilt as the percent of disease incidence, for *Paratrichodorus minor* as the density of nematodes per 100 cm³ of soil (samples collected 62 days after transplanting), for *Cyperus* species as the number of plants per 0.27-m² area that emerged through the plastic at the termination of the solarization period, and for marketable yield as metric tons per hectare.

[x] Clear, GI = clear, gas-impermeable solarization film; white = white on black, coextruded, low density polyethylene (LDPE); clear = clear LDPE solarization film; and IRT = a photoselective solarization film with transmission values of 15% for photosynthetically active radiation and 23% for total radiation.

[y] Main effect means for plastic film or soil treatment followed by the same letter (A, B, or C) do not differ according to the Waller-Duncan k-ratio t test ($P \leq 0.05$). Means for the interaction of soil treatment and plastic film followed by the same letter (a, b, c, or d) do not differ according to the Waller-Duncan k-ratio t test ($P \leq 0.05$).

[z] A mixture of methyl bromide + chloropicrin.

Soilborne diseases. There was a significant interaction between the effects of soil treatments and plastic film on the incidence of Fusarium wilt at the Georgia-94 site (Table 3). Plots receiving soil solarization or methyl bromide had significantly lower levels of disease than plots receiving metham sodium. Doubling the rate of metham sodium did not result in lower levels of disease (Table 4). Fusarium wilt was not detected at the other sites.

At the Georgia-94 site, neither soil treatment nor plastic had a significant effect on the incidence of bacterial wilt (Table 3). Doubling the rate of methyl bromide + chloropicrin resulted in significantly higher levels of bacterial wilt (Table 4). Doubling the rate of metham sodium did not affect the level of disease. Bacterial wilt was observed in validation sites I and II, but the incidence was below 1%.

Soil treatment and plastic film had a significant effect on the incidence of Southern blight at the Florida-95 site (Table 5). Disease levels were higher in the plots receiving only cabbage amendment and were lower in plots receiving soil solarization. In validation site II, the incidence of Southern blight was <0.1% in the solarized area and 3.7% in the areas treated with methyl bromide (Table 6).

Yellow and purple nutsedge. A significant interaction between soil treatment and plastic was observed at the Georgia-94 and Florida-95 sites. At the Georgia-94 site, nutsedge pressure was less severe, and both soil solarization and methyl bromide + chloropicrin provided significant control of mixed populations of yellow and purple nutsedge (Table 3). Doubling the dosage of methyl bromide + chloropicrin or metham sodium did not increase the level of control (Table 4). In the Florida-95 site, nutsedge pressure was more intense, and only treatments with solarization provided significant levels of control (Table 5).

Phytoparasitic nematodes. Soil treatment and plastic had a significant effect on the density of *Paratrichodorus minor* at the Georgia-94 site (Table 3). Significantly lower densities of *Paratrichodorus minor* were observed in the plots receiving solarization. Doubling the dosage of fumigants did not affect the density of *Paratrichodorus minor* (Table 4). In the Florida-95 site, soil treatment and plastic also had a significant effect on the density of *Paratrichodorus minor* (Table 7).

The density of *Paratrichodorus minor* was lower in areas receiving soil solarization in validation sites II and III (Table 6), but not in validation site I (Table 8). In all five sites examined, application of methyl bromide + chloropicrin failed to reduce the density of *Paratrichodorus minor* (Tables 3, 4, 6, 7, and 8).

A significant interaction between soil treatment and plastic was observed for the severity of root galling by *Meloidogyne* spp. at the Florida-95 site (Table 5). Treatments receiving methyl bromide + chloropicrin or the combination of soil solarization, 1,3-dichloropropene + chloropicrin, and a gas-impermeable plastic had significantly lower levels of root galling. Soil treatment had a significant effect on the density of *Meloidogyne* spp. at the Florida-95 site (Table 7). At validation site I, treatments did not significantly impact the density of *Meloidogyne* spp. (Table 8).

At the Florida-95 site, the density of *R. reniformis* was significantly lower in plots receiving methyl bromide + chloropicrin or 1,3-dichloropropene + chloropicrin (Table 7). The density of *R. reniformis* in validation site III was lower in plots treated with methyl bromide (Table 6).

Significant reductions in *Criconemella* and *Helicotylenchus* spp. were achieved using soil solarization, methyl bromide + chloropicrin, and 1,3-dichloropropene + chloropicrin (Table 7).

Marketable yield. A significant interaction between soil treatment and plastic was observed at the Georgia-94 and Florida-95 sites. No significant difference in yield was observed between plots receiving methyl bromide + chloropicrin or soil solarization at the Georgia-94 site except for plots that received cabbage + solarization using the photoselective plastic, which was significantly lower (Table 3). Doubling the rate of metham sodium did not affect yield. Doubling the rate of methyl bromide + chloropicrin resulted in significantly lower yields, because of the increased incidence of bacterial wilt. At the Florida-95 site, the interaction between plastic and soil treatment were complex. Yields in plots receiving solarization, solarization and methyl bromide + chloropicrin, or solarization and 1,3-dichloropropene + chloropicrin were significantly higher than yields in plots receiving soil treatments applied under white plastic (Table 5). At validation site I, yields in plots treated with a high rate of 1,3-dichloropropene + chloropicrin were significantly higher than yields in plots receiving only solarization (Table 8). At validation sites II and III, yields in areas treated with soil solarization were similar to yields in areas treated with methyl bromide (Table 6).

TABLE 4. Contrast among treatments at the Georgia-94 site under white plastic receiving reduced and full rates of methyl bromide + chloropicrin (MBC) or metham sodium (Metham-Na)

Treatment	Rate per m²	Control assessment[y]	$PR > F$[z]
Marketable yield			
Metham-Na	5.8 g	46.3	0.76
Metham-Na	11.6 g	41.9	
MBC	13.1 + 6.5 g	49.3	0.08
MBC	26.2 + 13.0 g	39.8	
Cyperus species			
Metham-Na	5.8 g	3.0	0.99
Metham-Na	11.6 g	7.7	
MBC	13.1 + 6.5 g	0.5	0.91
MBC	26.2 + 13.0 g	0.3	
Bacterial wilt			
Metham-Na	5.8 g	10.0	0.66
Metham-Na	11.6 g	18.5	
MBC	13.1 + 6.5 g	5.0	0.05
MBC	26.2 + 13.0 g	13.5	
Fusarium wilt			
Metham-Na	5.8 g	3.8	1.00
Metham-Na	11.6 g	5.7	
MBC	13.1 + 6.5 g	0.0	0.98
MBC	26.2 + 13.0 g	1.0	
Paratrichodorus minor			
Metham-Na	5.8 g	50.0	0.70
Metham-Na	11.6 g	58.0	
MBC	13.1 + 6.5 g	62.2	0.20
MBC	26.2 + 13.0	38.2	

[y] Assessment for marketable yield is the yield in metric tons per hectare, for *Cyperus* species is the number of plants emerging per 0.27-m² area, for bacterial wilt and Fusarium wilt is the percent of disease incidence, and for *Paratrichodorus minor* is the number of nematodes per 100 cm³ of soil.

[z] The significance probability value associated with the F value.

DISCUSSION

Soil solarization has been traditionally considered a soil disinfestation technique suitable for arid climates. In recent years, reports of its application potential in humid regions characterized by abundant rainfall and cloud cover have been published (8,9,19-20,30,33,34,37,39,43,44). In this study, precipitation occurred on 30 to 64% of the solarization days, yet marketable yields of fresh market tomato in plots receiving soil solarization were similar or greater than yields in plots receiving methyl bromide. In the large scale field plots, yields in areas receiving solarization were similar to yields in methyl bromide-treated areas, validating the application potential of soil solarization.

Although the yield potential of treatments receiving soil solarization was demonstrated, it should be noted that not all soilborne pests were suppressed when soil solarization was employed alone. Soil solarization failed to reduce densities of *R. reniformis* and *Meloidogyne* spp. and the severity of root galling in the Florida-95 experiment and in validation site I. In the Florida-95 site, a mix-

ture of *M. incognita* and *M. javanica* was identified. Significant reductions in *M. javanica* and *M. incognita* by soil solarization have been reported (1,39,40,48,51). Reductions in *R. reniformis* by soil solarization also have been reported (1,6,9,26). The lack of control of *Meloidogyne* spp. and *R. reniformis* may be due to nematode recolonization of treated soil. In several studies in which soil solarization suppressed initial populations, recolonization was shown to occur (1,6,26,40). At the Florida-95 site, nematode samples were not collected until 80 days after transplanting, allowing sufficient time for recolonization to occur. Strip solarization, in which 50% of the field was untreated, may have further enhanced recolonization. In a previous study performed in the same production

TABLE 5. Effects[u] of soil treatments and solarization with various plastic films on soilborne pests and yield of fresh market tomato at the Florida-95 site

Soil treatment	Rate per m²	Plastic film[v,w] Clear, GI	White	Clear	Mean[w]
Southern blight[x]					
None	...	2.0	11.0	4.2	5.8 B
Pebulate	0.1 g	5.6	8.5	4.0	6.1 B
Cabbage	6.8 kg	9.5	19.2	5.5	11.4 A
1,3-DCPC[y]	16.2 + 3.4 g	3.2	4.2	1.8	3.1 B
MBC[z]	13.1 + 6.5 g	3.2	2.0	3.0	2.8 B
Mean		4.8 B	9.0 A	3.7 B	
Root gall ratings					
None	...	3.6 abcd	2.8 def	3.2 bcde	3.2
Pebulate	0.1 g	4.0 abc	4.0 abc	4.5 a	4.2
Cabbage	6.8 kg	3.0 cde	4.5 a	4.3 ab	3.9
1,3-DCPC	16.2 + 3.4 g	0.8 gh	2.4 ef	1.8 fg	1.6
MBC	13.1 + 6.5 g	0.2 h	0.4 h	0.8 gh	0.4
Mean		2.3	2.8	2.9	
Cyperus species					
None	...	0.1 c	33.4 b	0.9 c	11.4
Pebulate	0.1 g	0.0 c	25.2 b	0.6 c	8.6
Cabbage	6.8 kg	0.0 c	56.1 a	0.6 c	18.9
1,3-DCPC	16.2 + 3.4 g	0.0 c	61.6 a	0.5 c	20.7
MBC	13.1 + 6.5 g	0.0 c	48.6 a	0.4 c	16.3
Mean		< 0.1	45.0	0.6	
Marketable yield					
None	...	42.8 ab	17.5 efg	30.3 bcd	30.2
Pebulate	0.1 g	27.0 cde	6.8 g	35.2 bc	23.0
Cabbage	6.8 kg	30.8 bcd	11.2 fg	41.1 ab	27.7
1,3-DCPC	16.2 + 3.4 g	36.0 bc	20.6 def	37.7 abc	31.4
MBC	13.1 + 6.5 g	50.2 a	14.1 fg	41.3 ab	35.2
Mean		37.3	14.1	37.4	

[u] Effects are stated for Southern blight as the percent of disease incidence, for root gall rating as an estimate of the number of galls per root system using a scale of 0 to 5 in which 0 = no galls and 5 = >100 galls (52), for *Cyperus* species as the number of plants per 0.27-m² area emerging through the plastic at the termination of the solarization period, and for marketable yield as metric tons per hectare.
[v] Clear, GI = clear, gas-impermeable solarization film; white = white on black, coextruded, low density polyethylene; and clear = clear, low density polyethylene solarization film.
[w] Main effect means for plastic film or soil treatment (A and B) followed by the same letter do not differ according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$). Means for the interaction of soil treatment and plastic film followed by the same letter (a to h) do not differ according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).
[x] Incidence of Southern blight caused by *Sclerotium rolfsii*.
[y] A mixture of 1,3-dichloropropene + chloropicrin.
[z] A mixture of methyl bromide + chloropicrin.

TABLE 6. Effect of soil solarization on soilborne pests and marketable yield of tomato in validation sites II and III

Plastic[r]	Soil treatment	Rate[s]	Southern blight[t]	*Paratrichodorus minor*[u]	*Rotylenchulus reniformis*[u]	*Meloidogyne* species[u]	Root gall index[v]	Marketable yield[w]
Validation site II[x]								
Clear	None	...	<0.1%	11.8	...	0.5	0.3	54.4
White	MBC[y]	45 g	3.7%	35.5	...	0.0	0.2	50.9
Validation site III[z]								
Clear	None	...	...	31.8	44.5	...	...	60.6
White	MBC	45 g	...	45.0	0.5	...	...	61.4

[r] Clear = clear, low density polyethylene solarization film and white = white on black, coextruded low density polyethylene.
[s] Per square meter.
[t] Disease incidence.
[u] Number of nematodes per 100 cm³ of soil.
[v] Estimates of the number of galls per root system using a scale of 0 to 5 in which 0 = no galls and 5 = >100 galls (52).
[w] Metric tons per hectare.
[x] Solarization performed on 0.48 ha (12 rows, 210 m long).
[y] A 98:2 mixture of methyl bromide + chloropicrin.
[z] Solarization performed on 0.42 ha (7 rows, 324 m long).

region, broadcast solarization provided season-long reduction in populations of *R. reniformis* (9). Evidence for late-season recolonization is supported by the fact that although high numbers of *R. reniformis*, *Meloidogyne* spp., and severe root galling were found in the Florida-95 experiment and in validation sites I and II, yield marketable fruit was not affected. Several studies in which colonization occurred also showed no effect of the nematode damage on yield (1,26). Strip solarization, as practiced here, was

TABLE 7. Effects of soil treatments and solarization using various plastic films on the density of phytoparasitic nematodes per 100 cm³ of soil at the Florida site[v]

Soil treatment	Rate per m²	Plastic film[w]			Mean[x]
		Clear, GI	White	Clear	
Meloidogyne species					
None	...	2,254	315	418	1,096 A
Pebulate	0.1 g	532	566	2,598	1,232 A
Cabbage	6.8 kg	1,219	1,140	978	1,112 A
1,3-DCPC[y]	16.2 + 3.4 g	46	619	635	434 AB
MBC[z]	13.1 + 6.5 g	<1	6	7	4 B
Mean		870	529	927	
Rotylenchulus reniformis					
None	...	126	203	353	228 AB
Pebulate	0.1 g	222	104	353	226 AB
Cabbage	6.8 kg	375	354	416	382 A
1,3-DCPC	16.2 + 3.4 g	54	62	54	57 BC
MBC	13.1 + 6.5 g	4	4	22	10 C
Mean		156	145	239	
Paratrichodorus minor					
None	...	16	15	10	13 A
Pebulate	0.1 g	3	12	3	6 A
Cabbage	6.8 kg	7	20	11	13 A
1,3-DCPC	16.2 + 3.4 g	3	28	18	16 A
MBC	13.1 + 6.5	2	16	20	13 A
Mean		6 B	18 A	12 AB	
Criconemella species					
None	...	6	64	10	27 AB
Pebulate	0.1 g	18	121	58	66 AB
Cabbage	6.8 kg	34	192	32	86 A
1,3-DCPC	16.2 + 3.4 g	1	22	0	8 B
MBC	13.1 + 6.5 g	0	5	0	2 B
Mean		12 B	81 A	20 B	
Helicotylenchus species					
None	...	2 a	101 a	11 ab	38
Pebulate	0.1 g	9 a	62 a	14 a	28
Cabbage	6.8 kg	3 a	83 a	4 ab	30
1,3-DCPC	16.2 + 3.4 g	0 a	0 b	1 b	<1
MBC	13.1 + 6.5 g	<1 a	0 b	<1 b	<1
Mean		3	49	6	

[v] Samples collected 80 days after transplanting.
[w] Clear, GI = clear, gas-impermeable solarization film; white = white on black, coextruded, low density polyethylene; and clear = clear, low density polyethylene solarization film.
[x] Main effect means for plastic film or soil treatment (A, B, and C) followed by the same letter do not differ ($P \leq 0.05$) according to Duncan's multiple range test. Means for interactions between plastic film and soil treatments followed by the same letter (a and b) do not differ ($P \leq 0.05$) according to Duncan's multiple range test.
[y] A mixture of 1,3-dichloropropene + chloropicrin.
[z] A mixture of methyl bromide + chloropicrin.

TABLE 8. Effect of soil solarization and chemical fumigants on soilborne pests and marketable yield of tomato at the validation site III

Plastic[s]	Soil treatment	Rate[t]	*Paratrichodorus minor*[u]	*Meloidogyne* species[u]	Root gall ratings[v]	Marketable yield[w]
White	MBC[x]	45. 0 g	31.0 a[y]	18.0 a	0.0 c	52.0 ab
White	1,3-DCPC[z]	32.4 + 6.9 g	19.5 a	2.5 a	0.2 bc	60.3 a
Clear	MBC	22.4 g	17.2 a	706.5 a	1.0 b	51.6 ab
Clear	1,3-DCPC	16.2 + 3.4 g	24.8 a	151.0 a	0.1 b	45.6 b
Clear	None	...	31.8 a	385.0 a	1.8 a	44.4 b

[s] White = white, low density polyethylene and clear = clear, low density polyethylene solarization film.
[t] Per square meter.
[u] Density of nematodes per 100 cm³ of soil.
[v] Estimates of the number of galls per root system using a scale of 0 to 5 in which 0 = no galls and 5 = >100 galls (52).
[w] Metric tons per hectare.
[x] A 98:2 mixture of methyl bromide + chloropicrin.
[y] Means in each column followed by the same letter do not differ ($P \leq 0.05$) according to the Waller-Duncan *k*-ratio *t* test (root gall ratings and marketable yield) or Duncan's multiple range test (*Paratrichodorus minor* and *Meloidogyne* spp.).
[z] A mixture of 1,3-dichloropropene + chloropicrin.

fective in reducing populations of *Paratrichodorus minor*, which commonly recolonizes fumigated soil, although trichodorid recolonization tends to occur from residual populations in deeper soil layers (55) rather than from nonfumigated strips.

Soil solarization provided significant reductions in the incidence of Southern blight and Fusarium wilt and was generally as effective as fumigation with methyl bromide + chloropicrin. Reductions in the incidence of diseases caused by *S. rolfsii* have been reported in other studies; although, in some instances, the effect varied from year to year (22,43,51). Reductions in the incidence of Fusarium wilt of tomato, cumin, and cotton by soil solarization also have been reported (15,32,36,41). Soil solarization resulted in significant levels of disease suppression even though it failed to significantly reduce populations of *F. oxysporum* f. sp. *lycopersici* in a previous study (8). Suppression of Fusarium wilt in the absence of a direct effect on pathogen survival is considered to be related to the involvement of fluorescent pseudomonads and other microorganisms in solarized soils (15).

The inability of soil solarization to control bacterial wilt agrees with earlier studies (8,9). Previous studies have shown a synergistic interaction between fumigation with methyl bromide + chloropicrin and soil solarization on survival of *Ralstonia solanacearum* and the incidence of bacterial wilt (8,9). This interaction was not observed in this study, perhaps because strip solarization left untreated areas that served as inoculum sources.

The efficacy of some soil disinfestation tactics was enhanced through the combination of soil solarization and improvements in plastic technology. For example, soil solarization and 1,3-dichloropropene + chloropicrin did not significantly reduce the severity of root galling (Table 5) when used alone. When the treatments were applied under a gas-impermeable plastic, the level of root galling was significantly reduced. While not significant at $P < 0.05$, a 10-fold reduction in the density of *Meloidogyne* spp. was also observed using this combination. Stapleton et al. (50) did not observe additional reductions in the density of *M. incognita* when soil solarization was combined with 1,3-dichloropropene. In this study, significant reductions occurred only when the gas-impermeable film was added. Polyethylene is only marginally effective in preventing chemical fumigants, including methyl bromide and 1,3-dichloropropene, from escaping into the atmosphere (12). Estimates of emissions of methyl bromide between 40 and 99% have been reported under LDPE plastic (18,29). The gas-impermeable "Bromotec" plastic (Lawson Mardon Packaging) consists of two layers of LDPE sandwiched around a nylon core. The nylon core gives the film its impermeable properties and has been shown to reduce methyl bromide emissions to levels less than 3% (7).

Soil amended with cabbage residue prior to solarization did not produce any additional benefits in the suppression of soilborne pests. Other studies have observed beneficial effects from combining cabbage residue with soil solarization treatments (16,34). It is possible that the concentration of antifungal volatile compounds evolved from solarized soil amended with cabbage residue was not high enough to provide additional control under the field conditions in this study. Retention of volatiles through the use of a gas-impermeable film did not improve efficacy. In this study, the cabbage residue was scattered onto the plots and then mixed with the soil as the raised beds were prepared. It was not ground into powder and uniformly mixed as was the case in the study by Gamliel and Stapleton (16). Large pieces of undecomposed cabbage may not have been affected by the heat treatment, and, rather than emitting biotoxic volatiles, may have served as a substrate for *S. rolfsii*, leading to higher levels of Southern blight in the cabbage-amended plots.

Other studies have investigated soil solarization performed on raised beds (24,25,33,34,44,47). This is the first study in which solarization of raised beds was shown to eliminate the border effect that results from a decrease in soil temperatures at the edge of the solarized area (23). With a raised bed, the plastic film on the sides of the bed provides additional surface area for solar radiation to penetrate the soil and functions as a barrier to prevent heat escaping from the edges of the treated area.

The soil solarization treatments were fully compatible with the regional fresh market fall tomato production system. The plastic was applied during the routine application window, and growers did not have to modify their application procedures. The cost of the clear LDPE was half of the cost of white on black, coextruded LDPE. The only additional procedure required of the solarization process was painting the plastic white at the end of the solarization period. The cost of paint was $135 per hectare. Per hectare costs of the standard soil disinfestation procedure, including plastic film and methyl bromide + chloropicrin, is approximately $1,535. The per hectare cost of the soil solarization treatment, including the paint, is approximately $548. Thus, soil solarization was more cost effective, resulting in savings approaching $1,000 per hectare. The solarization film provided an added benefit as it was also used as a production mulch after the solarization treatment was completed. Full integration of soil solarization into standard, raised-bed, plastic mulch production systems has been attempted in only a few instances (24,25,44).

Without any soil disinfestation treatment, marketable yields averaged 20.4 metric tons per hectare, which was a 50% reduction from the Florida state-wide average of 40.1 metric tons per hectare observed between 1991 and 1995 (3). Although less than the 61.4% reduction in production reported by Spreen et al. (46), this study further substantiates the claim that failure to obtain alternatives to methyl bromide for management of soilborne pests will result in major economic hardships for Florida vegetable producers. For example, using a 5-year average market price of $7.95 per 11.35-kg box, the 50% reduction in marketable yield represents an annual loss of $281 million from fresh market tomato production in Florida.

In this study, soil solarization was shown to be ideally suited to function as a framework in which to establish an IPM program for soilborne pests. Soil solarization integrated well into the raised-bed, plastic mulch production system, was cost effective, provided significant management of many of the key pests, enhanced the efficacy of other pest management tactics, and permitted reduced dosages of chemical fumigants. The decline in the availability of chemical fumigants coupled with an increase in the availability of biological control agents and new developments in plastic technology will continue to support the utilization of soil solarization in an IPM program for soilborne pests.

ACKNOWLEDGMENTS

This research was supported, in part, by the Gadsden County Tomato Growers Association and the United States Department of Agriculture, Specific Cooperative Agreement 58-6617-4-019. Florida Agricultural Experiment Station Journal Series R-05256. We thank Y. Katan for critical review of this manuscript and N. T. Garguilo, M. Fletcher, W. Maxwell, and S. Suber for use of their tomato production fields.

LITERATURE CITED

1. Abdel-Rahim, M. F., Satour, M. M., Mickail, K. Y., El-Eraki, S. A., Grinstein, A., Chen, Y., and Katan, J. 1988. Effectiveness of soil solarization in furrow-irrigated Egyptian soils. Plant Dis. 72:143-146.
2. Anonymous. 1993. The Biological and Economic Assessment of Methyl Bromide. The National Agricultural Pesticide Impact Assessment Program (NAPIAP), USDA, Washington, DC.
3. Anonymous. 1996. Florida Agricultural Statistics, Vegetable Summary. Florida Department of Agriculture & Consumer Services, Orlando, FL.
4. Bewick, T. A. 1989. Use of soil sterilants in Florida vegetable production. Acta Hortic. 255:61-72.
5. Cantliffe, D. J., Hochmuth, G. J., Locascio, S. J., Stansly, P. A., Vavrina, C. S., Polston, J. E., Schuster, D. J., Seal, D. R., Chellemi, D. O., and Olson, S. M. 1995. Production of solanaceae for fresh market under field conditions: Current problems and potential solutions. Acta Hortic.

412:229-244.
6. Cartia, G., Greco, N., and Cipriano, T. 1989. Effect of solarization and fumigants on soilborne pathogens of pepper in greenhouse. Acta Hortic. 255:111.
7. Chakrabarti, B., Wontner-Smith, T., and Bell, C. H. 1995. Reducing methyl bromide emissions from soil fumigation in greenhouses. Pages 25-1 to 25-3 in: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Methyl Bromide Alternatives Outreach, Fresno, CA.
8. Chellemi, D. O., Olson, S. M., and Mitchell, D. J. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. Plant Dis. 78:1167-1172.
9. Chellemi, D. O., Olson, S. M., Scott, J. W., Mitchell, D. J., and McSorley, R. 1993. Reduction of phytoparasitic nematodes on tomato by soil solarization and genotype. J. Nematol. 25(suppl.):800-805.
10. Clean Air Act. 1990. Title VI—Stratospheric Ozone Protection. Pub. L. 101-549, Sect. 6001-618, 104 Stat. 2649, 6272. As amended. U.S. Congress, Washington, DC.
11. Crop Protection Coalition. 1995. Status of methyl bromide alternatives research activities. Crop Protection Coalition, Fresno, CA.
12. Daponte, T. L. F. 1995. Barrier films: Hytibar. Acta Hortic. 382:56-66.
13. Federal Register. 1993. Fed. Registr. 58:65018-65082.
14. Frank, Z. R., Ben-Yephet, Y., and Katan, J. 1986. Synergistic effect of metham and solarization in controlling delimited shell spots of peanut pods. Crop Prot. 5:199-202.
15. Gamliel, A., and Katan, J. 1993. Suppression of major and minor pathogens by fluorescent pseudomonads in solarized and nonsolarized soil. Phytopathology 83:68-75.
16. Gamliel, A., and Stapleton, J. J. 1993. Characterization of antifungal volatile compounds evolved from solarized soil amended with cabbage residues. Phytopathology 83:899-905.
17. Gamliel, A., and Stapleton, J. J. 1993. Effect of chicken compost or ammonium phosphate and solarization on pathogen control, rhizosphere microorganisms, and lettuce growth. Plant Dis. 77:886-891.
18. Gan, J., Yates, S. R., Wang, D., and Ernst, F. F. 1995. Reducing fumigant volatilization through optimized application and soil management. Pages 26-1 to 26-2 in: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Methyl Bromide Alternatives Outreach, Fresno, CA.
19. Garibaldi, A., and Gullino, M. L. 1991. Use of solarization in marginally suitable climates. Pages 253-265 in: Soil Solarization. J. E. DeVay, J. J. Stapleton, and C. L. Elmore, eds. FAO Plant Prot. Prod. Pap. 109.
20. Ghini, R., Bettiol, W., Spadotto, C. A., de Moraes, G. J., Paraiba, L. C., and Mineiro, J. L. 1993. Soil solarization for the control of tomato and eggplant Verticillium wilt and its effect on weed and micro-arthropod communities. Summa Phytopathol. 19:183-189.
21. Gilreath, J. P., Jones, J. P., and Noling, J. W. 1995. Fumigant/herbicide combinations for polyethylene mulched tomato. Pages 38-1 to 38-2 in: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Methyl Bromide Alternatives Outreach, Fresno, CA.
22. Grinstein, A., Katan, J., Abdul Razik, A., Zeydan, O., and Elad, Y. 1979. Control of *Sclerotium rolfsii* and weeds in peanuts by solar heating of the soil. Plant Dis. Rep. 63:1056-1059.
23. Grinstein, A., Kritzman, G., Hetzroni, A., Gamliel, A., Mor, M., and Katan, J. 1995. The border effect of soil solarization. Crop Prot. 14:315-320.
24. Hartz, T. K., Bogle, C. R., and Villalon, B. 1984. Response of bell pepper (*Capsicum annum*) to soil solarization. (Abstr.) HortScience 19:209.
25. Hartz, T. K., DeVay, J. E., and Elmore, C. L. 1993. Solarization is an effective disinfestation technique for strawberry production. HortScience 28:104-106.
26. Heald, C. M., and Robinson, A. F. 1987. Effects of soil solarization on *Rotylenchulus reniformis* in the Lower Rio Grande Valley of Texas. J. Nematol. 19:93-103.
27. Jacobsen, B. J., and Backman, P. A. 1993. Biological and cultural plant disease controls: Alternatives and supplements to chemicals in IPM systems. Plant Dis. 77:311-315.
28. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
29. Jin, Y., and Jury, W. A. 1995. Methyl bromide diffusion and emission through soil columns under various management techniques. J. Environ. Qual. 24:1002-1009.
30. Kassaby, F. Y. 1985. Solar-heating soil for control of damping-off diseases. Soil Biol. Biochem. 17:429-434.
31. Katan, J. 1993. Replacing pesticides with nonchemical tools for the control of soilborne pathogens—A realistic goal. Phytoparasitica 21:95-99.
32. Katan, J., Fishler, G., and Grinstein, A. 1983. Short- and long-term effects of soil solarization and crop sequence on Fusarium wilt and yield of cotton in Israel. Phytopathology 73:1215-1219.
33. Keinath, A. P. 1995. Reductions in inoculum density of *Rhizoctonia solani* and control of belly rot on pickling cucumber with solarization. Plant Dis. 79:1213-1219.
34. Keinath, A. P. 1996. Soil amendment with cabbage residue and crop rotation to reduce gummy stem blight and increase growth and yield of watermelon. Plant Dis. 80:564-570.
35. Locascio, S. J., Dickson, D. W., and Kucharek, T. A. 1995. Fumigant alternative to methyl bromide for polyethylene mulched tomato. Pages 38-1 to 38-2 in: Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Methyl Bromide Alternatives Outreach, Fresno, CA.
36. Lodha, S. 1995. Soil solarization, summer irrigation and amendments for the control of *Fusarium oxysporum* f. sp. *cumini* and *Macrophomina phaseolina* in arid soils. Crop Prot. 14:215-219.
37. McSorley, R., and Parrado, J. L. 1986. Application of soil solarization to rockdale soils in a subtropical environment. Nematropica 16:125-140.
38. Ohr, H. D., Sims, J. J., Grech, N. M., Becker, J. O., and McGiffen, M. E. Jr. 1996. Methyl iodide, an ozone-safe alternative to methyl bromide as a soil fumigant. Plant Dis. 80:731-735.
39. Overman, A. J. 1985. Off-season land management, soil solarization and fumigation for tomato. Proc. Soil Crop Sci. Soc. Fla. 44:35-39.
40. Porter, I. J., and Merriman, P. R. 1983. Effects of solarization of soil on nematode and fungal pathogens at two sites in Victoria. Soil Biol. Biochem. 15:39-44.
41. Ramirez-Villapudua, J., and Munnecke, D. E. 1987. Control of cabbage yellows (*Fusarium oxysporum* f. sp. *conglutinans*) by solar heating of field soils amended with dry cabbage residues. Plant Dis. 71:217-221.
42. Ramirez-Villapudua, J., and Munnecke, D. E. 1988. Effect of solar heating and soil amendments of cruciferous residues on *Fusarium oxysporum* f. sp. *conglutinans* and other organisms. Phytopathology 78:289-295.
43. Ristaino, J. B., Perry, K. B., and Lumsden, R. D. 1991. Effect of solarization and *Gliocladium virens* on sclerotia of *Sclerotium rolfsii*, soil microbiota, and the incidence of Southern blight of tomato. Phytopathology 81:1117-1124.
44. Ristaino, J. B., Perry, K. B., and Lumsden, R. D. Soil solarization and *Gliocladium virens* reduce the incidence of Southern blight (*Sclerotium rolfsii*) in bell pepper in the field. Biocontrol Sci. Technol. In press.
45. Smith, S. A., and Taylor, T. G. 1994. Production cost for selected vegetables in Florida. Univ. Fla. Coop. Ext. Serv. Circ. 1146.
46. Spreen, T. H., VanSickle, J. J., Moseley, A. E., Deepak, M. S., and Mathers, L. 1995. Use of methyl bromide and the economic impact of its proposed ban on the Florida fresh fruit and vegetable industry. Univ. Fla. Inst. Food Agric. Sci. Bull. 898.
47. Standifer, L. C., Wilson, P. W., and Porch-Sorbet, R. 1984. Effects of solarization on soil weed seed populations. Weed Sci. 32:569-573.
48. Stapleton, J. J., and DeVay, J. E. 1983. Response of phytoparasitic and free-living nematodes to soil solarization and 1,3-dichloropropene in California. Phytopathology 73:1429-1436.
49. Stapleton, J. J., and DeVay, J. E. 1995. Soil solarization: A natural mechanism of integrated pest management. Pages 309-322 in: Innovative Approaches to Integrated Pest Management. R. Reuveni, ed. CRC Press, Boca Raton, FL.
50. Stapleton, J. J., Lear, B. L., and DeVay, J. E. 1987. Effect of combining soil solarization with certain nematicides on target and nontarget organisms and plant growth. Ann. Appl. Nematol. 1:107-112.
51. Stevens, C., Khan, V. A., Collins, D., Rodriguez-Kabana, R., Ploper, L. D., Adeyeye, O., Brown, J., and Backman, P. 1992. Use of soil solarization to reduce the severity of early blight, Southern blight and root-knot in tomatoes. (Abstr.) Phytopathology 82:500.
52. Taylor, A. L., and Sasser, J. N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University, Raleigh.
53. Tjamos, E. C., and Fravel, D. R. 1995. Detrimental effects of sublethal heating and *Talaromyces flavus* on microsclerotia of *Verticillium dahliae*. Phytopathology 85:388-392.
54. Volin, R. B., and McMillan, R. T., Jr. 1973. Response of the tomato cultivar 'Walter' grown on mulch to soil fumigants. Proc. Fla. State Hortic. Soc. 86:159-163.
55. Weingartner, D. P., Shumaker, J. R., and Smart, G. C., Jr. 1983. Why soil fumigation fails to control potato corky ringspot disease in Florida. Plant Dis. 67:130-134.
56. Williamson, C. E., Tammen, J., Hannon, C. I., and Denmark, J. C. 1955. Some tests with soil fumigants. Proc. Fla. State Hortic. Soc. 68:370-373.

DEL AGRICULTURAL
Ozone Solutions for the Agricultural Industry

Complete Understanding of Ozone Use & Technology
Introduction to DEL Agricultural

- Formed in 1975
- Ozone technology leaders for over 20 years
- Multiple ozone system sizes - ranging from 1 g/hr to 750 g/hr
- EPA registered and OSHA compliant
- Expert engineering and manufacturing teams
- Authoritative procedural publications adopted by health agencies throughout the United States
- Systems selected by FDA and USDA
- Over 1,000,000 ozone systems sold worldwide

DEL Agricultural Capabilities

- Original Manufacturing
- System Design Consultation
- Custom Application System Sizing
- In-House Engineering Team
  - Electrical Engineering
  - Mechanical Engineering
  - Design Engineering
- Technical Training
- Field Service
- Bilingual English/Spanish Standard Operating Procedures, Operation Manuals & Ozone Safety Manual

History of Ozone

- **1840** - Discovered by Schönbein
- **1893** - Used as a disinfectant in drinking water
- **1909** - Used as a food preservative for cold storage of meats
- **1939** - Was found to prevent the growth of yeast & mold during the storage of fruits

History of GRAS Status of Ozone

- **1982** - FDA GRAS declaration for ozone use in bottled water
- **1995** - FDA GRAS for ozone use in bottled water renewed without change
- **1997** - Industry Expert Panel declares ozone GRAS and meets FDA requirements. Regulators have the option to later add control on ozone use.
- **1999** - USDA rejects an ozone use protocol for meats, cites 1982 GRAS declaration for water where FDA stated "any other use must be regulated by a Food Additive Petition."
- **2000** - Food Additive Petition, that addresses both water and air use of ozone, under preparation. FDA estimates approval will occur within six months of submission of the Petition

Status of Ozone in the Food Industry

- **1982** - O3 declared GRAS for treatment of bottled water (Federal Register, Vol. 47, No. 113, November 5, 1982)
- **1997** - Expert panel report : Evaluation of the history and safety of ozone in processing foods for human consumption. Vol. 2 : Abstracts. Electric Power Research Institute, Palo Alto, CA. R & D Enterprise, Inc. / GRAS self declaration (Federal Register, v. 62 #74, April 19, 1997)
- **Currently under FDA review for expansion of ozone use under the Food Additive Petition. Approval expected 3rd quarter of 2000. No interim plans by FDA to reject use of ozone.**

EPA - Disinfection By-Product Rules (DBPR)

- **2000 - 2004:** Implementation Stage
- Ozone is EPA listed as DBPR compliant

Movement to Ozone as a Primary Sanitizer for the USA

- Increased environmental consciousness has resulted in an accelerated movement away from multi-chemical based sanitation treatment.
- External events, including water availability and cost, more stringent permit requirements for local and industrial companies.
- Waste water concerns.
- Need to reduce operating costs.
- Advancement of ozone-based technology to a safe, environmentally-friendly approach to sanitation treatment.

Properties of Ozone (O_3)

- A gas - triatomic form of oxygen
- Most powerful oxidizing agent available for conventional water treatment - highly reactive
- Unstable - must be generated and used on-site
    - The weak bond holding ozone's third oxygen atom is what causes the molecule to be unstable and thus, very effective as an oxidizer.
- Partially soluble in water, but more so than oxygen
- Ultimately reverts to environmentally friendly oxygen

How Ozone Works

- An oxidation reaction occurs upon any collision between an ozone molecule and a molecule of an oxidizable substance (i.e. bacteria, fungi (mold & yeast), viruses, forms of iron & manganese, etc.)
- The weak bond splits leaving oxygen as a by-product.
- During an oxidation reaction organic molecules are destroyed and dissolved metals are no longer soluble.

Ozone Antimicrobial Action

- Ozone is an effective biocide against:
    - Virus
    - Bacteria
    - Bio-Film
    - Fungi (including mold and yeast)
    - Protozoa
    - Other higher life forms such as small insects, worms, mites, and parasites

Relative Strength of Ozone - Oxidation Potential

Oxidizing Agent	EOP (volt)	EOP vs. Cl_2
Fluorine	3.06	2.25
Hydroxyl Radical	2.80	2.05
Oxygen (atomic)	2.42	1.78
OZONE	**2.08**	**1.52**
Hydrogen Peroxide	1.78	1.30
Hypochlorite	1.49	1.10
Chlorine	1.36	1.00
Chlorine Dioxide	1.27	0.93
Oxygen (molecular)	1.23	0.90

Relative Strength of Ozone - CT Values for Water

Microorganism	Disinfectant			
	Ozone (pH 6 to 7)	Preformed Chloramine (pH 8 to 9)	Chloride Dioxide (pH 6 to 7)	Free Chlorine (pH 6 to 7)
E. coli	0.02	95-180	0.4-0.75	0.034-0.05
Polio 1	0.1-0.2	770-3740	0.2-6.7	1.1-2.5
Rotavirus	0.006-0.06	3810-6480	0.2-2.1	0.01-0.05
***G. lamblia* cysts**	0.5-0.6	—	—	—
***G. muris* cysts**	1.8-2.0	1400	7.2-18.5	30-630
Source: Hoff (1987)				

Ozone vs. Chemicals
Chemical Processing Negatives

- Dangerous storage
- Dangerous for human contact (skin, eyes and inhalation)
- Inadequate sanitation (microorganisms may become tolerant and unaffected)
- Environmental issues
- Beneficial bacteria in leech fields or settling ponds can be killed
- Overall environmental negative affects

Ozone vs. Chemicals
Cold Ozone-Enriched Water Advantages

- Ozone is not stored - generated on-site as needed
- Ozone management systems eliminate inhalation worries
- Microorganisms can't build up an ozone tolerance
- Ozone used properly cannot endanger the environment
- Ozone will decompose to oxygen before entering sensitive leech or settling ponds
- Ozone leaves no chemical or residual byproducts to spoil product quality

Ozone vs. Thermal

Thermal Processing Negatives

- Cost to produce heat
- Time to produce heat
- Time to reduce heat
- Wear and tear on equipment and dangerous to workers

Ozone vs. Thermal

Cold Ozone-Enriched Water Advantages

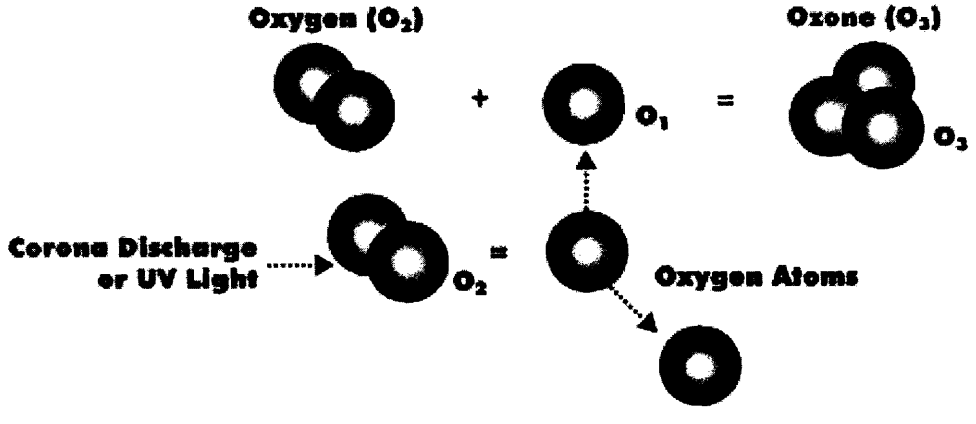
- Saves on heating costs
- Instant "ON" saves time
- No cool down - saves time
- Gentle on equipment surfaces, gaskets and seals

Ozone Generation Technology

- **Ultraviolet (Light Energy)** - This is how ozone is produced via the Sun's ultraviolet rays.
  - Simple, cheap, low output capacity
  - Residential or very low demand systems only
- **Corona Discharge (Electrical Energy)** - This is how ozone is produced via Lightning. Large scale production of ozone requires this method.
  - Relatively more complex, high output concentrations
  - Commercial and industrial applications

How Ozone is Made

- Oxygen molecules (O_2) are split by high voltage electrodes resulting in two individual oxygen atoms (O_1).
- Oxygen atoms (O_1) + (O_1) unite with other oxygen molecules (O_2) to produce OZONE (O_3).
- Hence: (O_1) + (O_2) = (O_3):



DEL Ozone System Overview

- Ozone generator w/ oxygen concentrator
- Stainless steel Nema 4x water-proof enclosure, mobile
- Safety interlocks
- Stainless steel water pump

- Ozone management system (eliminates ozone off-gas)
- Diagnostic gauges
- Pre-wired, Pre-plumbed
- EPA registered (EPA Estab. No. 071472-CA-001)
- Built to UL safety standards
- OSHA safety compliant

SOME CHEMICAL, CULTURAL, AND BIOLOGICAL ALTERNATIVES TO METHYL BROMIDE FUMIGATION OF SOIL FOR STRAWBERRY

J. M. Duniway[a]*, J. J. Hao[a], D. M. Dopkins[a], H. Ajwa[b], and G. T. Browne[c]
[a]Department of Plant Pathology, University of California, Davis, CA 95616
[b]USDA-ARS, Water Management Research Laboratory, Fresno, CA 93727
[c]USDA-ARS, Dept. of Plant Pathology, University of California, Davis, CA 95616

Chemical alternatives to methyl bromide are being tested in replicated field experiments at a coastal site near Watsonville, CA. Strawberry was grown each year, *Verticillium dahliae* and *Phytophthora* spp. were present in the soil, and bed fumigation treatments were applied to the same ground in early October of each of the last 2 years. Two-row beds were shaped, drip lines installed, and small cloth pouches containing soil with known populations of *V. dahliae* or vermiculite pieces of cultured *Phytophthora cactorum* were buried under plant row locations at depths of 15 and 50 cm. Beds were subsequently fumigated and covered with standard black polyethylene mulch or black virtually impermeable plastic film (VIF) (Hytibar, Klerk=s Plastics). Shank-applied treatments (three shanks/bed, 15-20 cm deep, rates given per unit of treated bed area which was 58% of the total area) included methyl bromide/chloropicrin (MBC) 67/33 at 325 lb/a, chloropicrin at 200 and 300 lb/a, and Telone C-35 at 283 and 425 lb/a. Treatments applied to beds under plastic in water emulsions through drip lines were chloropicrin at 200 lb/a and Telone C-35 at 283 and 425 lb/a. Inoculum pouches were recovered and Selva was transplanted through the plastic mulch one month after fumigation. Conventional practices for annual strawberry production and pest management for the area were followed, including sprinkler irrigation initially and drip irrigation in the production season. Berries were picked for fresh market at least weekly for several months by normal grower practice.

MBC killed all inoculum buried at 15 and 50 cm. With the exception of Telone C-35 at the lower rate, other shank-applied treatments reduced buried *V. dahliae* inoculum to very low or undetectable levels. The same treatments killed most of the *P. cactorum* buried at 15 cm but were only effective in doing so at the 50 cm depth where VIF plastic was used. With the exception of chloropicrin at 200 lb/a, all drip-applied treatments killed both fungi at a depth of 15 cm but not at 50 cm. This survival probably occurred because the volume of water used to deliver fumigants was insufficient for movement to the 50 cm depth. Disease incidence in the growing crop was variable, but both Verticillium wilt and Phytophthora root rot were controlled adequately in most fumigation treatments. All fumigation treatments effectively controlled weed growth through plant holes in the plastic mulch.

Shank fumigation of beds with MBC and VIF mulch more than doubled berry yields relative to nontreated soil, and MBC with standard mulch was only slightly less effective. With standard plastic mulch, chloropicrin at 200 lb/a and Telone C-35 at 283 and 425 lb/a, when shank- or drip-applied, gave yields as high as or higher than those obtained with MBC. Use of VIF plastic mulch, however, increased yields significantly in all chloropicrin treatments and in some Telone C-35 treatments. These results are similar to those obtained in 1997-98 when VIF mulch improved yields significantly in a variety of shank-applied bed fumigation

treatments, but differ from those obtained in 1998-99 when VIF effects were small. The results show that bed fumigations with the materials and methods used can be effective in the presence of significant disease pressures from soilborne pathogens, but the specific methods and rates of application need further research to be optimized.

We are continuing to research microbiological differences associated with the enhanced growth and productivity of strawberries in fumigated soils where the response is not due to control of known, major pathogens. Populations of fluorescent Pseudomonads in soil increased quickly following fumigation and several isolates of *Pseudomonas fluorescens*, *P. putida* and *P. chlororaphis* from strawberry rhizospheres in fumigated soils were beneficial when inoculated to strawberry transplanted into natural soils in the greenhouse (1). Bare-root runner plants (Selva) were inoculated with some of these bacteria in the fall of 1999 and transplanted into field plots treated with MBC, chloropicrin (200 lb/a), or not treated. None of the inoculations increased strawberry yields in MBC-treated soil, and some actually decreased yield significantly. While only one isolate increased yield in nontreated soil, three isolates increased yields significantly in chloropicrin treated soil. Periodic reinoculations during crop growth did not increase growth or yields over those obtained following one inoculation at transplanting.

Cultural methods for the management of Verticillium wilt are also under investigation. Five experiments on strawberry rotations with broccoli, Brussels sprouts, and/or rye have been completed on nonfumigated soils. At the Watsonville site with high populations of *V. dahliae* present, none of the rotations reduced the incidence of Verticillium wilt in the subsequent strawberry crop significantly, but physical removal of residues from the preceding strawberry crop did reduce disease. One-year rotations out of strawberry, however, increased subsequent strawberry yields by 18-44% relative to continuous strawberry. High-nitrogen organic amendments were incorporated into nonfumigated soil several weeks before planting to test their effects on Verticillium wilt. Feather meal applied to beds (4 tons/a of treated area) reduced disease incidence in Camarosa during both 1999 and 2000, while blood meal (4 and 8 tons/a), fish meal (8 tons/a), and compost (8-12 tons/a) reduced disease in only one of the two years. Amendments applied to beds, however, also caused phytotoxicity and, therefore, did not give increases in yield proportional to levels of disease reduction. Broadcast applications of blood or fish meal at 4 tons/a or feather meal at 2 tons/a before bed shaping reduced Verticillium wilt development during 2000 without causing phytotoxicity in the variety Aromas. Although current California strawberry varieties are all susceptible to Verticillium wilt, some (e.g. Camarosa) were significantly more susceptible than others (e.g. Selva, Chandler) when compared over several years in naturally infested soil.

Reference Cited: 1. Xiao, C. L., and Duniway, J. M. 1998. Bacterial population responses to soil fumigation and their effects on strawberry growth. Phytopathology 88:S100 (Abstract).
Acknowledgments: Research supported largely by the California Strawberry Commission, the University of California (UC) Statewide IPM Project, and the UC Sustainable Agricultural Research and Education Program.

Francis, AW. 1997
McGraw Hill Encycl. Science Technology 8th Ed v.12 McGraw Hill NY

there are other enzymes that repair oxidative damage to deoxyribonucleic acid (DNA). Indeed, analysis of human urine for oxidized pyrimidines, which are removed from DNA during such repair, indicates the occurrence of at least 1000 such events per cell per day.

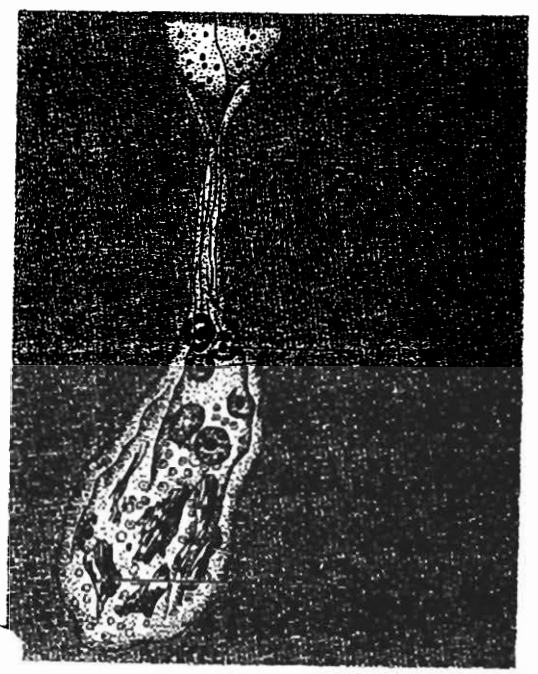
Thus, the apparent comfort in which aerobic organisms live in the presence of an atmosphere that is 20% O_2 is due to a complex and effective system of defenses against this peculiar gas. Indeed, these defenses are easily overwhelmed, and overt symptoms of oxygen toxicity become apparent when organisms are exposed to 100% O_2. For example, a rat maintained in 100% O_2 will die in 2 to 3 days.

Irwin Fridovich

Bibliography. I. B. Afanas'ev, *Superoxide Ion: Chemistry and Biological Implications*, 1989; I. Fridovich, Superoxide dismutases: An adaptation to a paramagnetic gas, *J. Biol. Chem.*, 264:7761–7764, 1989; H. Jonje, Genetic toxicology of oxygen, *Mutation Res.*, 219:193–208, 1989; A. Touati, Molecular genetics of superoxide dismutases, *Free Radical Biol. Med.*, 5:393–402, 1988.

Oxymonadida

An order of class Zoomastigophorea in the phylum Protozoa. These are colorless flagellate symbionts in the digestive tract of the roach *Cryptocercus* and of certain termites. They are xylophagous; that is, they ingest wood particles taken in by the host. Seven or more genera of medium or large size have been identified, the organisms varying from pyriform to ovoid in shape. At the anterior end a pliable necklike rostrum attaches the organism to the host intestinal wall, but they are sometimes free (see **illus.**). They can be either uni- or multinucleate. These organisms are termed karyomastigonts and each gives rise to two pairs of flagella in the unattached cells, two flagella to each blepharoplast. In the rostrum there is an axostylar apparatus, fibrils which pass to, and emerge at the posterior part of, the body. The nuclei contain long threadlike persistent chromosomes which appear to pass at random onto an intranuclear spindle. Isogamous (union of similar gametes) sexual processes have been described for three genera. In some cases, at least, these parallel the molting of the host.



An oxymonad, *Microrhopalodina inflata*.

A peculiar feature is the investment of the cell cuticle with bacteria and spirochetes, often resulting in a thick coating. The genera described have a global distribution. *See* CILIA AND FLAGELLA; PROTOZOA; SARCOMASTIGOPHORA; ZOOMASTIGOPHOREA.

James B. Lackey

Bibliography. L. R. Cleveland et al., The wood feeding roach *Cryptocercus*: Its protozoa and the symbiosis between protozoa and roach, *Mem. Amer. Acad. Arts Sci.*, 17:185–342, 1934; S. P. Parker (ed.), *Synopsis and Classification of Living Organisms*, 2 vols., 1982.

Ozone

A powerfully oxidizing allotropic form of the element oxygen. The ozone molecule contains three atoms (O_3), while the more common oxygen molecule has two atoms (O_2).

Ordinary oxygen is a colorless gas and condenses to a very pale blue liquid, whereas ozone gas is decidedly blue, and both liquid and solid ozone are an opaque blue-black color, similar to that of ink. Even at concentrations as low as 4%, the blue color of ozone gas mixed with air or other colorless gas in a tube 1 in. (2.5 cm) or more in diameter and 4 ft (1.2 m) or more long can be seen by looking lengthwise through the tube.

Properties and uses. Some properties of ozone are given in the **table**. Ozone has a characteristic, pungent odor familiar to most persons because ozone is formed when an electrical apparatus produces sparks in air. Ozone is irritating to mucous membranes and toxic to human beings and lower animals. U.S. Occupational Safety and Health Administration standards for industrial workers exposed to ozone on a daily basis limit ozone concentration to 0.1 part per million on the average, with a maximum of 0.3 ppm for short exposures.

High ozone concentrations in liquid- and gas-phase mixtures can decompose explosively when initiated by an electric spark or other high-level energy source. Controlled decomposition to reduce ozone to desirable low concentrations can be accomplished catalytically.

Ozone is a more powerful oxidizing agent than

Some properties of ozone

Property	Value
Density of the gas at 0°C (32°F), 1 atm (10⁵ Pa) pressure	2.154 g/liter
Density of the liquid at −111.9°C	1.54 g/ml
Density of the solid at −193°C	1.573 g/ml
Boiling point at 1 atm (10⁵ Pa) pressure	−111.9°C (−169.4°F)
Melting point of the solid	−192.5°C (−314.5°F)
Wavelength range of maximum absorption in visible spectrum	550–620 nm
Wavelength range of maximum absorption in the ultraviolet spectrum	240–290 nm

oxygen, and oxidation with ozone takes place with evolution of more heat and usually starts at a lower temperature than when oxygen is used. In the presence of water, ozone is a powerful bleaching agent, acting more rapidly than hydrogen peroxide, chlorine, or sulfur dioxide. *See* OXIDIZING AGENT.

Ozone is utilized in the treatment of drinking-water supplies. Odor- and taste-producing hydrocarbons are effectively eliminated by ozone oxidation. Iron and manganese compounds which discolor water are diminished by ozone treatment. Compared to chlorine, bacterial and viral disinfection with ozone is up to 5000 times more rapid. After treatment, the residual chlorine content leaves a characteristic undesirable taste and odor. In addition, chlorine may yield chloroform and other trihalomethane (THM) compounds which are potentially carcinogenic. *See* WATER TREATMENT.

Plants that use oxygen in aerobic digestion of sewage can add ozone treatment at reduced cost. Ozone can be produced more economically from pure oxygen. By proper integration of the facilities, oxygen not transformed into ozone in its generator passes through the ozonization tank into the aerobic digester with very high efficiency. *See* SEWAGE TREATMENT.

Ozone undergoes a characteristic reaction with unsaturated organic compounds in which the double or triple bond is attacked, even at temperatures as low as −150°F (−100°C), with the formation of ozonides; these ozonides can be hydrolyzed, oxidized, reduced, or thermally decomposed to a variety of compounds, chiefly aldehydes, ketones, or carboxylic acids. Double (C=C) bonds are almost always ruptured in this reaction. Commercially ozonolysis (ozonation followed by decomposition of the ozonide) is employed in the production of azelaic acid and certain chemical intermediates used in the drug industry. *See* OZONOLYSIS.

Natural occurrence. Ozone occurs to a variable extent in the Earth's atmosphere. Near the Earth's surface the concentration is usually 0.02–0.03 ppm in country air, and less in cities except when there is smog; under smog conditions in Los Angeles ozone is thought to be formed by the action of sunlight on oxygen of the air in the presence of impurities, and on bad days the ozone concentration may reach 0.5 ppm or more for short periods of time.

At vertical elevations above 12 mi (20 km), ozone is formed by photochemical action on atmospheric oxygen. Maximum concentration of 5×10^{12} molecules/cm^3 (more than 1000 times the normal peak concentration at Earth's surface) occurs at an elevation of 19 mi (30 km).

Intercontinental air transports cruise at altitudes of 7.5 to 11 mi (12 to 17 km). On flights through northern latitudes, significant concentrations (up to 1.2 ppm) of ozone have been encountered. At these levels ozone can cause coughing and chest pains, especially for cabin attendants who are actively working. Carbon filters and catalytic ozone-decomposing equipment have been installed to eliminate the problem.

Absorption of solar ultraviolet radiation by ozone provides enough energy to raise the temperature of the stratosphere (6–30 mi or 10–50 km) significantly above that of the upper troposphere. This increase of temperature with increasing height forms a stable layer resistant to vertical mixing. Gases injected into the stratosphere above 12 mi (20 km) may remain 2 years or longer.

By absorbing most of the short-wavelength light, the ozone layer protects human and other life forms. The layer is thinnest at the Equator, where it permits more ultraviolet radiation to reach ground levels in the torrid zone. This is believed to account for the high incidence of skin cancer in equatorial areas.

The dissociation of ozone to oxygen is catalyzed by several chemicals, especially nitrogen oxides and chlorine. Cosmic rays form nitric oxide in the stratosphere. As solar activity causes Earth's magnetic field to increase, cosmic rays are deflected away from Earth. Consequently, there is less nitric acid and more ozone immediately following the maximum phase of the solar activity cycle.

Volcanic eruptions and cosmic rays result in increased levels of chemicals which dissociate ozone. Above-normal levels of these natural events in previous geologic ages are believed to have reduced the ozone layer to 10% of normal. The resulting increase in ultraviolet radiation reaching the Earth's surface may account for the sudden

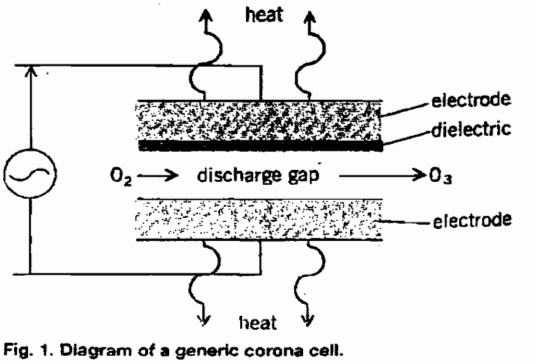


Fig. 1. Diagram of a generic corona cell.

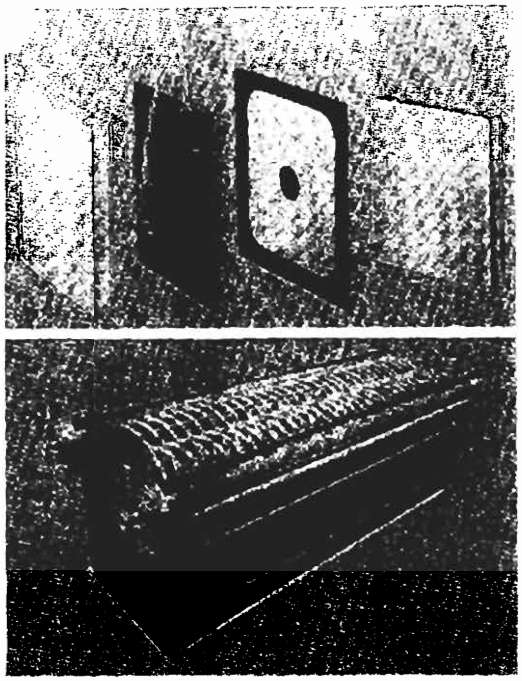


Fig. 2. Lowther cell for ozone generation: (a) expanded view of a single cell; (b) 30-cell module.

extinction of some species.

Human activities also influence the ozone ayer. Nuclear explosions in the atmosphere, and supersonic aircraft cruising at altitudes around 12 mi (20 km), inject nitric oxide into the stratosphere. A still larger effect may be developing from the release of certain relatively stable fluorocarbons, especially $CFCl_3$ and CF_2Cl_2. This type of compound is believed to remain intact for many years in the atmosphere, permitting the gradual vertical transport from the surface into the stratosphere. Intense photochemical activity decomposes the fluorocarbon molecule, releasing chlorine atoms, each of which may destroy many ozone molecules. *See* ATMOSPHERIC OZONE; FLUOROCARBON.

Preparation. The only method used to make ozone commercially is to pass gaseous oxygen or air through a high-voltage, alternating-current electric discharge called a silent electric discharge. First, oxygen atoms are formed as in reaction (1).

$$O_2 \rightarrow 2O \quad (1)$$

Some of these oxygen atoms then attach themselves to oxygen molecules as in reaction (2). The excess

$$O + O_2 + M \rightarrow O_3 + M \quad (2)$$

energy in the newly formed ozone is carried off by any available molecule (M) of gas, thus stabilizing the ozone molecule.

The corona discharge principle employed in all types of commercial ozone generators involves applying a high-voltage alternating current between two electrodes which are separated by a layer of dielectric material and a narrow gap through which the oxygen-bearing gas is passed (**Fig. 1**). The dielectric is necessary to stabilize the discharge over the entire electrode area, so that it does not localize as an intense arc.

A substantial fraction of the electrical energy is converted to heat. The low volume of gas flowing between the electrodes does not have sufficient capacity to remove this heat. Some external heat sink is necessary, since the decomposition of ozone is accelerated by increasing temperature.

The Lowther cell (**Fig. 2***a*) is an example of a modern, plate-type, air-cooled ozone generator. An individual cell is a gastight sandwich consisting of an aluminimum heat dissipator, a steel electrode coated with a highly stable ceramic dielectric, a spacer to set the width of the discharge gap, a second ceramic-coated steel electrode with an oxygen inlet, and an ozone outlet passing through a second aluminum heat dissipator. Individual cells are stacked into 30-cell modules (Fig. 2*b*), which are grouped with power supplies and controls into packaged ozonators (**Fig. 3**). In the concentric-tube type the oxygen or air to be ozonized passes through the annular space (about 2–3 mm across) between two tubes, one of which must be made of a dielectric material, usually glass, and the other may be either glass or a metal which does not catalyze ozone decomposition, such as aluminum or stainless steel. The internal surface of the inner tube and the external surface of the outer tube, when made of glass, are in contact with an electrical conductor such as metal foil, an electrically conducting paint, or electrically conducting water; these conductors act as electrodes. Between 5000 and 50,000 V at a

Fig. 3. Packaged ozone generator.

frequency between 50 and 10,000 Hz is then applied across the electrodes. In some commercial ozone generators the inner and outer tubes are both water-cooled. The latter represents, a simpler type of construction, but does not permit as high an input of electrical power as when both tubes are cooled.

The concentration of ozone in the gas stream leaving commercial ozone generators is usually 1–10% by weight. The yield of ozone is better when oxygen is used instead of air. Other factors which increase the yield of ozone in the silent electric discharge are thorough drying of the oxygen or air before it enters the ozonizer, refrigeration, increasing the pressure to a little above atmospheric, and increasing the frequency of the discharge.

A practical method has been developed for distribution of ozone in small quantities convenient for laboratory use. The ozone is dissolved in a liquefied fluorocarbon. The mixture is maintained at low temperature by a jacket filled with dry ice. Under these conditions, ozone decomposition proceeds very slowly, allowing sufficient time for transport to the user and a modest storage time. Rather high concentrations of ozone may be introduced safely into the laboratory in this manner.

Analytical methods. The analytical determination of ozone is usually carried out in the laboratory by bubbling the gas through a neutral solution of potassium iodide, acidifying the solution, and titrating the iodine thus liberated with standard sodium thiosulfate solution. Ozone in a gas stream may be determined automatically and continuously by passing the gas through a cell with transparent windows and measuring the absorption of either visible light or of ultraviolet radiation beamed through the cell. *See* OXIDATION-REDUCTION; OXYGEN.

Arthur W. Francis

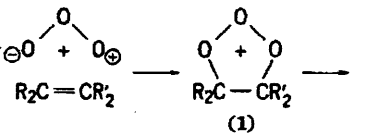
Bibliography. S. B. Majumdar and O. J. Sproul, Technical and economic aspects of water and wastewater ozonization, *Water Res.*, 8:253–260, May 1974; J. B. Murphy and J. R. Orr (eds.), *Ozone Chemical Technology*, 1975; National Academy of Science, *Protection Against Depletion of Stratospheric Ozone by Chlorofluorocarbons*, 1979; National Academy of Science, *Stratospheric Ozone Depletion by Halocarbons*, 1979.

Ozonolysis

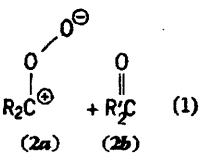
A process which uses ozone to cleave unsaturated organic bonds. General olefin cleavage was first extensively studied by C. Harries, beginning in 1903, as a technique to determine the structure of unsaturated compounds by identification of the cleavage products.

Generally, ozonolysis is conducted by bubbling ozone-rich oxygen or air into a solution of the reactant. The reaction is fast at modera temperatures. Intermediates are usually not isolate but are subjected to further oxidizing conditions to produce acids or to reducing conditions to form alcohols or aldehydes. An unsymmetrical olefin is capable of yielding two different products whose structures are related to the groups substituted on the olefin and the position of the double bond.

The presently accepted mechanism of ozonolysis involves the initial formation of an unstable 1,2,3-trioxacyclopentane "primary ozonide" (**1**) by a 1,3-dipolar cycloaddition of ozone, as shown in one of its resonance structures, with an olefin, in reaction (1). Intermediate (**1**) readily decomposes



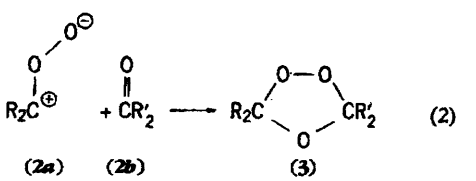
(1)



(1)

(**2a**) (**2b**)

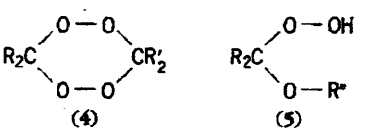
to a zwitterion key intermediate, carbonyl oxide (**2a**), and a carbonyl (**2b**). An important reaction of intermediate (**2a**) is with ketones, for example (**2b**), to form a 1,2,4-trioxacyclopentane (**3**), called ozonide, as in reaction (2). The intermediate



(2)

(**2a**) (**2b**) (**3**)

(**2a**) can also dimerize to the diperoxide (**4**) or polymerize to polymeric ozonides and peroxides. The zwitterion produces oxyperoxides of the general structure (**5**), where the reaction media



(**4**) (**5**)

contains water (R″=H), ethanol (R″=OCH_2CH_3), or acetic acid (R″=$OOCCH_3$).

Before World War I, ozonolysis was applied commercially to the preparation of vanillin from isoeugenol. Today the only major application of the technique in the United States is in the manufacture of azelaic and pelargonic acids from oleic acid. *See* ALKENE; OZONE.

Robert K. Barnes

Bibliography. P. S. Bailey, *Ozonation in Organic Chemistry*, vol. 1, 1978; M. Horvath, L. Bilitzk, and J. Huttner, *Ozone*, 1985.

Four systemic nematicides namely, aldicarb, carbofuran (3,10% ai), oxamyl (granules, liquid), fenamiphos and the nonsystemic, ethoprop were evaluated for the control of the root-knot nematode, *M. javanica*. The experiment was conducted under the green house conditions on eggplant, *Solanum melongena* grown in 15 cm plastic pots filled with sandy soil. The concentrations used were 0.1 and 0.2 g / kg soil added as side dressing one week after nematode inoculation (except for ethoprop, which added a week before or after nematode inoculation). Results indicated that mocap accomplished the best significant reduction in the numbers of galls and egg-masses (86 – 96%, 65 – 91%; respectively). Aldicarb and fenamiphos came after ethoprop without significant differences. Oxamyl granules achieved moderate degree of nematode control. Results of carbofuran (3, 10% ai) were unsatisfactory. Oxamyl liquid, aldicarb and ethoprop, in that order, were the best in improving plant growth criteria.

Nematode faunal profiles of soil ecosystems. H. FERRIS (1), T. Bongers (2), and R. G. M. de Goede (3). (1) Department of Nematology, University of California, Davis CA 95616; (2) Laboratory of Nematology, Wageningen University, Wageningen, Netherlands; (3) Sub-department of Soil Quality, Wageningen University, Wageningen, Netherlands. Phytopathology 91:S134. Publication no. P-2001-0037-SON.

Nematodes are partitioned into functional guilds based on feeding habit, opportunistic response to environmental enrichment and sensitivity to perturbation. Abundance within each guild is weighted by the indicator importance of that guild for the enrichment or structure characteristic of the system. Soil foodwebs are categorized as enriched but unstructured (A), enriched and structured (B), resource-limited and structured (C), or resource-depleted with minimal structure (D). Systems differ in predatory regulation of opportunistic taxa, amount of sequestered carbon, level and nature of enrichment, decomposition channels, and C:N of organic input. Category A systems are represented by annual-cropped agriculture with frequent disturbance; B systems by organically-driven, relatively undisturbed perennial systems; C systems by grasslands and forests with little disturbance or extrinsic enrichment; and D systems by stressed annual agricultural systems and contaminated sites.

Molecular barcodes for soil nematode identification. R. M. FLOYD, A. Papert, and M. L. Blaxter. ICAPB, University of Edinburgh. Phytopathology 91:S134. Publication no. P-2001-0038-SON.

We have developed a molecular barcoding system for identification of soil nematodes by DNA sequencing. PCR is carried out on individual nematodes, and the 5' segment of the small subunit ribosomal RNA (SSU) gene is amplified and sequenced. Resulting sequences (typically 450-500 bases) are aligned and clustered using a neighbour-joining algorithm. Groups of similar or identical sequences are designated as molecular operational taxonomic units (MOTU). A Scottish upland *Agrostis-Festuca* grassland soil was sampled, using both random and culture-based methods. The MOTU discovered could readily be assigned to classical, morphologically defined taxonomic groups using a database of SSU sequences from named nematode species. The MOTU technique allows a rapid assessment of nematode taxonomic diversity in soils. Correlation with a database of sequences from known species offers a route to application of the technique in ecological surveys addressing biological as well as genetic diversity.

Cloning putative parasitism genes expressed in the esophageal gland cells of the soybean cyst nematode. B. GAO (1), R. Allen (1), T. Maier (2), E. L. Davis (3), T. J. Baum (2), and R. S. Hussey (1). Depts. of Plant Pathology, (1) University of Georgia, Athens, GA 30602; (2) Iowa State University, Ames, IA 50011; (3) North Carolina State University, Raleigh, NC 27695-7616. Phytopathology 91:S134. Publication no. P-2001-0039-SON.

Secretions produced in nematode esophageal gland cells play key roles in plant parasitism. Contents microaspirated from the gland cells and from the intestinal region of parasitic stages of the soybean cyst nematode were used to construct separate cDNA libraries. Suppression subtractive hybridization (SSH) between gland cell and intestinal cDNA isolated genes expressed preferentially in the glands. SSH product was used to construct a gland cell library macroarray containing 3,000 cDNA clones. Gland contents were also used to construct a full-length cDNA library macroarray, representing 6,144 clones. Twenty-three unique cDNA sequences were identified from the SSH macroarray and full-length cDNAs of 21 of these clones were obtained from the full-length cDNA macroarray. Proteins encoded by 10 clones contained a signal peptide and PSORT II predicted 8 to be extracellular. mRNA in situ hybridization localized several clones within the dorsal or subventral glands of the nematode.

Biological soil suppression affects both sexes of *Heterodera schachtii*. Y. GAO and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521. Phytopathology 91:S134. Publication no. P-2001-0040-SON.

In a local soil suppressive for beet cyst nematode, previous studies have suggested that fungal parasitism of females, cysts and eggs caused the nematode population decline. This project focussed on the population of males, as mating is required for successful reproduction of *H. schachtii*. Pots with five week-old seedlings of the host plant Swiss chard were infested with 2000 second-stage juveniles and incubated at 20°C in an environmental growth chamber. After the nematodes had completed two generations, the soil was amended with 20 percent (v/v) suppressive or conducive soil. *H. schachtii* populations were assayed at 200 and 400 degree days after amendment application. Both male and cyst populations were significantly less abundant in suppressive than in conducive soil at both sampling dates. The results indicate that a reduction in the male population may contribute to the *H. schachtii* population decline in the suppressive soil.

Hot water and ozone treatments of Easter lily for management of lesion nematode, *Pratylenchus penetrans*. D. D. GIRAUD (1), B. B. Westerdahl (2), L. J. Riddle (3), C. E. Anderson (2), and A. Pryor (4). (1) University of California (UC), Cooperative Extension, Eureka, CA 95503; (2) Dept. Nematology, UC, Davis, CA 95616; (3) Easter Lily Research Foundation, Brookings, OR 97415; (4) SoilZone, Inc., Davis, CA 95616. Phytopathology 91:S134. Publication no. P-2001-0041-SON.

Easter lily bulbs for greenhouse forcing are produced in Del Norte County, CA and Curry County, OR. *P. penetrans* infestation of soil and roots is a serious detriment to production. In 3 years of field trials, hot water(HW) and ozone(O3) treatments of bulblet planting stock were tested alone, and in combination with commercial chemical standards and compared to untreated controls. Each trial consisted of 3 replicates of 40 treatments. Several treatments performed better than untreated, but not as well as commercial standards in all evaluation criteria. For example, HW treatment at 49 centigrade(C) for 35 minutes(M) or 46 C for 90 M, consistently reduced nematode populations within roots but did not substantially improve growth of bulbs. In contrast, O3 that was produced by a conventional electrical discharge generator did not reduce nematode numbers but improved bulb growth in some treatments.

Endoglucanase expression in plant-nematode interactions. M. GOELLNER, X. Wang, and E. L. Davis. Dept. of Plant Pathology, North Carolina State University. Phytopathology 91:S134. Publication no. P-2001-0042-SON.

The formation of specialized feeding cells within host roots by cyst and root-knot nematodes, termed syncytia and giant-cells, respectively, requires extensive cell wall remodeling. Cell wall degrading enzymes have been implicated in cell wall modifications observed during feeding cell formation; however, it is unclear whether the enzymes are of nematode or plant origin. Antisera specific to beta-1,4-endoglucanases (EGases) produced by the tobacco cyst nematode localized secreted nematode EGases in plant cortical tissue during migration, but not within syncytia. Tobacco EGase expression was upregulated in both root knot and cyst nematode-infected roots. Three full-length tobacco cDNA clones each encoding structurally divergent EGases belonging to glycosyl hydrolase family 9 have been characterized. *In situ* mRNA hybridization localized tobacco EGase transcripts within syncytia, giant-cells, root tips, and lateral root primordia. Tobacco EGases are likely an integral component of a complex array of cell wall enzymes recruited during nematode parasitism for the development of feeding cells in plant roots.

Impact of green manure crops on sustainable management of sugar beet cyst nematode. S. L. HAFEZ and P. Sundararaj. University of Idaho. Parma Research and Extension Center, 29603 U of I Ln, Parma, Idaho 83660, USA. Phytopathology 91:S134. Publication no. P-2001-0043-SON.

A series of experiments were conducted for three years to study the efficacy of green manure crops for management of sugarbeet cyst nematode, *Heterodera schachtii*, under field conditions. In all experiments, oil radish and white mustard cultivars were seeded at the rate of 25 lb/acre in fall and incorporated twelve weeks later as the field was prepared for sugarbeet. In the first year, mustard 'Concerta' produced 35% more above ground biomass than radish 'Colonel' and the viable cysts declined 29% and 19% in oil radish and mustard treatments, respectively. Planting of oil radish and mustard produced significantly more beet yield and sugar per acre than the untreated check. In the second year, radish 'Adagio' produced significantly more above ground

Phytopathology Vol. 91 No. 6 (Supplement) 2001 pg S134

Cleaning of Drip Lines

MIF Home

Trickle-L Archives

Drip Directory

Future Meetings

Digital Gallery

Related Links

Soil Moisture Sensors

Irrigation On The Net

Chlorination is an effective way for cleaning the lines from microbial growth. However, there are several points to be considered:

1. The type of chlorinating agent: Chlorine gas, is the most effective and generally less costly than other agents, especially for large acreage. Special precautions should be taken due to the toxicity of chlorine fumes. Sodium hypochlorite liquid, is a safer chlorinator than chlorine gas, but degrades over time. So if it sits in storage for a long time it may lose its effectiveness. Calcium hypochlorite granules or tablets, it is more stable than sodium hypochlorite. A stock solution is prepared, then injected into the system in a manner similar to sodium hypochlorite but it does not dissolve completely in water. The prepared stock solution should be injected upstream from the filter to avoid emitters clogging by undissolved material. It is advisable to inject chlorinating agents upstream from the filter .

2. The mode of chlorination and concentration of chlorine in irrigation water: Continuous chlorination could be done on maintenance basis (e.g. every irrigation) if the microbial count in the water is high (e.g canal water). Maintenance chlorination requires chlorine concentration at the end of the far most lateral to be 1-2 ppm of active (not total) chlorine. Shock treatment for sever clogging problems, or when microbial growth is not particularly due to the irrigation water but may be due to other factors such as residual nitrogen fertilizer that was not regularly flushed out of the system. This treatment is done as frequent as may be necessary, and requires chlorine concentration in irrigation water of 10-30 ppm for 30-60 minutes. The chlorine concentration and duration of treatment depend on the severity of the problem. Insufficient chlorine concentration in irrigation water, with either treatments, could diminish the effectiveness of chlorination.

3. The pH of the irrigation water: High pH (7.8+, e.g. ground water) of irrigation water encourages the precipitation of calcium carbonate and iron oxide, both are serious emitter cloggers. A recent water analysis will be helpful in identifying the clogging potential. If the pH of irrigation water is inherently high, you may need to acidify and chlorinate concurrently. If this is the case, chlorine and acid injectors should be 2-3 feet apart on the line, BUT NEVER MIX CHLORINE AND ACID IN THE SAME CONTAINER.

4. Flushing the lines: It is a good idea to flush the lines, starting with the larger pipes to the smaller ones and ending with the laterals, before concluding the season. It is also advisable to flush the fertilizer out of the system before the end of each irrigation run to minimize the chances of microbial growth in the laterals. This is a brief of some of the high points regarding effectiveness of chlorination for cleaning drip lines. If you need further details please let me know, I will be glad to help. F

by Farouk A. Hassan

Ozone

I found your infornation very interesting. Could you please comment on the use of ozone generators as a sorce of an oxidizing agent. No dangerous fumes and some even claim better crop results. I know that their use has been approved for use in packing houses to disinfect wash water. I would like your thoughts on this.

by Chuck Warner

Your interest and your e-mail are appreciated. I was doing some search on the use of ozone, the subject of your enquiry, that is why my response to you was delayed. You are probably trying to compare ozone to chlorine. Cost wise chlorination of irrigation water costs about $7-$10 per acre, while ozonation may cost between $20 and $30 per acre.

There is no dangerous fumes with ozone as the gas is generated on the spot in a closed system and dissolved in water under pressure, while undissolved gas is collected and disposed of by means of a special separator to avoid accumulation of gas bubbles in the system. This is particularly important for pressurized irrigation systems. Both chlorine and ozone are oxidizing agents and will cause the precipitation of iron in irrigation water. This requires their injection upstream from the filter to avoid clogging of emitters. Both chlorine and ozone will be more effective in acidified water. Continuous use of chlorine without sufficient leaching may increase its concentration in the soil.

Some crops are sensitive to high chlorine in the soil. Ozone in irrigation water may destroy some soil microorganisms but will disintegrate and would not have the harmful effect of high chlorine levels on crops. Ozone is a more effective disinfectant with less harmful specific effect on crops than chlorine. Whether or not the use of ozone would result in a better crop that justifies the extra cost, this is a question that requires further studies under field conditions. I hope this will be of help.

by Farouk A. Hassan

by Chuck Warner

Your interest and your e-mail are appreciated. I was doing some search on the use of ozone, the subject of your enquiry, that is why my response to you was delayed. You are probably trying to compare ozone to chlorine. Cost wise chlorination of irrigation water costs about $7-$10 per acre, while ozonation may cost between $20 and $30 per acre.

There is no dangerous fumes with ozone as the gas is generated on the spot in a closed system and dissolved in water under pressure, while undissolved gas is collected and disposed of by means of a special separator to avoid accumulation of gas bubbles in the system. This is particularly important for pressurized irrigation systems. Both chlorine and ozone are oxidizing agents and will cause the precipitation of iron in irrigation water. This requires their injection upstream from the filter to avoid clogging of emitters. Both chlorine and ozone will be more effective in acidified water. Continuous use of chlorine without sufficient leaching may increase its concentration in the soil.

Some crops are sensitive to high chlorine in the soil. Ozone in irrigation water may destroy some soil microorganisms but will disintegrate and would not have the harmful effect of high chlorine levels on crops. Ozone is a more effective disinfectant with less harmful specific effect on crops than chlorine. Whether or not the use of ozone would result in a better crop that justifies the extra cost, this is a question that requires further studies under field conditions. I hope this will be of help.

by Farouk A. Hassan

Weed Science. 1983. Volume 31:857–861

Ozone-Herbicide Interactions on Sorghum (*Sorghum bicolor*) and Velvetleaf (*Abutilon theophrasti*) Seedlings[1]

KRITON K. HATZIOS and YAW-SHING YANG[2]

Abstract. The potential interactive effects between the herbicides chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl] benzenesulfonamide}, PP009 {butyl 2-[4-[5-(trifluoromethyl-2-pyridinyl)oxy] phenoxy] propanoate}, and BAS 9052 OH {2-[1-(ethoxyimino)-butyl]-5-[2-(ethylthio)-propyl]-3-hydroxy-2-cyclohexene-one} and the air pollutant ozone (O_3) on the growth of sorghum [*Sorghum bicolor* (L.) Moench. 'Funk G623®'] and velvetleaf (*Abutilon theophrasti* Medic. #[3] ABUTH) were examined. All three herbicides were applied postemergence either before or after a single 6-h fumigation of sorghum and velvetleaf seedlings with O_3 at 0, 0.1, and 0.2 ppmv. Chlorsulfuron was applied at 0, 0.06, or 0.12 kg ai/ha, while PP009 and BAS 9052 OH were applied at 0, 0.6, and 1.2 kg ai/ha. Two weeks after treatment, dry weight responses of velvetleaf seedlings revealed that PP009 interacted synergistically while chlorsulfuron and BAS 9052 OH interacted antagonistically with O_3. The sequence of O_3 fumigation and herbicide treatment appeared to be an important factor determining the type of interactive effects of these herbicides with O_3. The interactive effects of all three herbicides with O_3 on sorghum seedlings were additive regardless of the sequence of O_3 fumigation and herbicide treatment.

Additional index words. Chlorsulfuron, PP009, BAS 9052 OH, air pollutant, synergism, antagonism, ABUTH.

INTRODUCTION

The recent development of postemergence grass herbicides with excellent crop selectivity is considered a major advancement in modern herbicide technology. Among these herbicides are chlorsulfuron, PP009, and BAS 9052 OH. Chlorsulfuron applied either preemergence or postemergence effectively controls many broadleaf and certain grass weeds in small grains (11). PP009 controls many annual and perennial grasses at rates ranging from 0.125 to 2.0 kg/ha (16). BAS 9052 OH is very effective on both annual and perennial grass weeds when applied postemergence (12, 14).

Recently, concern has been expressed about the effects of deteriorating air quality on plant productivity (10). Among the numerous atmospheric pollutants, the effects of ozone (O_3) and sulfur dioxide (SO_2) on plants have attracted particular attention (2, 3, 23). Air pollutants have been reported to interact with several agricultural practices including chemical weed control (1, 6, 7, 19). However, research on the potential interactive effects of herbicides and air pollutants on vegetation has been limited. Ozone may interact with selected herbicides on certain crop plants, thereby modifying either the plant response to herbicides (1, 15, 18, 22) or the metabolism of herbicides in fumigated plants (5, 6, 7, 8, 9). For example, pebulate (*S*-propyl butylethylthiocarbamate), chloramben (3-amino-2,5-dichlorobenzoic acid), and metribuzin [4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one] have been reported to interact synergistically with O_3 on selected cultivars of tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.), respectively (1, 15). In other studies, the herbicides benefin (*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine), diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide), and isopropalin (2,6-dinitro-*N,N*-dipropylcumidine) were reported to interact antagonistically with O_3 on selected tobacco cultivars (18, 22). Preliminary studies by Hodgson (5) indicated that fumigation of intact corn (*Zea mays* L.) and sorghum plants with O_3 altered the metabolism of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] in those plants. Subsequent studies by Hodgson and co-workers illustrated that the metabolism of the herbicide diphenamid in tomato and pepper (*Capsicum frutescens* L.) plants was quantitatively altered when these plants were fumigated with O_3 (6, 7, 8, 9).

This study was designed to investigate potential interactions between postemergence applications of the herbicides chlorsulfuron, PP009 or BAS 9052 OH and O_3 on sorghum, a grass crop with intermediate tolerance to O_3, and on velvetleaf, a broadleaf weed with low to intermediate ozone tolerance.

MATERIALS AND METHODS

Plant material and herbicide application. Sorghum[4] plants were grown from seed in a 2:2:1, v/v/v mixture of potting medium[5], vermiculite and peat. A controlled-release fertilizer (14-14-14) and an agricultural fertilizer (4-3-9) were added to the potting mixture to supplement nutrient levels. Three seeds were planted/473-ml plastic cup. Seeds of velvetleaf collected from a field located in Blacksburg, VA, were mechanically scarified and planted in the same mixture at the rate of three seeds/plastic cup. After planting, the plastic cups were placed in a greenhouse with charcoal-filtered air, a 14-h photoperiod, and day/night temperatures of

[1] Received for publication March 7, 1983. Contribution No. 486, Dep. Plant Pathol. and Physiol., Virginia Polytech. Inst. and State Univ., Blacksburg.

[2] Asst. Profs., Dep. Plant Pathol. and Physiol., Virginia Polytech. Inst. and State Univ., Blacksburg, VA 24061.

[3] WSSA-approved computer code from Important Weeds of the World, 3rd ed. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

[4] Funk Seeds Int., 1000 W. Washington, Bloomington, IL 61701.

[5] Weblite®, Weblite Corp., P.O. Box 308, Blue Ridge, VA 24064.

29/22 C. When sorghum and velvetleaf seedlings were 2 and 3 weeks old, respectively, they were treated with the herbicides 1 day before and 1 day after O_3 fumigation. Formulated products of all three herbicides were applied postemergence with a link-belt sprayer at 205.9 kPa in 935 L/ha spray volume. Chlorsulfuron was applied at 0, 0.06, and 0.12 kg ai/ha while PP009 and BAS 9052 OH were applied at 0, 0.6, and 1.2 kg ai/ha.

Fumigation of seedlings with O_3. One day before and 1 day after herbicide application, sorghum and velvetleaf seedlings were fumigated with O_3 for 6 h between 1000 and 1600 h eastern standard time in three continuous stirred tank reactor (CSTR) exposure chambers. These chambers were constructed by using a cylindrical metal frame covered totally with a transparent teflon film; and they utilize a dynamic, negative-pressure, single-pass airflow design that permits the simultaneous injection of several gaseous pollutants. A detailed description of the construction and operation of these chambers has been given by Heck et al. (4). Sorghum and velvetleaf seedlings in the first chamber were exposed only to charcoal-filtered air (0 ppmv of O_3). Seedlings in the other two chambers were exposed to 0.1 or 0.2 ppmv ± 0.01 ppmv of O_3. Ozone was generated by a UV, O_3 generator[6] and concentrations within the chambers were monitored with a chemiluminescent ozone analyzer[7]. The O_3 monitor was calibrated every 2 weeks with an automated ozone calibrator[8]. The relative humidity in all CSTR chambers was maintained at 65 ± 5% by means of a steam generator[9], and the chamber air temperature was 30 ± 2 C. After O_3 fumigation, the seedlings were returned to the greenhouse.

Data collection and statistical analysis. Two weeks after herbicide application and/or O_3 fumigation, sorghum and velvetleaf shoots were harvested, dried in a forced-air oven at 50 C for 48 h, and weighed. Data presented are the means of two experiments with two replications and three subreplications in each experiment and are expressed as the average shoot dry weight per plant per cup. These data were analyzed for variance as a three-by-three (herbicide by ozone) factorial experiment for each herbicide in a completely randomized design. The standard errors of the observed mean responses were calculated. Statistically significant interactions for each combination treatment were identified by the F-test for a two-by-two comparison of that treatment with the control and the separate levels of each herbicide and O_3 involved, as described by Nash (13). In addition, the expected responses for each combination treatment were calculated, assuming no interactions, by subtracting the observed response of the control from the sum of the observed responses of the separate level of each herbicide and O_3 involved (21). For example, in Table 1, the expected response of velvetleaf seedlings to the combined treatment of chlorsulfuron at 0.06 kg/ha and O_3 at 0.1 ppmv is calculated as follows: $(0.38 + 2.07) - 2.33 = 0.12$. In Tables 1 and 2 the calculated expected responses for each treatment combination are given in parentheses. The expected responses for each treatment combination were then compared to the observed responses for the same treatment combination and the interactive effects were characterized by Putnam and Penner (17) as follows. When the observed value is less than the expected value, synergism is indicated. When the observed value exceeds the expected value, antagonism is indicated. Finally, when the observed and expected values are similar, the interactive effect is additive. In the discussion that follows, differences between observed and expected responses for each treatment combination are viewed as biologically important only when the observed response for a given treatment combination between the herbicide and ozone or antioxidants was significant by the F-test. In all other cases, differences between observed and expected responses are not viewed as biologically important and the interactive effects are characterized as additive.

RESULTS AND DISCUSSION

Of the three herbicides examined, chlorsulfuron was the only one that significantly reduced the dry weights of velvetleaf seedlings as indicated by the standard errors of the means (Table 1). The effects of PP009 and BAS 9052 OH on velvetleaf seedlings were not significant with the exception of BAS 9052 OH which, when applied alone at 1.2 kg/ha decreased the dry weight of velvetleaf seedlings slightly. These results might be expected since only chlorsulfuron has been reported to possess strong activity on broadleaf plants (11) while all three herbicides are effective on grasses (12, 14, 16). In the absence of herbicide treatments, fumigation with O_3 at 0.1 ppmv did not significantly reduce the dry weights of velvetleaf seedlings. However, when the concentration of O_3 was increased to 0.2 ppmv, significant reductions in velvetleaf shoot dry weights were evident (Table 1). When the seedlings were first fumigated with O_3, the interactive effects of chlorsulfuron with O_3 on velvetleaf seedlings were additive (Table 1). When the velvetleaf seedlings were treated with chlorsulfuron prior to fumigation with O_3, chlorsulfuron at 0.06 and 0.12 kg/ha interacted antagonistically with O_3 at 0.2 ppmv. The interactive effects of PP009 and O_3 on velvetleaf seedlings that were first treated with the herbicide and then fumigated were additive. However, a significant synergistic interaction was observed between all levels of PP009 and O_3 on velvetleaf seedlings first fumigated with O_3 and then treated with the herbicide (Table 1). BAS 9052 OH at 0.6 kg/ha interacted antagonistically with 0.2 ppmv of O_3 only on velvetleaf seedlings receiving a prior fumigation with O_3 (Table 1). BAS 9052 OH at 1.2 kg/ha interacted antagonistically with 0.2 ppmv of O_3 on velvetleaf seedlings regardless of the sequence of O_3 fumigation and herbicide application.

All levels of each herbicide significantly reduced the dry weights of sorghum seedlings as revealed by the standard errors of the means (Table 2). Ozone fumigation caused no

[6] Laboratory ozonator (Model T-408). Welsbach Ozone Systems Corp., Philadelphia, PA 19129.
[7] Model 8002. Bendix Process Inst. Div., Lewisburg, WV 24901.
[8] Photocal 3000. Columbia Sci. Ind., Austin, TX 78766.
[9] Automatic Stream Products Corp., Long Island, NY 11101.

Table 1. Shoot dry-weight responses of velvetleaf treated with chlorsulfuron, PP009, and BAS 9052 OH before or after fumigation with ozone[a].

Herbicide	Rate	Type of response	Shoot weight (Sequence herbicide/ozone) Ozone concentration, (ppmv) 0	0.1	0.2	Shoot weight (Sequence ozone/herbicide) Ozone concentration, (ppmv) 0	0.1	0.2
	(kg/ha)		(g)					
Chlorsulfuron	0.00	Observed	2.33 ± 0.34	2.07 ± 0.21	1.83 ± 0.08	2.85 ± 0.25	2.56 ± 0.21	2.32 ± 0.19
	0.06	Observed	0.38 ± 0.07	0.33 ± 0.07	0.48** ± 0.05	1.27 ± 0.55	0.70 ± 0.13	0.63 ± 0.1
		Expected		(0.12)	(−0.12)		(0.98)	(0.74)
	0.12	Observed	0.40 ± 0.07	0.35 ± 0.05	0.43** ± 0.03	0.85 ± 0.14	0.43 ± 0.12	0.50 ± 0.1
		Expected		(0.14)	(−0.10)		(0.56)	(0.32)
PP009	0.0	Observed	2.20 ± 0.2	2.15 ± 0.3	2.05 ± 0.13	2.67 ± 0.13	2.75 ± 0.13	2.40 ± 0.15
	0.6	Observed	2.07 ± 0.2	2.07 ± 0.33	1.95 ± 0.45	2.37 ± 0.1	2.15** ± 0.14	1.75** ± 0.16
		Expected		(2.02)	(1.92)		(2.45)	(2.10)
	1.2	Observed	1.97 ± 0.2	1.83 ± 0.33	1.68 ± 0.22	2.47 ± 0.14	2.05** ± 0.65	1.30** ± 0.12
		Expected		(1.92)	(1.82)		(2.55)	(2.20)
BAS 9052 OH	0.0	Observed	2.45 ± 0.3	2.35 ± 0.31	2.00 ± 0.29	2.87 ± 0.33	2.80 ± 0.18	2.28 ± 0.27
	0.6	Observed	2.42 ± 0.14	2.35 ± 0.48	1.90 ± 0.44	2.20 ± 0.21	2.13 ± 0.11	2.48** ± 0.25
		Expected		(2.32)	(1.97)		(2.13)	(1.61)
	1.2	Observed	1.87 ± 0.35	2.93 ± 0.14	2.05** ± 0.33	2.25 ± 0.25	2.08 ± 0.19	2.38** ± 0.24
		Expected		(1.77)	(1.42)		(2.18)	(1.66)

[a]Mean weight values are from four replications ± standard error of each mean. Expected values for each combination treatment are given in parentheses and were calculated, assuming no interactions, by subtracting the observed response of the control from the sum of the observed responses of the separate level of each herbicide and O_3 involved. Asterisks indicate significant interactions at the 1% (**) level of probability as determined by F-values for each combination term calculated for 2-by-2 comparisons of that treatment with the control and the separate level of herbicide and ozone involved.

significant reduction in the dry weights of sorghum seedlings. Use of the F-test for each treatment combination of any herbicide with O_3, calculated by means of two-by-two comparisons, detected no significant interactions between the herbicides chlorsulfuron, PP009, BAS 9052 OH and O_3. In general, the interactive effects of all rates of the three herbicides and O_3 on sorghum seedlings were additive regardless of the order of herbicide application and O_3 fumigation (Table 2).

The exact mechanisms of the synergistic and antagonistic interactions between the three herbicides and O_3 on velvetleaf are unknown at the present time. Synergistic interactions between O_3 and selected herbicides could result from either a O_3-induced alteration of herbicide degradation in certain plants (5, 6, 7, 9) or a herbicide-induced predisposition of specific plants to greater injury by O_3 (1, 15). Since the synergistic interaction between PP009 and O_3 was only evident on velvetleaf seedlings that were first fumigated with O_3, it could be postulated that O_3 adversely influences the mechanisms responsible for the tolerance of velvetleaf to the PP009. In susceptible grasses, PP009 is hydrolyzed to its free acid, which is believed to be the active form of this herbicide (20). Exposure of soybean [*Glycine max* (L.) Merr.] leaves and Ponderosa pine (*Pinus ponderosa* Dougl.) seedles to O_3 has been reported to increase the activity of several enzymes involved in phenol metabolism such as phenylalanine-ammonia lyase and polyphenoloxidase (22). Ozone-injured plants and water-stressed plants possess several features in common (2), and stimulation of the activity of enzymes involved in hydrolysis and metabolic degradation has been reported to occur in plants exposed to moderate or severe water stress (24). Therefore, it could be suggested that exposure of velvetleaf seedlings to O_3 could have stimulated the activity of enzymes that hydrolyze PP009 to its active form. Further research is needed to substantiate such a hypothesis.

Antagonistic interactions between the herbicides chlorsulfuron or BAS 9052 OH and O_3 on velvetleaf seedlings are also difficult to explain. Reports by other investigators (18, 22), have demonstrated that selected herbicides can protect plants such as tobacco against O_3 injury. However, the exact mechanisms through which these herbicides neutralize the effects of O_3 on tobacco and other plants are unknown.

Ambient diurnal summertime concentrations of O_3 in the eastern United States often range from 0.06 to 0.08 ppmv, with occasional peak concentrations exceeding the National Ambient Air Quality Standards (NAAQS) of 0.12 ppmv. Studies have indicated that the NAAQS for photochemical oxidants such as O_3 were frequently violated in each year from 1975 through 1981 even though the NAAQS for O_3 was changed from 0.08 ppmv/h once/yr to 0.12

Table 2. Shoot dry-weight responses of sorghum treated with chlorsulfuron, PP009, and BAS 9052 OH before or after fumigation with ozone[a]

Herbicide	Rate	Type of response	Shoot weight (Sequence herbicide/ozone)			Shoot weight (Sequence ozone/herbicide)		
			Ozone concentration, (ppmv)			Ozone concentration, (ppmv)		
			0	0.1	0.2	0	0.1	0.2
	(kg/ha)		(g)					
Chlorsulfuron	0.00	Observed	2.30 ± 0.35	2.12 ± 0.28	2.27 ± 0.47	2.25 ± 0.36	2.25 ± 0.46	1.85 ± [illegible]
	0.06	Observed	0.55 ± 0.09	0.47 ± 0.12	0.55 ± 0.13	0.60 ± 0.2	0.48 ± 0.11	0.55 ± [illegible]
		Expected		(0.37)	(0.52)		(0.60)	(0.20)
	0.12	Observed	0.37 ± 0.03	0.35 ± 0.03	0.23 ± 0.08	0.60 ± 0.16	0.35 ± 0.12	0.43 ± [illegible]
		Expected		(0.19)	(0.34)		(0.60)	(0.20)
PP009	0.0	Observed	1.80 ± 0.32	1.90 ± 0.54	1.90 ± 0.5	1.87 ± 0.45	1.95 ± 0.33	1.70 ± [illegible]
	0.6	Observed	0.17 ± 0.05	0.25 ± 0.06	0.28 ± 0.05	0.30 ± 0.07	0.38 ± 0.03	0.35 ± [illegible]
		Expected		(0.27)	(0.27)		(0.38)	(0.13)
	1.2	Observed	0.20 ± 0.001	0.15 ± 0.05	0.10 ± 0.04	0.32 ± 0.11	0.28 ± 0.07	0.33 ± [illegible]
		Expected		(0.30)	(0.30)		(0.40)	(0.15)
BAS 9052 OH	0.0	Observed	2.07 ± 0.39	1.88 ± 0.4	1.90 ± 0.45	2.30 ± 0.43	2.20 ± 0.36	2.30 ± [illegible]
	0.6	Observed	0.30 ± 0.10	0.30 ± 0.06	0.33 ± 0.09	0.28 ± 0.05	0.20 ± 0.001	0.20 ± [illegible]
		Expected		(0.11)	(0.13)		(0.18)	(0.28)
	1.2	Observed	0.29 ± 0.07	0.28 ± 0.05	0.18 ± 0.03	0.23 ± 0.03	0.23 ± 0.02	0.20 ± [illegible]
		Expected		(0.20)	(0.12)		(0.13)	(0.23)

[a]Mean weight values are from four replications ± standard error of each mean. Expected values for each combination treatment are given in parentheses and were calculated, assuming no interactions, by subtracting the observed response of the control from the sum of the observed responses of the separate level of each herbicide and O_3 involved.

ppmv/h once/yr in February of 1979 (25). Thus, O_3 concentrations at the levels used in this study could or do occur in certain areas of United States.

ACKNOWLEDGMENTS

The expert technical assistance of Ms. Celestia M. Mauer is greatly appreciated. Thanks are due to BASF Wyandotte Corp., Parsippany, NJ; E. I. duPont de Nemours and Co., Inc., Wilmington, DE; and ICI Americas, Inc., Goldsboro, NC, for providing formulated products of the herbicides.

LITERATURE CITED

1. Carney, A. W., G. R. Stephenson, D. P. Ormrod, and C. G. Ashton. 1973. Ozone-herbicide interactions in crop plants. Weed Sci. 21:508–511.
2. Heath, R. L. 1975. Ozone. Pages 23–55 *in* J. B. Mudd and T. T. Kozlowski, eds. Responses of Plants to Air Pollution. Academic Press, NY.
3. Heath, R. L. 1980. Initial events in injury to plants by air pollutants. Annu. Rev. Plant Physiol. 31:395–431.
4. Heck, W. W., R. B. Philbeck, and J. A. Dunning. 1978. A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants: Principles, specifications, construction, and operation. ARS-S-181 U.S. Dep. Agric., Agric. Res. Serv., Raleigh, NC. 32 pp.
5. Hodgston, R. H. 1970. Alteration of triazine metabolism by ozone. Abstr. Weed Sci. Soc. Am. No. 28.
6. Hodgson, R. H., D. S. Frear, H. R. Swanson, and L. A. Regan. 1973. Alteration of diphenamid metabolism in tomato by ozone. Weed Sci. 21:542–549.
7. Hodgson, R. H., K. E. Dusbabek, and B. L. Hoffer. 1974. Diphenamid metabolism in tomato: Time course of an ozone fumigation effect. Weed Sci. 22:205–210.
8. Hodgson, R. H. and B. L. Hoffer. 1977. Diphenamid metabolism in pepper and an ozone fumigation effect. I. Absorption translocation and the extent of metabolism. Weed Sci. 25:324–330.
9. Hodgson, R. H. and B. L. Hoffer. 1977. Diphenamid metabolism in pepper. II. Herbicide metabolite characterization. Weed Sci. 25:331–337.
10. Lawrence, J. A. and L. H. Weinsten. 1981. Effects of air pollutants on plant productivity. Annu. Rev. Phytopathol. 19:257–271.
11. Levitt, G., H. L. Ploeg, R. C. Weigel, Jr., and D. T. Fitzgerald. 1981. 2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino carbonyl] benzenesulfonamide, a new herbicide. J. Agric. Food Chem. 29:416–418.
12. McAvoy, W. J., L. W. Hendrik, M. A. Veenstra, W. J. Sciarappa, M. Schroeder, and A. Tasker. 1980. Selective postemergence perennial grass control with BAS 9052 OH. Abstr. Weed Sci. Soc. Am. No. 28.
13. Nash, R. G. 1981. Phytotoxic interaction studies – Techniques for evaluation and presentation of results. Weed Sci. 29:147–155.
14. Pearson, J. O. 1980. Postemergence graminicide for broadleaf crops. Abstr. Weed Sci. Soc. Am., No. 245.
15. Phatak, S. C. and T.J.A. Proctor. 1976. Ozone and metribuzin interaction in tomatoes. Abstr. Weed Sci. Soc. Am., No. 173.
16. Plowman, R. E., W. C. Stonebridge, and J. N. Hawtree. 1980. Fluazifop-butyl – A new selective herbicide for the control of annual and perennial grass weeds. Proc. Br. Crop Prot. Conf. – Weeds. 1:29–37.
17. Putnam, A. R. and D. Penner. 1974. Pesticide interactions in higher plants. Residue Rev. 50:73–110.
18. Reilly, J. J. and L. D. Moore. 1982. Influence of selected herbi-

cides on ozone injury in tobacco (*Nicotiana tabacum*). Weed Sci. 30:260–263.
19. Rich, S. 1975. Interactions of air pollution and agricultural practices. Pages 335–360 *in* J. B. Mudd and T. T. Kozlowski, eds. Responses of Plants to Air Pollution. Academic Press, NY.
20. Rosser, S. W., P. S. Zorner, W. W. Witt, and G. L. Olson. 1982. Uptake of fluazifop-butyl and accumulation of its free acid metabolite in johnsongrass. Proc. South. Weed Sci. Soc. 35:339.
21. Steel, R.G.D. and T. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, NY. 481 pp.
22. Sung, S. S. and L. D. Moore. 1979. The influence of three herbicides on the sensitivity of greenhouse-grown flue-cured tobacco (*Nicotiana tabacum*) plants to ozone. Weed Sci. 27:167–173.
23. Tingey, D. J. 1974. Ozone induced alteration in the metabolite pools and enzyme activities of plants. Pages 40–57 *in* W. M. Dugger, ed. Air Pollution Effects on Plant Growth. Am. Chem. Soc. Symp. Ser., No. 3. Am. Chem. Soc. Washington, DC.
24. Todd, G. W. 1972. Water deficits and enzyme activity. Pages 177–216 *in* T. T. Kozlowski, ed. Deficits and Plant Growth, Vol. III. Academic Press, NY.
25. Yang, Y. S. and B. I. Chevone. 1982. Characterization of ambient oxidant pollutants in the Blue Ridge mountains of Virginia. Phytopathology 72:712 (Abstr.).

Weed Science. 1983. Volume 31:861–865

Response of Wheat (*Triticum aestivum*) and Rotation Crops to Chlorsulfuron[1]

BILL D. BREWSTER and ARNOLD P. APPLEBY[2]

Abstract. Chlorsulfuron {2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl] benzenesulfonamide} at rates of 140 g ai/ha and lower did not reduce wheat (*Triticum aestivum* L. 'Stephens') grain yield, but soil residues from 35 g/ha injured snap beans (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L. 'DuPuits'), sweet corn (*Zea mays* L. 'Jubilee'), Italian ryegrass (*Lolium multiflorum* Lam.), sugarbeets (*Beta vulgaris* L. 'Giant Western'), and rape (*Brassica napus* L. 'Dwarf Essex'). Chlorsulfuron at 35 g/ha reduced foliage weight of sugarbeets seeded 26 months after application. Phytotoxic levels of the herbicide were present 10 to 20 cm deep, 168 days after application to a silt loam soil. Chlorsulfuron reduced alfalfa petiole length and fresh weight in the greenhouse when mixed in soil at rates as low as 0.025 ppb (w/w).

Additional index words. Herbicide persistence, herbicide soil residues, bioassay.

INTRODUCTION

Chlorsulfuron is a broad-spectrum herbicide which selectively controls many annual weeds and Canada thistle [*Cirsium arvense* (L.) Scop. #[3] CIRAR] in fall-sown small grains (2, 4, 9, 10, 12, 13, 14). However, several research workers have found injury to succeeding crops from chlorsulfuron residues in the soil. In Idaho, Thill (16) showed that several crops, except spring wheat, were injured 1 yr following a 10 g/ha application. Evans and Gunnell (4) in Utah reported that barley (*Hordeum vulgare* L.) stand was reduced 20 to 80% when seeded 1 yr after chlorsulfuron applications of 35 to 140 g/ha on spring barley. Norris et al. (10) in California found several crops to be sensitive to chlorsulfuron residues.

Since crop rotation is a common practice in many diversified farming areas, herbicide residues in the soil are of concern. The purpose of this study was to determine tolerance levels of winter wheat to chlorsulfuron, and to investigate the effect of chlorsulfuron soil residues on rotation crops by field and greenhouse bioassays.

MATERIALS AND METHODS

Field experiments were conducted at Corvallis, Oregon, during 1978 to 1980. The soil was a Woodburn silt loam, a member of the fine-silty, mixed-mesic Aquualtic Argixerolls. Soil pH was 5.8, organic matter was 2.8%, and cation exchange capacity was 20 meq/100 g. The soil contained 14% sand, 70% silt, and 16% clay.

The climate in Corvallis is characterized by mild, rainy winters and cool, dry summers. Rainfall and temperature means are presented in Table 1. Rotation crops were sprinkler irrigated at a rate of approximately 25 mm/week.

[1] Received for publication November 2, 1982 and in revised form June 20, 1983. Contribution of the Oregon Agric. Exp. Stn., Oregon State Univ., Tech. Paper No. 6599.

[2] Sr. Instr. and Prof., respectively, Crop Sci. Dep., Oregon State Univ., Corvallis, OR 97331.

[3] WSSA-approved computer code from Important Weeds of the World, 3rd ed., 1983. Available from WSSA, 309 West Clark St., Champaign, IL 61820.

ozone fumigation best in Sandy Soils

Hayes (2000)

Small Business Innovation Research

Final Report: Ozone Biocidal Properties and Stimulation of Trichoderma Harzianum (strain T-22) when Applied in Combination as an Environmentally Benig Alternative for Methyl Bromide

EPA Grant Number: 68D99035

Title: Ozone Biocidal Properties and Stimulation of Trichoderma Harzianum (strain T-22) when Applied in Combination as an Environmentally Benig Alternative for Methyl Bromide

Investigators: Christopher Hayes

Institution: BioWorks, Inc.

EPA Project Officer: Dr. Jim Gallup

Project Period: 10/1/99 - 11/30/00 (including extensions)

Project Amount: $70,000

Research Category: Pollution Prevention

Project Summary:

Project Description:

The purpose of the research was to examine the use of a commercially available biofungicide in conjunction with ozone as a possible replacement for Methyl Bromide. Methyl Bromide use is rapidly being phased out in agriculture because of concerns about its long-term effects on the environment. By 2005, Methyl Bromide will no longer be commercially available. Alternatives need to be tested to determine the most effective options for the grower.

In this study, ozone and a commercially available biofungicide were used in various combinations to determine their effectiveness as a replacement for Methyl Bromide. One or two applications of ozone at various concentrations applied alone or in combination with a registered biofungicide was used to treat strawberry plots at two sites. The parameters used to measure effectiveness included yield and total plant death, as a measurement of disease control. The chemical, Telone, as well as nontreated plots were added as a check.

The gas ozone was applied either once, as a pre-plant treatment, or applied a second time during midseason. The amount of ozone used varied, from low (25 lbs./A) to medium (100 lbs./A) to high amounts (400 lbs./A). Two different formulations, a granular and a wettable powder, of a commercially available biofungicide, Trichoderma harzianum, were used with the various ozone applications.

The overall objectives of the research were to examine the conditions under which ozone and a commercially-produced biofungicide would protect against soil pathogens in strawberry plots. Originally, the following research objectives were proposed:

1. Determine the optimal range of rates under which the biofungicide was stimulated by ozone applications.
2. Compare ozone and a commercial biofungicide combination against replacement chemicals to be used in strawberry production.
3. Perform laboratory surveys of the possible effects of ozone and the biofungicide on other soilborne microflora.
4. Extrapolate the field trial results to verify the economics of the process on a larger scale.

Summary of Findings:

In general, a limited positive yield response was observed with the biofungicide applied alone when compared to untreated control. One application of ozone, in conjunction with the biofungicide, showed a uniformly positive response in No. 1 berry yields and total yields compared to untreated controls. Midseason applications of ozone showed a positive response when compared to untreated controls at one site, but had a negative response when compared to untreated controls at the other site. Telone treatments consistently gave the best yields, both in No. 1 berry yields and total yields, regardless if the biological was applied. Telone treatments more than doubled yields compared to untreated controls.

The single application of ozone caused only a slight decline in microbial populations of aerobic bacteria, anaerobic bacteria, actinomycetes, nitrogen-fixing bacteria, yeasts and molds, and Verticillium spp. The biofungicide was found to be present on the roots, but its concentration was low. The concentration of the biofungicide did not increase with a mid-season ozonation, which contrasts to what has been observed in past studies. Plant death was reduced for most treatments when compared to nontreated controls. The least amount of plant death was recorded on the Telone treated plots.

Conclusions:

Tests were conducted to determine if ozone, when used as a biocide, followed by a commercial biofungicide could be used as an alternative to methyl bromide. In the past, this combination of products has been shown to be highly effective. The chemical, Telone, was included in the test as a control since growers have commercially used it. Ozone was applied at three different rates either once or again during mid-season. In all plots, biofungicide application followed

ozonation. The biofungicide was applied as a granule in the planting hole, or roots were dipped into a dry powder before placement in the field.

In all cases, yield and percentage plant survival was greatest in the chemical treated plots. Some of the plots receiving ozone and the biofungicide also gave yield increases and increased plant survival when compared to the untreated controls, but fell short of the levels recorded for the chemical.

Root washings demonstrated that the biofungicide had colonized the roots by both methods of application. Root washings after mid-season ozonation showed no increase in the level of the biofungicide on the roots, a direct contradiction to earlier trials. Using ozone alone or in conjunction with the biofungicide were not as favorable as we have seen in prior years' trials at the same site. We believe the differences were primarily due to the much lower ozone injection flow rate used when injecting ozone in this year's trials. This year ozone was injected at the rate of 30 cfh/20 ft of bed through 0.5 gph drip tubing. In prior years' trials in Watsonville, ozone was injected at the rate of 280 cfh/20 ft. of bed through "modified" 4.0 gph tubing. It was decided to use lower flow rates at MBA this year based on the desire to integrate the ozone technology with current farming practices which primarily use 0.5 gph tubing or tape for irrigation. Also, 3 straight years of successful trials injecting ozone at these lower flow rates in sandy soils in tomato, carrot, and broccoli trials have been done in trials conducted with Dr. Becky Westerdahl of UCD at the UC Extension Research Station in Irvine California. Laboratory trials had also been conducted that indicated that lower ozone gas flow rates were equivalent to higher flow rates in killing nematodes in sandy soils. However, all of the work was done with nematodes and in very sandy soils. Also, in retrospect it was observed that all of these crops are fairly rapidly growing compared to strawberries. It may very well be that the zone of effect of ozone has to be much larger in strawberries to provide a longer period of protection and this could not be completely provided with the lower flow rates used this year.

Since the biofungicide is most effective as a preventative against soil phytopathogenic fungi, it must be used in conjunction with something else especially in high disease conditions, which occurred at the test sites. Record rainfall during the trial period favored the development of high disease pressure. This, in combination with suboptimal kill by the ozonation, reduced protection by the biofungicide.

In this study, ozone was used to first reduce or eliminate most of the microflora in the plots. Following ozonation were treatments with the biofungicide. In most cases, this combination

gave increased yields and a decrease in plant loss when compared to the nontreated plots. In all cases, the chemical treated plots had better yields and lower plant loss than the combination treatment. In lab studies, it was shown that the use of the smaller diameter pipe restricted ozone movement in the soil. Improper initial knock-down, followed by the high disease pressure created by the excessive rain observed after planting were too much for the combination technique. Even the chemical plots experienced plant loss due to high disease pressure.

The results of this study suggest that the combination technique employed in this study is a viable alternative to the commercial use of Methyl Bromide. Indeed, they warrant additional research to determine the optimum method to introduce ozone into the soil profile and to optimize the amount of the biofungicide needed in field situations. Passed performance with the ozone/biofungicide combination has been shown to be effective at controlling root rot and increasing yield. Additional studies are needed to optimize the system.

Publications:
None

Commercialization Keywords:
Biocontrol, Methyl Bromide, Ozonation, Strawberries, Telone

Subject: ozone as an antimicrobial agent, crop production aid
Date: **Wed, 13 Feb 2002** 10:05:20 -0600

From: **Michael Herman** <mrherman@hamilton.net>
To: Kim Burton <Kim.Burton@jmsmucker.com>,Owusu Obandele <obandele@subr.edu>,Robert Pooler Bob.Pooler@usda.gov

CC: Emily Brown Rosen <ebr@omri.org>,"info@omri.org" <info@omri.org>, "David.Miesbach@NDEQ.State.NE.US" <David.Miesbach@NDEQ.State.NE.US>, Andy Christiansen <achristiansen1@unl.edu>, Chris Arnold <Carnold@t-tape.com>, Ernie Wilmink <keywater@worldnet.att.net>, Freddie Lamm <flamm@oznet.ksu.edu>

Kim Burton, National Organic Standards Board, Materials Committee Chair
Owusu Obandele, National Organic Standards Board, Crops Committee Chair
Robert Pooler, National Organic Program, USDA

I would like to request that you approve the use of ozone as an antimicrobial agent under the classification 'crop production aid', specifically as it pertains to the maintenance of a subsurface drip irrigation system in an organic production system. As I have looked for an effective and compliant maintenance program, I sense that the subsurface drip irrigation system has not been considered by the organic community. Therefore, I am submitting the following for your review and consideration.

PREVENTIVE MAINTENANCE FOR SUBSURFACE DRIP IRRIGATION

When using subsurface drip irrigation, preventive maintenance is highly recommended simply because one cannot visually inspect emitters, and the major cause of failure is clogging of the emitters. There are three categories of clogging: physical, chemical and biological. The physical is addressed by a filtering system. The chemical is addressed by acids. The biological is addressed by chlorine. The recommendation by Kansas State University is if the microbiological load is high, a low concentration of 1 to 2 ppm of chlorine should be injected continuously. If the load is not high, a shock treatment of 10 to 30 ppm is recommended.

In our particular case, the source of water is a well and it meets and surpasses the EPA requirement for drinking water. The engineer, Chris Arnold, has recommended, as a preventative measure, to shock treat at the end of the irrigation season. As the National List now stands, chlorine is acceptable as long as it meets the requirement of the Safe Drinking Water Act, which is no more than 4 ppm. Am I to assume that I could continuously inject up to 4 ppm throughout the irrigation season, but I could not inject 10 ppm for 13 minutes at the end of the irrigation season? No one has recommended the use of the other approved crop production aids for cleaning subsurface drip irrigation systems because of the question of their effectiveness. In my search of the Safe Drinking Water Act, I have also discovered that there is an Underground Injection Control Program that is regulated by the states, in our case the Nebraska Department of Environmental Quality, and a subsurface drip irrigation system falls under the UIC program. In addition, chlorine is considered a pesticide and is also under the Chemigation Program. As of yet, I have had no decision from the NDEQ on the use of chlorine or ozone.

A final ruling by the FDA in June of 2001 now allows the use of ozone as an antimicrobial agent for food treatment, storage and processing. It is considered an alternative to the use of chlorine and chlorine

dioxide as an antimicrobial agent. Municipalities are switching from chlorine to ozone for treatment in their drinking water and swimming pools.

Michael R. Herman, The Grain Place, Inc., 1904 North Highway 14, Marquette, NE. 68854, 402-854-3195

cc:
Freddie Lamm, Research Agricultural Engineer, KSU Northwest
Research-Extension Center, 105 Experiment Farm Road, Colby, Kansas USA
67701-1697, Phone: 785-462-6281 FAX: 785-462-2315, E-Mail:
flamm@oznet.ksu.edu, SDI Website: http://www.oznet.ksu.edu/sdi/

Chris Arnold, T-Systems International, 12180 East Iowa Dr., Aurora, CO.80012 970-378-0247,
Mobile 303-882-0209, Carnold@t-tape.com

T-Systems International, 7545 Carroll Road, San Diego, Ca. 92121-2401,800-765-1860 www.t-tape.com

Nebraska Department of Environmental Quality, David Miesbach,
David.miesbach@NDEQ.State.NE.US, 402-471-2186
Ernie Wilmink, 'The Key' Water and Air International, Inc., West Highway 91,Lindsay, Ne. 68644, 800-539-6220, consultant in Custom Ozone Technology keywater@worldnet.att.net

ohioline.ag.ohio-state.edu -- your Link to information, News, and Education

Bulletin

Ornamental Plants -- Annual Reports and Research Reviews 1999

Special Circular 173-00

New Approaches to Control of Plant Pathogens in Irrigation Water

Harry A. J. Hoitink and Matthew S. Krause

Dispersal of plant pathogens such as *Pythium* and *Phytophthora* spp. with irrigation water has caused problems in nurseries for decades. Many procedures have been tested to control this problem. They include chlorination, ozone treatment, heating, ultraviolet (UV) irradiation and others. None of these procedures has been adopted successfully in nurseries. The volume of water used in nurseries simply is too large for some methods. Other methods have proved ineffective or have even aggravated the problem.

In the late 80s, a very old but effective procedure known as slow sand filtration was introduced into nurseries and greenhouses in Geisenheim, Germany, through the research of Dr. W. Wohanka. He determined that this system effectively filtered bacteria and fungi from irrigation water. Several plant pathologists from Europe and Australia have verified the effectiveness and the practical value of this filtration procedure for nursery irrigation systems.

Slow-sand-filtration systems that filter 200,000 gals/day are being used in nurseries today. In Germany, a survey of growers revealed that the quality of nursery crops was improved after these filters had been installed. German and Dutch nurseries have used this technology extensively during the past five years. These nurseries, however, predominantly use container media prepared with Sphagnum peat. Peat media are conducive to Phytophthora root rots, and this allows for the development of high pathogen populations. Most American nurseries use media prepared with tree barks or other types of composted products, and these mixes, when formulated properly, suppress Phytophthora root rots. Unfortunately, these suppressive effects can be overcome by high salinity and this allows *Phytophthora* spp. to increase in populations.

During prolonged periods of dry weather, the volume of irrigation water available in nurseries often becomes limiting. In addition, the salinity goes up, and it is under these conditions that Phytophthora root rots become most serious. In conclusion, during periods of drought when high-in-salinity irrigation water is recycled onto crops susceptible to Phytophthora root rots, the potential for transmission of these pathogens with infested irrigation water to noninfected plants becomes a

problem. Slow sand filtration is designed to control this problem.

Crops in the Ericaceae family unfortunately are affected not only by root rots but also by Phytophthora dieback diseases. They affect the foliage, the stem, and less so, the root system. The *Phytophthora* spp. that cause these diseases – such as *P. cactorum, P. citricola, P. citrophthora, P. parasitica,* etc. – sporulate heavily on lesions during high-humidity and high-temperature (75°F or higher) weather conditions. They are spread from plant to plant as inoculum in splashing irrigation water droplets. The plant surface must be wet for at least two hours for infections to occur. Thus, under overhead irrigation, these pathogens can spread within crops even if filtered water were to be used. These dieback diseases do not occur in dry summers in nurseries when leaves dry quickly after overhead sprinkler irrigation.

One way to reduce the severity of Phytophthora die-back diseases is to produce such plants under drip irrigation. Ohio nurserymen have begun to produce rhododendron and lilac, which are highly susceptible to Phytophthora dieback, under drip irrigation in pot-in-pot systems (above ground as well as buried pot systems) using container media naturally suppressive to root rots. The crops also are sprayed with fungicides to further suppress these problems (see OSU Extension Fact Sheet HYG-3073-99 entitled *Control of Phytophthora and Other Major Diseases of Ericaceous Plants*). This integrated approach has reduced the severity of both types of diseases. The question now becomes whether slow sand filtration can reduce symptoms further in plants that do not show symptoms (latent infections). An answer to this question is not available presently. However, it seems that slow sand filtration should be applied to those crops most susceptible to Phytophthora root rot and dieback diseases. Furthermore, large plants of such crops should be produced under drip irrigation in ground beds or in pot-in-pot systems. Finally, fungicides described in the previously mentioned fact sheet should be applied correctly to further reduce these diseases. Other factors, such as avoidance of puddles on the base where containers are placed, pre-filtration, and settling of irrigation water, should also be included as part of the overall management program.

Principles of Slow Sand Filtration

The principle of a slow sand filter is very simple. Water must be treated before entering a filter to remove as much silt and organic matter as possible. The pre-treated water then is filtered very slowly through a deep bed of sand (Figure 1). The flow rate depends on the size of the microorganism that needs to be removed. For *Phytophthora* spores this means a flow rate of two to three gallons per square foot filter surface area per hour. An irrigation pond, within which a 1,000 sq-ft filter is installed in the deepest end, could filter 50,000 to 75,000 gals of water per day.

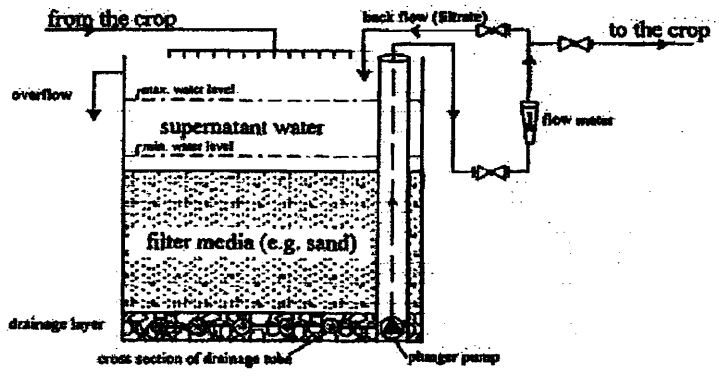


Figure 1. Diagram of a slow sand filter (adapted from W. Wohanka, Geisenheim, Germany).

Table1. Quality Requirements for Filter Sand (Adapted from W. Wohanka, Geisenheim, Germany).	
effective grain size	0.15-0.30mm (100 - 50 mesh)
uniformity coefficinet (UC)	< 3, maximum 5
silt content	< 1%
acid solubility	< 5 % after 30 min
effective grain size (d10): sieve opening through which 10% (by weight) of the grains will pass.	
uniformity coeffecient (UC): ratio between the sieve opening through which 60% (by weight) of the grains will pass and the effective grain size; UC = d60/d10	

Soon after the filtering process begins, a layer of brown material develops in the surface inch layer of the bed. *Pseudomonas, Trichoderma*, and other microorganisms already known as biocontrol agents in compost-amended container media, seem to slowly destroy the pathogens immobilized through filtration in this layer. Filters are not very effective until after this layer develops, and this requires several weeks. The layer must not be disturbed by water added to the top of the filter. Therefore, a three-feet or deeper layer of water is always maintained on top of the filter. Water must be added to the reservoir through a piping and sprinkler system that minimizes turbulence.

The depth of the sand bed should be three to four feet, and it must be at least two-feet deep. A depth of four-feet allows periodic cleaning of the filter by removal of the surface layer, if flow rates go down due to clogging. The type of sand that must be used is explained in Table 1. Underneath the sand are several layers of gravel and a drainage system. Rockwool (Grodan type 01251 or 012519) has performed better in nurseries than sand, because it becomes clogged less easily than sand filters. It is more costly, but gravel does not have to be used under Rockwool filters.

Below either type of filter, a system of conventional four-inch drainage tubes drains filtered water into a sealed concrete reservoir in the lowest point of the pond filtration system. Water is then pumped up out of this reservoir at a controlled flow rate to a storage facility or the irrigation system. A flow meter with feedback control is essential for effective operation of large pond systems.

The pump must always recirculate some water through the system when the irrigation system is not in use. Without circulation, anaerobic conditions develop in the filter, and this also destroys effectiveness.

The cost of slow sand filtration is very low as compared to other technologies. One California nursery has used a 200,000 gallon-per-day system for three years without problems and is installing a second to further reduce losses caused by plant pathogens for crops not yet treated with filtered water.

For information on *Phytophthora* control and slow-sand-filtration systems, see the references listed here.

1. Harry A. J. Hoitink and Stephen T. Nameth. 1999. *Control of Phytophthora and Other Major Diseases of Ericaceous Plants*. Ohio State University Extension Fact Sheet HYG-3073-99.

Web site:http://sardi.sa.gov.au/hort/floricul/curres.htm

2. **Web site giving additional design criteria for slow sand filtration:**
   http://sardi.sa.gov.au/hort/floricul/curres.htm

3. **Wohanka, W. 1995. Disinfection of recirculating nutrient solutions by slow sand filtration.** *Acta Horticulturae* **382:245-255.**

Back | Forward | Table of Contents

Kirk Othmer 4th ED Kroschwitz JI

absorbed by aqueous KI.

$$O_3 + 2I^- + H_2O \longrightarrow I_2 + O_2 + 2OH^-$$

The liberated iodine is measured spectrometrically or titrated with standard sodium thiosulfate solution ($I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$) following acidification with sulfuric acid; buffers are sometimes employed. The method requires measurement of the total gas volume used in the procedure. The presence of other oxidants, such as H_2O_2 and NO_x, can interfere with the analysis. The analysis is also technique-sensitive, since it can be affected by a number of variables, including temperature, time, pH, iodide concentration, sampling techniques, etc (140). A detailed procedure is given in Reference 141.

Ozone in the gas phase can be determined by direct uv spectrometry at 254 nm via its strong absorption. The accuracy of this method depends on the molar absorptivity, which is known to ±1%; interference by CO, hydrocarbons, NO_x, or H_2O vapor is not significant. The method also can be employed to measure ozone in aqueous solution, but is subject to interference from turbidity as well as dissolved inorganics and organics. To eliminate interferences, ozone sometimes is sparged into the gas phase for measurement.

Various colorimetric methods have been employed for measuring ozone residuals, although most of these are susceptible to significant interferences (142). The indigo trisulfonate method (143), however, has been approved by the Standard Methods Committee of the American Public Health Association (141) and the International Ozone Association for ozone residual measurement.

Electrochemical methods can involve the amperometric titration of liberated iodine with phenylarsine oxide, using a rotating Au/Cu electrode or excess phenylarsine oxide, and measuring the excess by direct pulse polarography (144). Ozone also can be measured directly by reduction to O_2 on a rotating Au/Cu electrode or a Ni/Ag–AgCl electrode pair (145). To eliminate interferences, membrane cells have been developed that consist of a semipermeable membrane such as Teflon or dimethyl silicone and a suitable sensor (146). Ozone diffuses into the cell through the porous membrane and is measured polarographically by employing electrode combinations such as gold–calomel or Au/Ag–AgCl. ORP cells consist of an indicating and a reference electrode combination, which generates a potential proportional to the ozone concentration. They are typically employed as a monitor or controller in water treatment applications.

Chemiluminescent analyzers are based on the light (chemiluminescence) emitted in the gas-phase reaction of ozone with ethylene, which is measured with a photomultiplier tube. The resulting current is proportional to the ozone concentration (see LUMINESCENT MATERIALS, CHEMILUMINESCENCE).

Laboratory, portable, and rugged industrial ozone meters employing uv, amperometry, or chemiluminescence are available for continuous or semicontinuous analysis of either gaseous or aqueous ozone.

Health and Safety

Depletion of the Ozone Layer. As a constituent of the atmosphere, ozone forms a protective screen by absorbing radiation of wavelengths between 200

and 300 nm, which can damage DNA and be harmful to life. Consequently, a decrease in the stratospheric ozone concentration results in an increase in the uv radiation reaching the earth's surfaces, thus adversely affecting the climate as well as plant and animal life. For example, the incidence of skin cancer is related to the amount of exposure to uv radiation.

Laboratory studies in 1974 (76,147) indicated that chlorine radicals from photodegradation of CFCs (chlorofluorocarbons) can destroy ozone. Because of these studies and the fact that the two main varieties ($CFC\text{-}11(CFCl_3)$ and $CFC\text{-}12(CF_2Cl_2)$) can persist in the atmosphere for 75–100 years, the United States in 1978 banned their use in aerosols such as hair sprays and certain deodorants. However, pressure to eliminate CFCs slackened until 1985, when the Antarctic ozone hole was discovered (148). The ozone hole is a dramatic seasonal thinning of the normal ozone concentration over the continent of Antarctica, attributed to catalytic decomposition of ozone by halogen radicals ($Cl^{\bullet}$ and $Br^{\bullet}$), which are formed by photodecomposition of CFCs and Halons (bromofluorocarbons). A similar but less severe reduction in ozone levels in the Arctic also has been observed. Reduced ozone levels extend to the lower latitudes and can pose a threat to human, animal, marine, and plant life as a result of increased energetic uv–B radiation (280–320 nm) reaching the surface of the earth. Data from Antarctica show that uv radiation soars under the ozone hole, where fully half of the atmospheric ozone is destroyed each spring. The global drop in amphibian population such as frogs, toads, and salamanders has been ascribed to increased uv radiation from ozone thinning (149).

Confirmation of the destruction of ozone by chlorine and bromine from halofluorocarbons has led to international efforts to reduce emissions of ozone-destroying CFCs and Halons into the atmosphere. The 1987 Montreal Protocol on Substances That Deplete the Ozone Layer (150) (and its 1990 and 1992 revisions) calls for an end to the production of Halons in 1994 and CFCs, carbon tetrachloride, and methylchloroform by January 1, 1996. In 1993, worldwide production of CFCs was reduced to 50% of 1986 levels of 1.13×10^6 and decreases in growth rates of CFC-11 and CFC-12 have been observed (151).

In order to meet the established goals, the industry has accelerated efforts to find alternatives to CFCs (152). In 1988, the first International CFC & Halon Alternatives Conference was held for the purpose of sharing technology. The U.S. industry is switching to hydrofluorocarbons (HFCs), which do not contain chlorine or bromine that can decompose ozone, for use in compressors of home refrigerators and almost all new car and truck air-conditioning systems, and to hydrochlorofluorocarbons (HCFCs) for industrial refrigeration units and in foam-blowing applications. The Montreal Protocol classifies HCFCs as transitional, setting a limit on their production in 1996 and gradually phasing them out. There is some concern that HCFCs may slow the ozone layer recovery (153). Under the stratospheric ozone protection provision of the Clean Air Act, the EPA has issued its final rule regarding the evaluation and regulation of ozone-depleting substitutes (154) (see FLUORINE COMPOUNDS, ORGANIC–FLUORINATED ALIPHATIC COMPOUNDS).

A smaller factor in ozone depletion is the rising levels of N_2O in the atmosphere from combustion and the use of nitrogen-rich fertilizers, since they

are the sources of NO in the stratosphere that can destroy ozone catalytically. Another concern in the depletion of ozone layer, under study by the National Aeronautics and Space Administration (NASA), is a proposed fleet of supersonic aircraft that can inject additional nitrogen oxides, as well as sulfur dioxide and moisture, into the stratosphere via their exhaust gases (155). Although sulfate aerosols can suppress the amount of nitrogen oxides in the stratosphere by converting N_2O_5 to HNO_3, the actual effect depends on where in the atmosphere the plane's exhaust gases finally accumulate.

Environmental Impact of Ambient Ozone. Ozone can be toxic to plants, animals, and fish. The lethal dose, LD_{50}, for albino mice is 3.8 ppmv for a 4-h exposure (156); the 96-h LC_{50} for striped bass, channel catfish, and rainbow trout is 80, 30, and 9.3 ppb, respectively. Small, natural, and anthropogenic atmospheric ozone concentrations can increase the weathering and aging of materials such as plastics, paint, textiles, and rubber. For example, rubber is degraded by reaction of ozone with carbon–carbon double bonds of the rubber polymer, requiring the addition of aromatic amines as ozone scavengers (see ANTIOXIDANTS; ANTIOZONANTS). An ozone decomposing polymer (noXon) has been developed that destroys ozone in air or water (157).

Although the naturally occurring concentration of ozone at earth's surface is low, the distribution has been altered by the emission of pollutants, primarily by automobiles but also from industrial sources, which lead to the formation of ozone. The strategy for controlling ambient ozone concentrations arising from automobile exhaust emissions is based on the control of hydrocarbons, CO, and NO via catalytic converters. As a result, peak ozone levels in Los Angeles, for instance, have decreased from 0.58 ppm in 1970 to 0.33 ppm in 1990, despite a 66% increase in the number of vehicles.

The EPA is reviewing and revising the Air Quality and Other Photochemical Oxidants (Criteria Document) and reevaluating the national ambient air quality standards (158). The EPA is also proposing that manufacturers ($\geq$10,000 lb O_3/yr) and users ($\geq$25,000 lb O_3/yr) submit data annually to the EPA on estimated ozone releases (159).

Human Exposure to Ozone. The toxicity of ozone is largely related to its powerful oxidizing properties. The odor threshold of ozone varies among individuals but most people can detect 0.01 ppm in air, which is well below the limit for general comfort. OSHA has established a time-weighted average permissible exposure level for workers for an eight-hour day of 0.10 ppm v/v (0.2 mg/m^3) and a short-term exposure limit of 0.30 ppm v/v (0.6 mg/m^3) for an exposure less than 15 minutes (160). The latter is based on observations showing that significant declines in pulmonary function can result from repeated intermittent exposures or from a single short-term exposure to ozone. The toxicity of gaseous ozone varies with concentration and exposure time (161). The symptoms experienced on exposure to 0.1–1 ppm ozone are headache, throat dryness, irritation of the respiratory passages, and burning of the eyes caused by the formation of aldehydes and peroxyacyl nitrates. Exposure to 1–100 ppm ozone can cause asthma-like symptoms such as tiredness and lack of appetite. Short-term exposure to higher concentrations can cause throat irritations, hemorrhaging, and pulmonary edema. Additional toxicity data is given in Reference 162.

The presence of naturally occurring ozone in the lower stratosphere creates a potential hazard for passengers and crew members of high flying aircraft (163,164). Ozone in the inlet air to the aircraft cabin, which can reach 1.2 ppm, is destroyed catalytically.

Ozone Disinfection By-Products. Ozonation of drinking water produces various by-products such as aldehydes, ketones, carboxylic acids, organic peroxides, epoxides, nitrosamines, *N*-oxy compounds, quinones, hydroxylated aromatic compounds, brominated organics, and bromate ion. Although some of these compounds are potentially toxic or carcinogenic, most bioassay-screening studies have shown that ozonated water induces substantially less mutagenicity than chlorinated water (165–167). However, further work is necessary to identify and screen (Ames test) ozonation by-products formed under typical water treatment conditions (168). Ozonation by-products are on the Drinking Water Priority List as candidates for future regulation (169). The Disinfection and Disinfection By-Products Rule proposed by the U.S. EPA will set limits for both disinfectants (excluding ozone) and disinfection by-products (eg, bromate) and require biofiltration following ozone use (118).

BIBLIOGRAPHY

"Ozone" in *ECT* 1st ed., Vol. 9, pp. 735–753, by V. A. Hann and T. C. Manley, The Welsbach Corp.; in *ECT* 2nd ed., Vol. 14, pp. 410–432, by T. C. Manley and S. J. Niegowski, The Welsbach Corp.; in *ECT* 3rd ed., Vol 16, pp. 683–713, by C. Nebel, PCI Ozone Corp.

1. C. Schönbein, *Compt. Rend. Hebd. Seances Acad. Sci.* **10**, 706 (1840).
2. A. G. Streng, *J. Chem. Eng. Data* **6**, 431 (1961).
3. D. Hanson and K. Mauersberger, *J. Chem. Phys.* **83**, 326 (1985).
4. L. F. Kosak-Channing and G. R. Helz, *Environ. Sci. Technol.* **17**, 145 (1983).
5. J. A. Roth and D. E. Sullivan, *Ind. Eng. Chem. Fundam.* **20**, 137, (1981); V. Tarinina and co-workers, *Zh. Obshch. Khim.* **53**, 1441 (1983); R. R. Munter, Deposited Doc. *VINITI*, 3996–3984 (1984).
6. R. Battino, ed., *Oxygen and Ozone, IUPAC Solubility Series*, Vol. 7, Pergamon, Oxford, U.K., 1981.
7. L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis*, John Wiley & Sons, Inc., New York, Vols. 1–17.
8. JANAF Thermochemical Tables, *J. Phys. Chem. Ref. Data* **14**(Suppl. 1) 1695 (1985).
9. J. A. Dean, ed., *Lange's Handbook of Chemistry*, 13th ed., McGraw-Hill Book Co., Inc., New York, 1985.
10. G. Hettner, R. Pohlman, and H. J. Schumacher, *Z. Physik* **91**, 372 (1934).
11. R. Atkinson and co-workers, *J. Phys. Chem. Ref. Data* **21**, 1125 (1992).
12. M. L. Kilpatrick and co-workers, *J. Am. Chem. Soc.* **78**, 1784 (1956).
13. R. Tramborubo and co-workers, *J. Chem. Phys.* **21**, 851 (1953).
14. M. Mack and J. S. Muenter, *J. Chem. Phys.* **66**, 5279 (1977).
15. P. C. Hiberty, *Israel J. Chem.* **23**, 10 (1983); R. D. Harcourt and co-workers, *J. Chem. Soc. Faraday Trans.* **82**, 495 (1986); P. Borowski, and co-workers, *J. Chem Phys.* **97**, 5568 (1992); W. Wu and co-workers, *Chin. J. Chem.* **11**, 490 (1993).
16. D. L. Cooper and co-workers, *J. Chem. Soc. Perkin Trans.* **2**, 1187 (1989); D. V. Kostikova and co-workers, *Dokl. Akad. Nauk* **296**, 914 (1987).
17. A. Glissman and H. J. Schumacher, *Z. Physik. Chem.* **21B**, 323 (1933).
18. J. A. Zaslowsky and co-workers, *J. Am. Chem. Soc.* **82**, 2682 (1960).

CONSULTANT REPORT

PIER
Public Interest Energy Research
CALIFORNIA ENERGY COMMISSION

Industrial/Agricultural/Water
End-Use Energy Efficiency

INTEGRATED AGRICULTURAL TECHNOLOGIES DEMONSTRATIONS

Larsen 1999

100pp

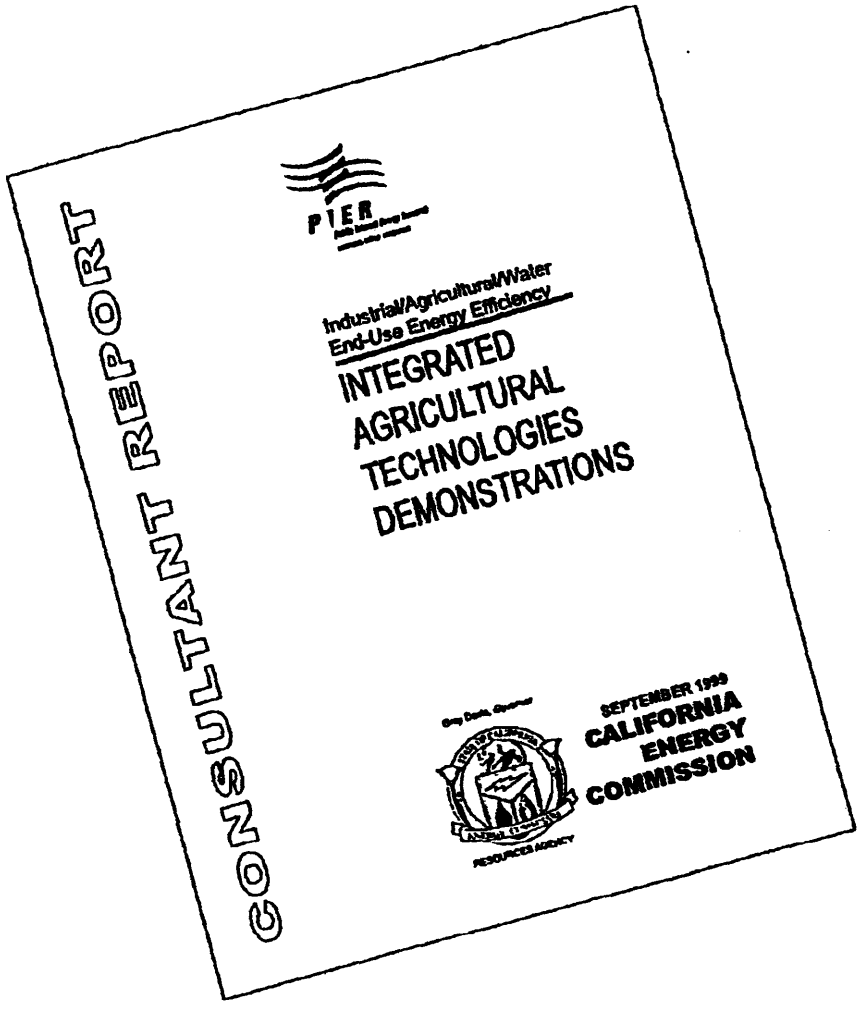
Gray Davis, *Governor*

SEPTEMBER 1999

CALIFORNIA ENERGY COMMISSION

RESOURCES AGENCY

P600-00-012



CALIFORNIA ENERGY COMMISSION

Prepared for:
CALIFORNIA ENERGY COMMISSION

Prepared by:
Lory E. Larson
EDISON TECHNOLOGY SOLUTIONS
Irwindale, CA

Contract No. 500-97-012
Project No. 03

Contract Amount: $320,000

Ricardo Amon, *Project Manager*
PLANNING AND PROCESS ENERGY OFFICE

Scott Matthews, *Deputy Director*
ENERGY EFFICIENCY DIVISION

Gary Klein, *Contract Manager*
ENERGY TECHNOLOGY DEVELOPMENT DIVISION

2.1.1 Project Objective

This project sought to demonstrate the effectiveness of ozone as a preplant soil fumigant to destroy a variety of soil-borne microorganisms. The objective of this research was to determine and demonstrate the efficacy of soil treatment with ozone in increasing yields in field trial scale applications in a geographically diverse variety of important California crops. Varying application dosages and duration were used in all trials and produce yield and quality from treated plots were compared to those from untreated control plots and, in some cases, plots treated with alternative fumigants. Where applicable, soil pathogen pressures and/or active soil-borne fungi and bacteria populations were determined and correlated with crop yield and treatment.

2.1.2 Project Approach

As a result of the pressing need to develop environmentally benign replacements to methyl bromide for soil fumigation, SoilZone, Inc. commenced field trials using ozone in 1997. This technology use root zone injection of ozone gas that was generated in the field, close to the injection site.

Based on the initial success of these independently evaluated trials involving carrots and tomatoes, SoilZone believed its ozonation technology had the potential to provide a sustainable and long-term alternative to methyl bromide for soil fumigation treatment. SoilZone subsequently requested and received matching research assistance contracts from the Electric Power Research Institute (EPRI) Agricultural Technology Alliance (ATA) and the California Energy Commission through Edison Technology Solutions (ETS) to perform ten field trials in California. Subsequently, field trials were conducted in conjunction with co-investigator Dr. Becky Westerdahl from the University of California, Davis (UCD).

Table 1 lists the crops, California location, research collaborators, and methods of ozone injection for these trials.

Table 1. Field Trial Collaborators

Crop	Test Location (California)	Research Collaborator	Ozone Injection Method
Tomatoes	Irvine	Dr. Becky Westerdahl, UCD	Buried Drip Tube
Tomatoes	Tulare	Edison AgTAC/EPRI-ATA	Buried Drip Tube
Carrots	Irvine	Dr. Becky Westerdahl,	Buried Drip Tube
Carrots	Tulare	Edison AgTAC/EPRI-ATA	Buried Drip Tube
Strawberries	Watsonville	Dr. John Duniway, UCD	Buried Drip Tube
Sugar Beets	Irvine	Dr. Becky Westerdahl, UCD	Buried Drip Tube
Broccoli	Santa Maria	Rancho Laguna Farms	Buried Drip Tube
Prune Replant	Orland	Steve Brown, Farmer	Injection Probe
Sweet Potatoes	Stevenson	Nakashima Farms	Buried Drip Tube
Peach Replant	Winton	Mallard Bend Farms	Injection Probe

All ozone injection through drip tubing used 1/2-inch PVC tubing with 12-inch emitter spacing. Tubing was buried 6 inches deep in bed centers except for strawberries where double injection tubes were used for each bed and buried 10 inches from bed edges.

The injection tubing used for the initial application was left in place throughout the duration of the trial and used for subsequent midseason applications of ozone in the Tulare carrot and tomato trials. In the Irvine tests, the same drip tubing was used both for ozone injection and for subsequent irrigation. Ozone for orchard replants was applied through a 1/2-inch steel injection probe with 3/8-inch emitter holes drilled between 8 and 18 inches in depth. A 6-foot square of plastic mulch was laid down over each injection site and sealed around the injector in the center and around the plastic edges with dirt. All applications used ozone produced in air unless otherwise indicated.

Table 2 tabulates the crop, ozone application method, and ozone application rate used for these trials.

Table 2. Ozone Application Methods

Crop, Location	Ozone Injection Method	Ozone Treatments (per acre or tree)
Tomatoes, Irvine	0.5 gallons per hour (gph) with 12 inch drip tube	250 lbs. O_3 with and without pre-irrigation
		250 lbs. O_3 in O_2
		50 lbs. O_3 w with and without 100 lbs. CO_2
Tomatoes, Tulare	4.0 gph with 12 inch drip tube	50 and 250 lbs. O_3
		50 lbs. O_3 with 1 x 25 lbs. midseason
Carrots, Irvine	0.5 gph with 12 inch drip tube	250 lbs. O_3 with and without pre-irrigation
		250 lbs. O_3 in O_2
		50 lbs. O_3 with and without 100 lbs. CO_2
Carrots, Tulare	4.0 gph with 12 inch drip tube	50 and 250 lbs. O_3
		50 lbs. O_3 with 2 x 15 lbs. midseason
		50 lbs. with 100 lbs. CO_2
Strawberries	4.0 gph with 12 inc. drip tube	400 lbs. O_3
		400 lbs. O_3 with 100 lbs. Trichoderma
Sugar Beets, Irvine	0.5 gph with 12 inch drip tube	250 lbs. O_3 with and without pre-irrigation
		250 lbs. O_3
		50 lbs. O_3 with and without 100 lbs. CO_2
Broccoli	2.0 gph with 12 inc. drip tube	50 and 250 lbs. O_3
Prune Replant	Probe Mulch	1.25 lbs. O_3/tree hole
Sweet Potatoes	2.0 gph with 12 inch drip tube	100 and 400 lbs. O_3
Peach Replant	Probe per mulch	1.25 lbs. O_3/tree hole

Plots were laid out in random blocks or in a manner ensuring equal spacing of different treatments from each other to minimize field edge effects. Table 3 tabulates the crop, plot size and number, moisture content of the receiving soil, and the ozone concentration used for this series of field trials.

Table 3. Treatment Block Size and Repetitions

Crop	No. of Repetitions and Plot Size Per Treatment	Application Soil (percent) Moisture	O_3 Concentration (percent w.w)
Tomatoes, Irvine	Six – 20 ft x 34 in	12 – 17	2.7 – 6.0
Tomatoes, Tulare	Six – 20 ft x 34 in	10 – 14	2.7 – 6.0
Carrots, Irvine	Six – 20 ft x 34 in	10 – 14	2.7 – 6.0
Carrots, Tulare	Six – 20 ft x 40 in	17-18	1.6 – 1.8
Strawberries	Three – 20 ft x 52 in	8-11	1.8 – 2.0
Sugar Beets	Six – 20 ft x 34 in	12 – 17	2.7 – 6.0
Broccoli	Six – 30 ft x 38 in	14-16	1.8 – 2.0
Prune Replant	Ten Trees – 20 ft on center	13-18	1.5 – 1.6
Sweet Potatoes	Six – 20 ft x 40 in	11.2	2.5 – 2.8
Peach Replant	Ten Trees – 20 ft on center	8-11	1.8 – 2.0

Field test results, for each of the crops involved, are reported in the following narrative.

2.1.2.1 1998 Irvine Tomato Field Trials

These trials were performed in a field heavily infested with root knot nematodes at the University of California South Coast Field Station in Irvine, California. The research was conducted in conjunction with Dr. Becky Westerdahl, University of California at Davis, Department of Nematology.

Ozone was injected in early July through underground drip tubing buried four to six inches deep in the center of 32-inch furrows. Various combinations of pre- and post-irrigation and application rates were used (Table 2).

Tomato seedlings were planted three weeks after the injection treatment and the total yield and number of root galls were compiled at the end of the September harvest. These results were compared to untreated controls and plots treated with other fumigants.

In these trials, yields from pre-irrigated plots treated with ozone at a rate of 250 lbs. per acre were approximately 44 percent higher than yields from the untreated control plots (Figure 1). The yield differential was equal to the metam sodium-treated plots and 17 percent greater than yields from the Telone-treated plots. Plots treated with ozone at 50 lbs. per acre with 100 lbs. per acre of carbon dioxide produced yields 30 percent greater than that of the untreated controls.

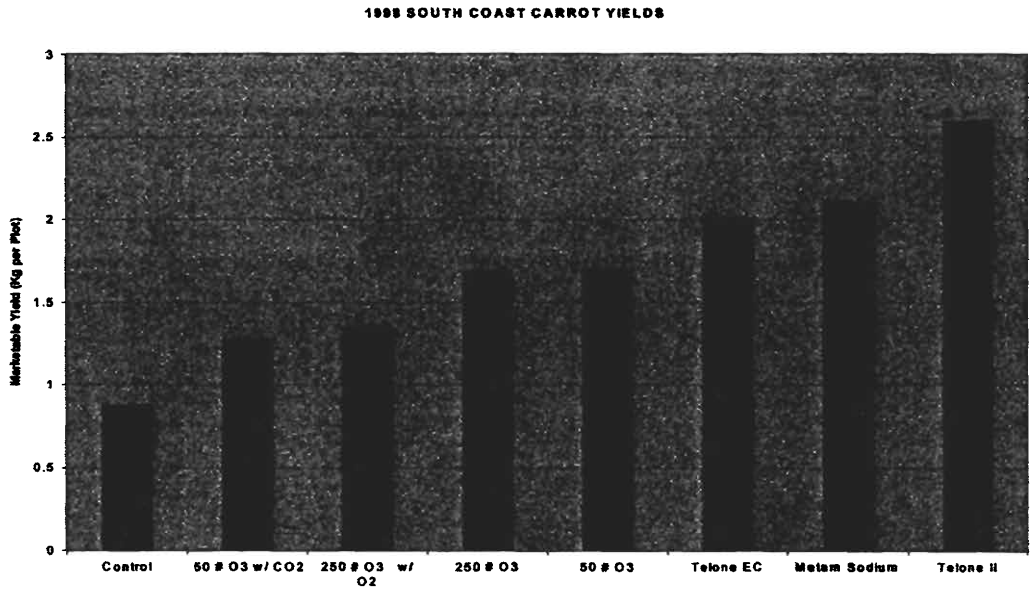


Figure 4. 1998 Irvine Field Trials – Marketable Carrot Yield

The total yield (including nematode damaged produce) was greatest in the 250 and 50 lbs. per acre ozonated plots possibly indicating increased nutrient uptake in these plots (Figure 5).

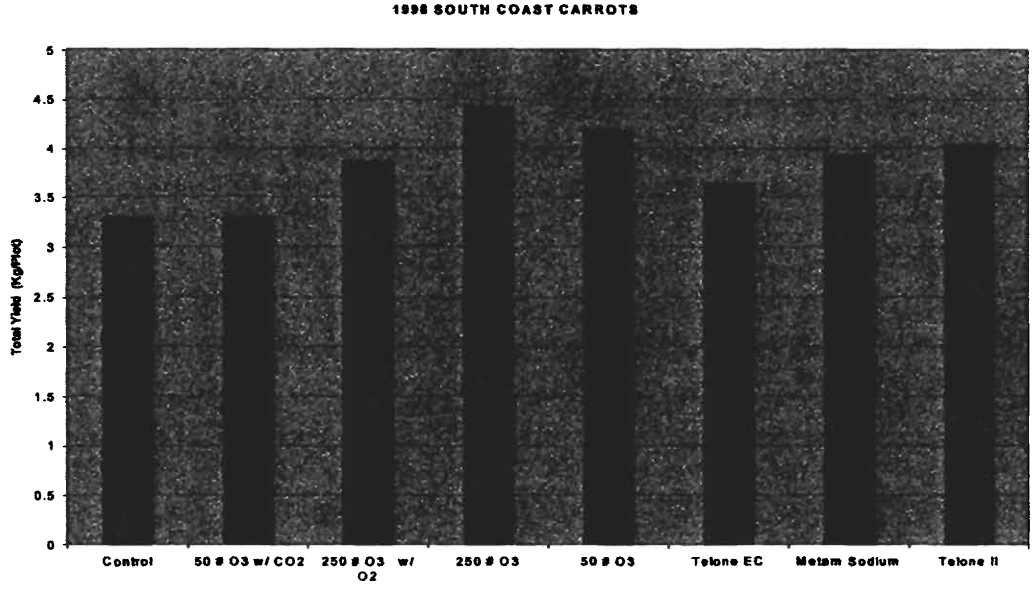


Figure 5. 1998 Irvine Field Trials – Total Carrot Yield

2.1.2.4 1998 Tulare Carrot Field Trials

These field experiments were performed, in conjunction with the EPRI, at the Southern California Edison AgTAC in Tulare, California, during the summer through winter of 1998.

Tomato seedlings were planted five days after treatment. An additional midseason application of 15 lbs. per acre was applied to half of the 50 lbs. per acre treatments and the total yields were compiled at the end of the harvest.

In these trials, single 50 lbs. per acre preplant ozone-treated plots experienced increased total fruit production of approximately 57 percent compared to untreated controls (Figure 3). Those plots, which also received a 15 lbs. per acre midseason dosage, had total marketable yield increases of 46 percent compared to yields in untreated control plots. The increased production with the absence of any soil-borne pathogen pressures again indicates a bio-stimulative component of soil ozonation.

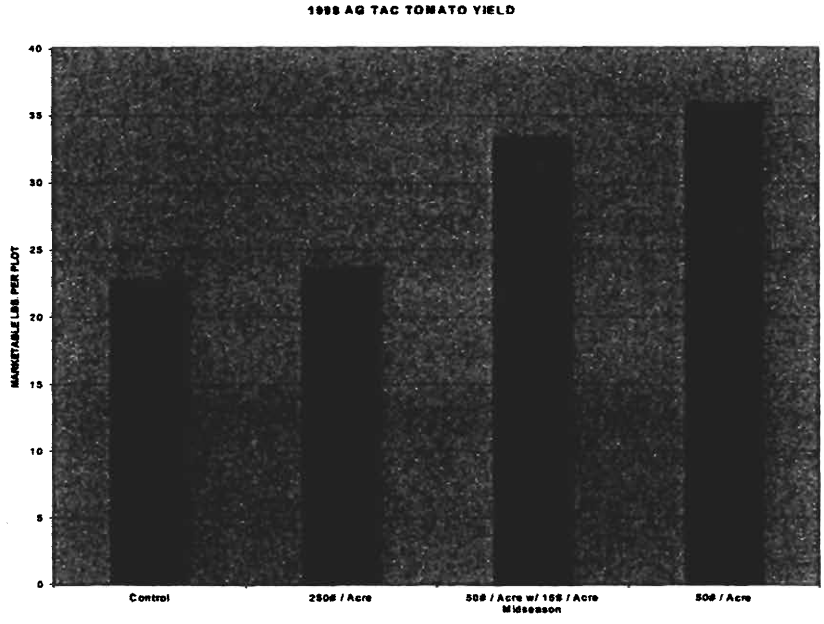


Figure 3. 1998 Tulare Field Trials – Tomato Yield

2.1.2.3 1998 Irvine Carrot Field Trials

These trials were performed in a manner similar to the tomato trials except that carrots were sown from seeds.

Upon harvest, plots that had been treated with either 50 or 250 lbs. per acre of ozone following pre-irrigation experienced a 92 percent increase in total marketable carrot yields compared to untreated controls (Figure 4). Ozone-reated plot production was only slightly less than Telone-emulsifiable concentrate and Vapam treated plots.

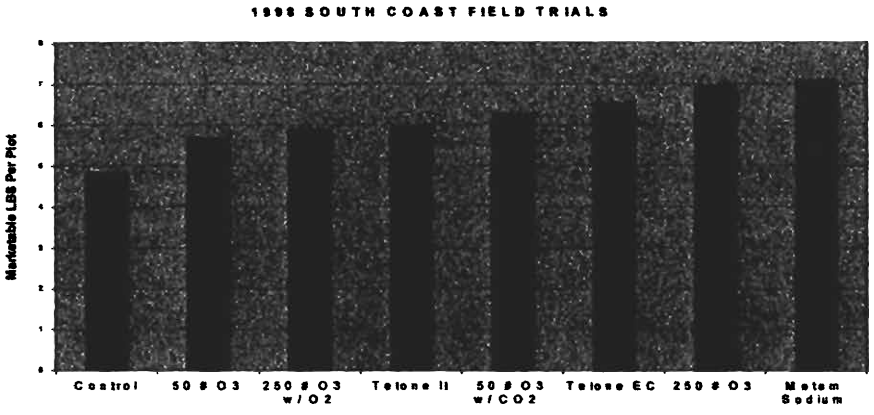


Figure 1. 1998 Irvine Field Trials – Tomato Yield

Nematode root galling was not appreciably lower in the ozone-treated plots than in the Telone-treated control plots despite the improved yields from the ozone treated plots (Figure 2). The increased yield may be the result of biostimulation; probably due to increased soil nutrient availability. These biostimulatory effects, combined with the biocidal aspects of ozone treatment are also important in plant yield.

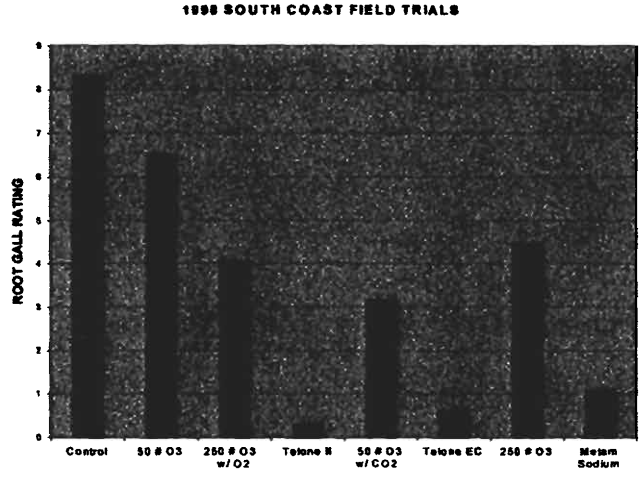


Figure 2. 1998 Irvine Field Trials – Tomato Root Galling

2.1.2.2 1998 Tulare Tomato Field Trials

Field experiments were also conducted during the spring and summer of 1998 at the Southern California Edison Agricultural Technology Application Center (AgTAC) in Tulare, California, in conjunction with the EPRI.

Ozone was injected at the rate of 250 lbs. or 50 lbs. per acre through underground drip lines buried about 6 inches deep in the center of 40-inch furrows.

Fields were free of known pathogens. Ozone was injected at the rate of 250 lbs. or 50 lbs. per acre (some co-extensively with 100 lbs. per acre CO_2) through underground drip lines buried about 6 inches deep in the center of 48-inch furrows. Carrot seeds were planted 5 days after treatment. Two additional midseason treatments of 15 lbs. per acre were applied to half of the plot that previously received the 50 lbs. per acre preplanting treatment.

Harvested carrots were segregated into marketable and non-marketable categories and weighed. When the comparison was restricted to similarly seeded and irrigated plots, plots pretreated with 50 lbs. O_3 and 100 lbs. CO_2 produced a 26 percent increase in total marketable carrot yield compared to the untreated control plots (Figure 6). Plots pretreated with 50 lbs. per acre of ozone and two midseason applications of 15 lbs. per acre produced a 15 percent increase in total marketable carrot yields compared to the untreated controls.

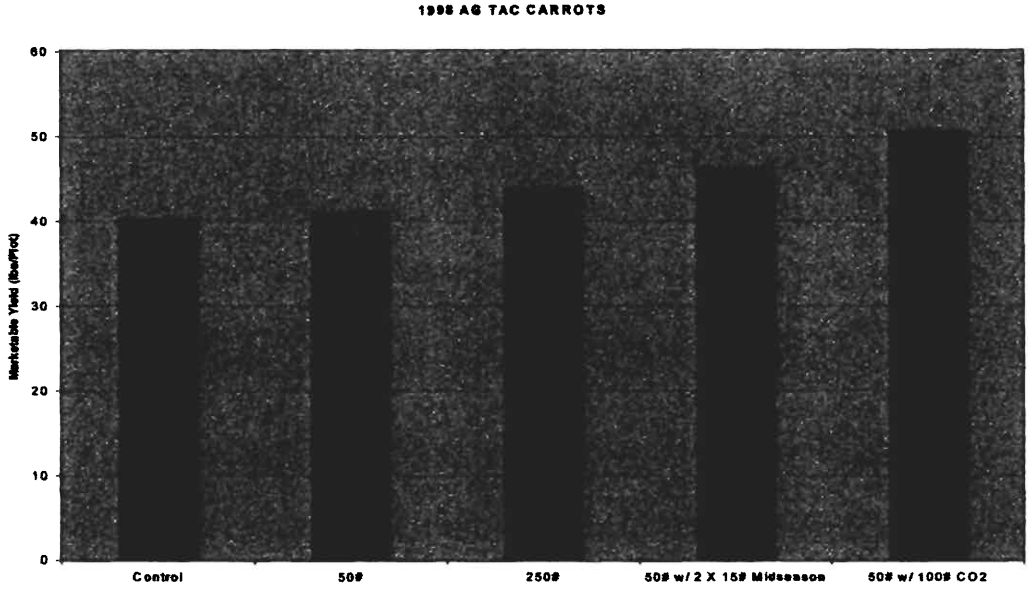


Figure 6. 1998 Tulare Field Trials – Carrot Yield (lbs. per plot)

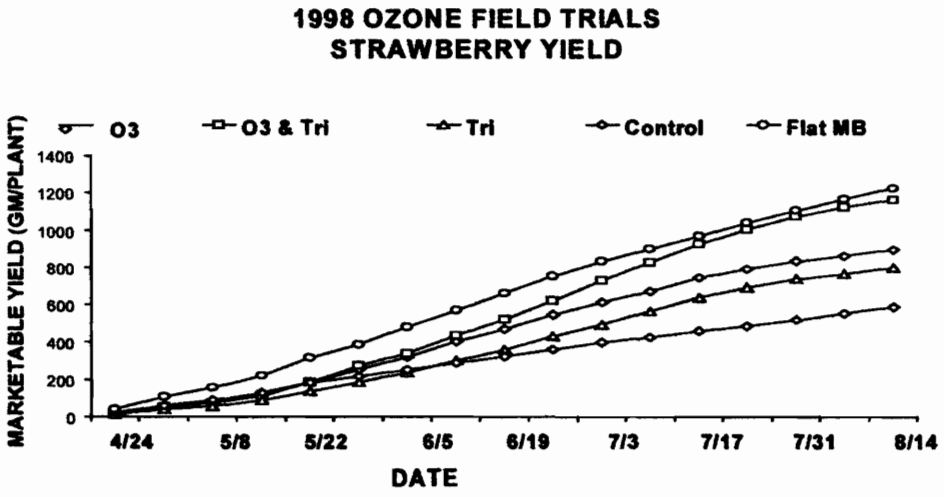
2.1.2.5 Strawberries

This experiment was performed at a site maintained by the U.S. Department of Agriculture (USDA) and the California Strawberry Commission in Watsonville, California, in conjunction with Dr. John Duniway of the UCD Department of Plant Pathology.

The soil at this site was heavily infested with *Verticillium* sp. fungi. Ozone was injected at the rate of 400 lbs. per acre through drip tubing buried about six inches deep in the center of 36-inch beds.

Ozonation applications were made with and without pre-inoculation with Trichoderma fungi. Strawberry transplant planting occurred five days later in November of 1997. In early June 1998, an additional midseason ozone application of 15 lbs. per acre was made to those plots that had been previously inoculated with the Trichoderma sp. fungi.

Cumulative yields totaled through the end of the growing season in early August showed the ozonated plots with Trichoderma fungi produced 97 percent greater marketable fruit than untreated controls (Figure 7). Yields from the ozone-treated plots were functionally equivalent to the methyl bromide/Chloropicrin treated plots. The plots that received ozone only were 51 percent greater than the untreated controls.



Collaborators: Dr. John Duniway, Department of Plant Pathology, UC Davis
Location: Watsonville, CA

Figure 7. 1997-98 Ozone Field Trials – Strawberry Yield

2.1.2.6 Irvine Sugar Beet Trials in Cyst Nematode Infested Soils

These trials were performed in a field heavily infested by cyst nematodes (Hetrodera schachtii) at the University of California's South Coast Field Station in Irvine, California. The research was conducted in conjunction with Dr. Becky Westerdahl of the UCD Department of Nematology.

Ozone was injected in early July both with and without pre- and post-irrigation at the rate of 250 lbs. per acre through underground drip tubing buried four to six inches deep in the center of 32 inch furrows.

Each treatment consisted of six 20-foot rows in randomized complete blocks. Sugar beets (variety HH103) were planted 1 week following the ozone injection and the total yield was compiled at the end of the late December harvest.

In these trials, the best ozone treatment at 50 lbs. per acre increased total sugar beet production by 10.8 percent compared to untreated controls and 13.2 percent compared to Telone treated plots (Figure 8).

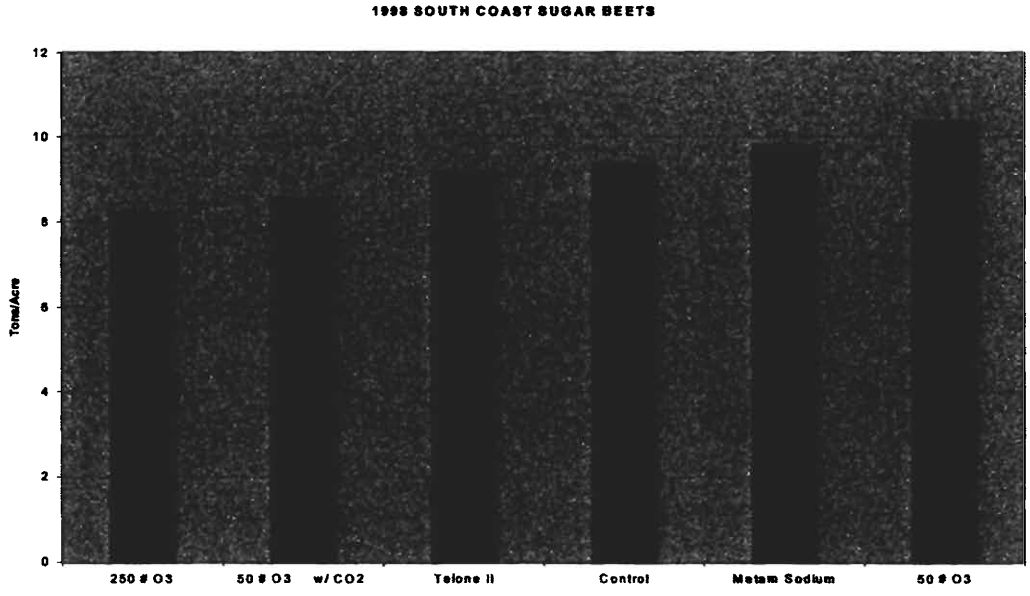


Figure 8. Ozone Field Trials-Sugar Beet Cyst Nematode Infestation

2.1.2.7 Irvine Sugar Beet Trials in Root Knot Nematode Infested Soils

These field experiments were also performed at the South Coast Field Station with Dr. Becky Westerdahl in parallel with the trials described in Section 2.1.2.6 except that plots were placed in soils highly infested with the root knot nematode (Meloidogyne javanica). The methods and dates of applications were identical to those described in Section 2.1.2.6.

In these trials, preplant treatment with 50 lbs. ozone combined with 100 lbs. CO_2 per acre experienced increased total production of approximately 2.5 percent compared to the untreated controls (Figure 9). Yields from the ozone-treated plots were 7.4 percent greater than those from the metam sodium-treated plots. Yields were, however, 20.8 percent less than those obtained from the Telone-treated plots.

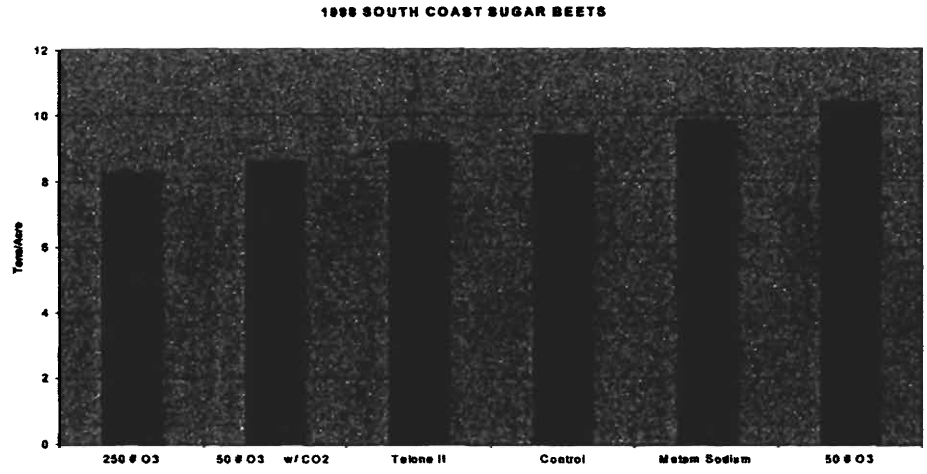
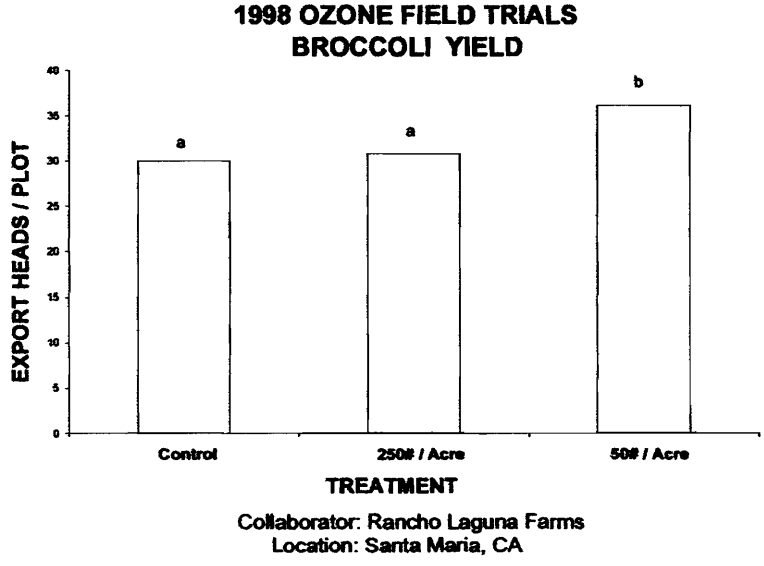


Figure 9. Irvine Field Trials Sugar Beet Root Knot Nematode Infestation

2.1.2.8 Broccoli

These experiments were performed in the summer of 1998 at a private farm in Santa Maria, California.

Ozone was injected through drip lines buried 8 inches deep, at 50 lbs. and 250 lbs. per acre rates, and broccoli seedlings were planted 5 days later. Upon harvest 10 weeks later, broccoli heads were segregated into export or domestic quality. Export heads are highly desirable because they command a 200 to 300 percent price premium over domestic quality heads. The 50 lbs. per acre ozone-treated plots produced a statistically significant 20 percent increase in the number of export quality heads produced compared to the untreated controls (Figure 10).



not dose related

Figure 10. 1998 Broccoli Trials

2.1.2.9 Prunes

These trials were conducted in an established 30-year-old prune orchard with severe lesion and ring nematode population pressures. Tree replant sites were excavated in the fall to remove the stumps and larger roots and the sites were re-mounded. Ozone was applied at the rate of 1.25 lbs. per hole through an injection probe with 3/8-inch emitter holes drilled between 8 and 18 inches in depth. Trees were planted 1 week later in March. The survival rate, tree diameter, and vigor were evaluated in November.

Trees planted in ozone treated holes had a 70 percent survival rate which was equal to or greater than the survival rates for trees grown in Enzone, Telone, and methyl bromide treated soils and in untreated soil (Figure 11).

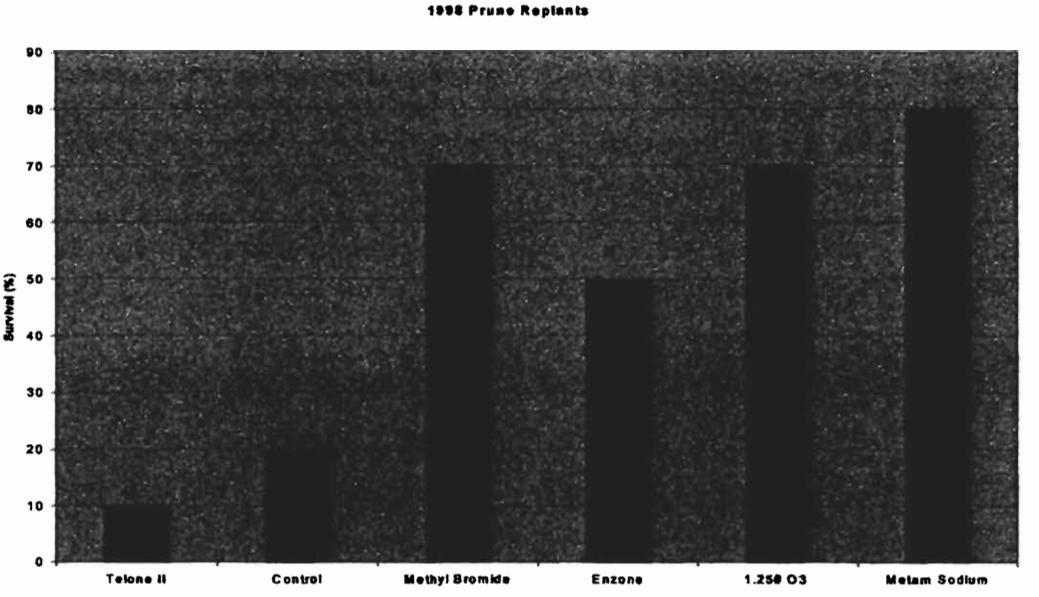


Figure 11. 1998 Ozone Field Trials-Prune Replants Survival

By multiplying the average vigor rating of the surviving trees by the survival percentage, an overall rating of treatment effectiveness is obtained. When so evaluated, trees planted in ozone-treated soil showed an overall rating substantially greater than trees planted in untreated soils or soils treated with methyl bromide, Enzone, or Telone. Metam sodium treated trees exceeded the ozone treatment trees' overall rating only slightly (Figure 12).

2.1.2.11 Peaches

These trials were conducted on the non-productive periphery of an established 25-year-old peach orchard. In the same manner as the prune trials, 1.25 lbs. of ozone was injected into an excavated and re-mounded tree site 5 days before trees were planted. The injection probe had eight 3/8-inch holes spaced around the probe at a buried depth of from 6 to 18 inches through which the ozone was injected into the probe. Within several weeks, an apparent phytotoxic effect was noticed in the trees that had been planted in ozonated soil.

The trunks of these trees turned a darker brown and the few leaflets that had formed were very small and very dark green. These are symptoms characteristic of nitrogen burn due to excess ammonia or nitrate nitrogen in the soil. Subsequent soil analysis revealed nitrate nitrogen increased in the ozonated tree holes from 26 parts per million (ppm) to 149 ppm and ammonia nitrogen increased from 2.1 ppm to as high as 16 ppm.

It was concluded the phytotoxicity was probably caused by an excess formation of nitrogenous compounds in the soil resulting from over ozonation. These tests will be repeated with lower ozone dosages in the future. A compounding factor in these trials was that many of the untreated control replants also showed poor vigor. It is believed that this was due to root fungus on the replant roots originating from improper handling after removal of the trees from cold storage. It is not known if similar results would have occurred if all the ozonated tree replants were not similarly afflicted (Figure 14).

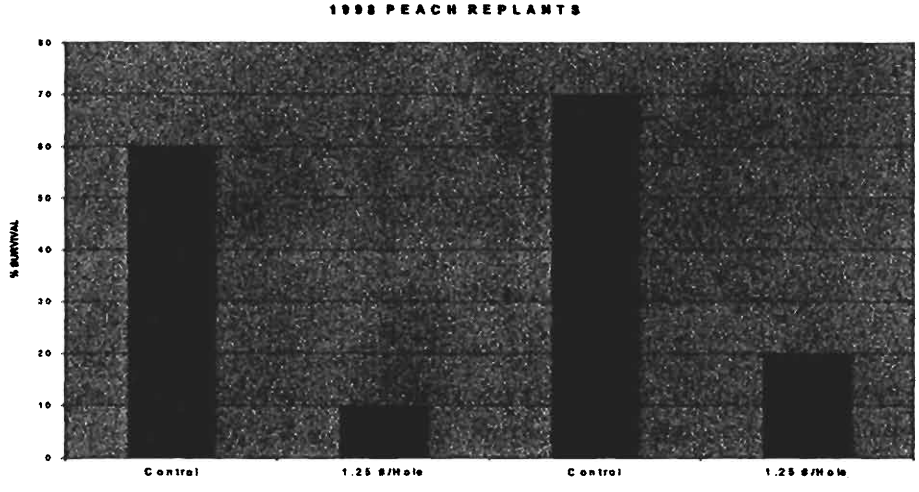


Figure 14. Ozone Field Trials – Surviving Peach Replants

2.1.3 Project Outcome

The results of these field trials generally demonstrated the broad effectiveness of ozone treatment of soil to increase plant yield and reduce the detrimental effects of soil pathogens on a variety of commercially important crops and soil types under a range of climatic conditions. Field test results indicate that soil treatment with ozone decreased soil pathogen pressures (due to ozone's ability to kill living organisms) and increased nutrient availability (due to ozone's

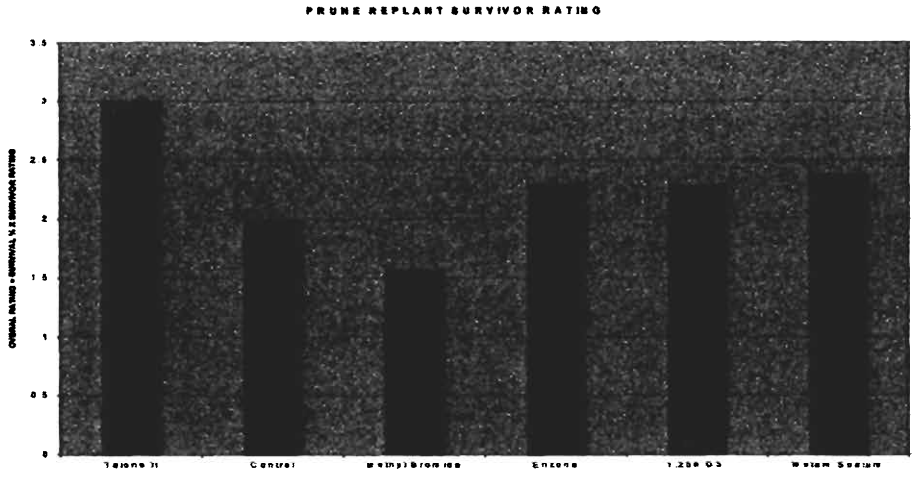


Figure 12. 1998 Ozone Field Trials-Prune Replants Treatment Effectiveness

2.1.2.10 Sweet Potatoes

These experiments were performed in May through October of 1998 at a private farm in Stevenson, California. Ozone injection was performed in early May at 100 lbs. or 400 lbs. per acre through drip lines buried 7 inches deep. Sweet potato seedlings were planted 5 days later.

Upon harvest in early October, potatoes were segregated into large marketable or small potatoes. The 100 lbs. per acre ozone-treated plots produced an average 15 percent increase in large potato weights compared to the untreated controls (Figure 13).

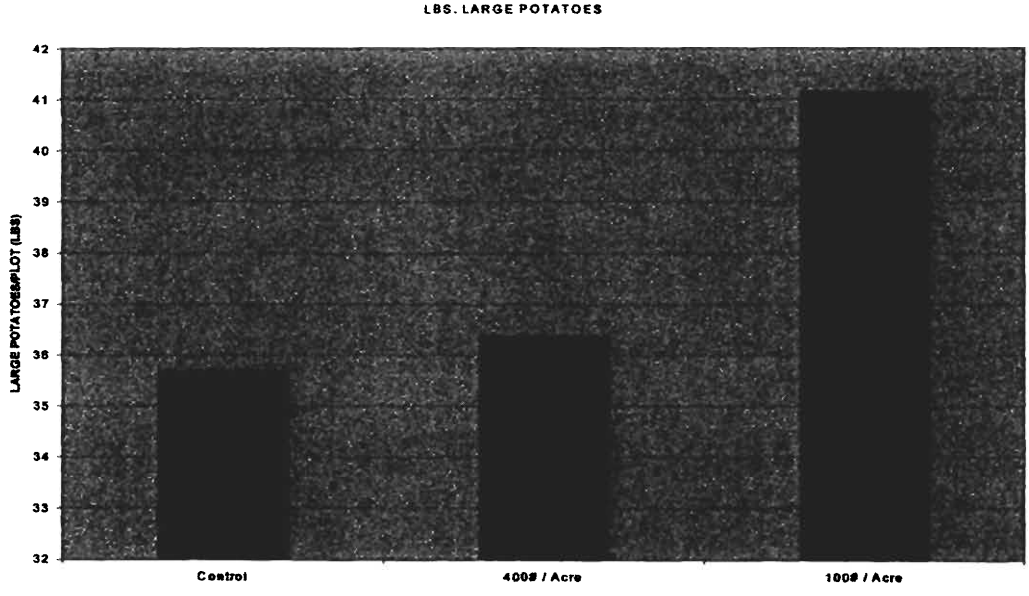


Figure 13. 1998 Ozone Field Trials – Sweet Potato Yield

Table 5 provides the increase or decrease in yield resulting from the best ozone treatment at each site compared to the alternative fumigants tested.

Table 5. Ozone Treatment Compared to Alternative Fumigant Crop Yield

Crop	Best Ozone Treatment (per acre or tree)	Crop Yield Compared to Alternative Fumigant Yield
Tomatoes, Irvine	250 lbs. O_3 with pre-irrigation	+17.1% versus Telone II
		-1.4% versus Vapam
Carrots, Irvine	50 lbs. O_3 with pre-irrigation	-20.8% versus Telone EC
		-19.2% versus Vapam
Strawberries	400 lbs. O_3 with 100 lbs. Trichoderma	-9.5% versus Methyl Bromide
Sugar Beets, Cyst Nematode Laden Soil	50 lbs. O_3 with pre-irrigation	+13.2% versus Telone II
		+5.8% versus Vapam
Sugar Beets, Root Knot Nematode Laden Soil	50 lbs. O_3 with pre-irrigation	+7.4% versus Vapam
		-20.8% versus Telone II
Prune Replant	1.25 lbs. O_3/tree hole	Overall survival/vigor rating -15.8% versus Vapam +39.4% versus Enzone +434% versus Telone II 45.8% versus Methyl Bromide

Modest levels of phytotoxicity were noted in the form of lower leaf burn on a number of plants in several plots following midseason applications of only 15 or 25 lbs. of ozone per acre in the tomato and carrot trials in Tulare. The application of ozone in midseason in tomatoes at Tulare resulted in a slightly lower yield than what was harvested from plots not receiving ozonation.

In contrast, the plots which received midseason ozonation applications in the strawberry trials in Watsonville, California, showed substantially increased growth compared to plots that received only a preplant treatment. Further work is needed to properly define the correct dosage levels to maximize yields without phytotoxicity.

The effects of mixing carbon dioxide with the ozone gas for injection as a preplant soil treatment were mixed. In the case of the tomato and sugar beet trials in root knot nematode laden soil in Irvine and the carrot trials in Tulare, the coextensive use of carbon dioxide resulted in increased yield. The opposite effect, however, was seen in the carrot trials and sugar beet trials in cyst nematode laden soils in Irvine.

Numerical (as compared to statistically significant) increases in total fungal biomass were noted in some trials but not in others. The underlying mechanisms of such stimulation are not known and further laboratory work is required in conjunction with field experiments to understand this occurrence.

The economic effectiveness of the ozone treatments is more difficult to ascertain than is the case for conventional agricultural fumigants. Conventional fumigants are sold and delivered to the grower for application by the pound. In the case of soil treatment with ozone, two additional cost components require consideration: the operating cost to power the generation equipment

oxidative effects on soil organics). Table 4 provides the increase or decrease in yield resulting from ozone treatments at each site compared to untreated controls.

Table 4. Ozone Treatment Crop Yield Compared to Untreated Control

Crop	Ozone Treatments (per acre or tree)	Crop Yield Compared to Untreated Control
Tomatoes, Irvine	250 lbs. O_3 with pre-irrigation	+44.2%
	250 lbs. O_3 without pre-irrigation	+35.1%
	50 lbs. O_3 with 100 lbs. CO_2	+30.0%
	250 lbs. O_3 in O_2	+22.1%
	50 lbs. O_3	+17.6%
Tomatoes, Tulare	50 lbs. O_3	+57.4%
	50 lbs. O_3 with 1x 25 lbs. midseason	+46.7%
	250 lbs. O_3	+3.7%
Carrots, Irvine	50 lbs. O_3	+92.2%
	250 lbs. O_3 with pre-irrigation	+92.0%
	250 lbs. O_3 in O_2	+53.6%
	50 lbs. O_3 with 100 lbs. CO_2	+45.4%
	250 lbs. O_3 without pre-irrigation	-9.1%
Carrots, Tulare	50 lbs. O_3 with 100 lbs. CO_2	+25.6%
	50 lbs. O_3 with 2 x 15 lbs. midseason	+14.8%
	50 lbs. O_3	+8.8%
	250 lbs. O_3	+2.0%
Strawberries	400 lbs. O_3 with 100 lbs. Trichoderma	+96.9%
	400 lbs. O_3	+51.5%
Sugar Beets, Cyst Nematode Laden Soil	50 lbs. O_3	+10.8%
	50 lbs. O_3 with 100 lbs. CO_2	-8.5%
	250 lbs. O_3	+12.2%
Sugar Beets, Root Knot Nematode Laden Soil	50 lbs. O_3 with 100 lbs. CO_2	+2.5%
	250 lbs. O_3	-18.9%
	50 lbs. O_3	-22.6%
Broccoli	50 lbs. O_3	+20.3%
	250 lbs. O_3	+2.8%
Prune Replant	1.25 lbs. O_3/tree hole	+300.7% overall survival/vigor rating
Sweet Potatoes	100 lbs. O_3	+15.3%
	400 lbs. O_3	+1.8%
Peach Replant	1.25 lbs. O_3/tree hole	Control survival: 70% Ozonated survival: 20%

and equipment-related rental cost (including labor, transportation, overhead and capital cost amortization of the ozone producing equipment).

The amortization component of the cost representing 1 lb. per day of ozone generating capacity is extremely variable. It primarily depends on whether the equipment is used on a continuous basis or only intermittently. It is estimated that a service company could provide onsite delivery of ozone for $3 to $4 per lb. with cash margins of approximately 30 percent consistent with industry norms.

Assuming the ozone delivery injection tubing is also used for irrigation during the growing season and that the delivered price of ozone is $3.50 per lb., an applied dosage of 50 lbs. of ozone per acre could be secured for a total price of $175 per acre. This is competitive with the cost of Vapam and Telone. Vapam costs $60 to $80 per lbs. and is applied at a rate of 150 to 200 gallons per acre for a total product cost of $90 to $160 per acre. With application costs at approximately $30 per acre, total costs would be $120 to $190 per acre.

Telone costs about $12 per gallon and about 12 to 14 gallons per acre is used. With an application rate of $50 per acre, this would result in a total applied cost of $194 to $218 per acre. Methyl bromide costs from $3 to $4 per lb. with application rates of from $150 to $300 lbs. per acre. Product costs of approximately $450 to $1,200 per lb. do not include application and tarping costs of $200 to $500 per acre for a net cost of $750 to $1,700 per acre.

A dosage of 250 lbs. per acre of ozone would cost $875 per acre, which is competitive with methyl bromide costs.

2.1.4 Conclusions and Recommendations

Field trial results demonstrated the broad effectiveness of ozone treatment of soil in increasing plant yield and reducing the detrimental effects of soil pathogens in a variety of crops and soil types under a range of climatic conditions. In every trial except the peach trial, substantial improvements in crop yield or plant vigor resulted from the ozone preplant application compared to untreated controls. In many cases where alternative fumigants were also tested, the best ozone treatment often exceeded one or more of the conventional fumigant treatments.

The study concludes that soil treatment with ozone results in decreased soil pathogen pressures (due to its biocidal effects) and increased nutrient availability (due to its oxidative effects on soil organics). Together as a preplant treatment, these benefits promote increased plant growth and yield without detrimental environmental effects.

Much additional work is necessary, however, to enable accurate prediction of the specific growth response achieved by ozonation in difference crops, soil types and climatic conditions.

Pergamon

Wat. Sci. Tech. Vol. 40, No. 4–5, pp. 235–245, 1999

0273–1223/99 $20.00 + 0.00

PII: S0273-1223(99)00505-3

ADVANCED TREATMENT AND DISINFECTION FOR MUNICIPAL WASTEWATER REUSE IN AGRICULTURE

L. Liberti and M. Notarnicola

Institute of Environment and Land Engineering, Polytechnic University of Bari, viale del Turismo 8, 74100 Taranto, Italy

ABSTRACT

In a 3-year joint research project, approved in 1995 by the European Commission, methods for advanced treatment and disinfection of municipal wastewater to permit reuse in agriculture were investigated. Pathogen inactivation, disinfection by-products (DBP) formation and the cost effectiveness of disinfection methods involving UV rays, ozone (O_3) and peracetic acid (PAA) were evaluated. The investigation was carried out on municipal effluents which had received different degrees of treatment (secondary, clarified, clarified-filtered) in a 100 m^3/h pilot plant that was designed, built and operated at West Bari (S. Italy) municipal wastewater treatment plant. Under the experimental conditions investigated, the WHO microbial guideline for unrestricted reuse of wastewater in agriculture (1,000 CFU/100ml for Faecal Coliforms) was easily achieved with all three disinfectants, while the corresponding Italian standard (2 CFU/100ml) was effectively met only with UV at an O&M cost in the range 17.5-35 EURO/1000m^3. Log-inactivation values ≥ 5 for both UV and PAA and ≥ 3 for O_3 were obtained; selected pathogens were affected by UV and, in part, by O_3. No DBPs were detected with UV and PAA, while limited formation of aldehydes was found with O_3.

KEYWORDS

Disinfection; municipal effluent; peracetic acid; ozone; UV rays; wastewater reuse.

INTRODUCTION

Following the discovery that chlorine may produce harmful disinfection by-products (DBP), alternative disinfectants are being considered worldwide for meeting the sanitary standards required for wastewater discharge and reuse (WEF, 1996; IWSA, 1997). With this objective, a 3-year (1995-1998) project linking research teams from Israel, Malta, Morocco, Spain, UK and Italy as the leading country, supported in part by the Commission of the European Community, has been carried out on various technical and health care aspects of wastewater treatment for agriculture reuse. The research programme of the foreign partners of the project was centered on advanced disinfection with hydrogen peroxide and oligo-metals (Israel), identification and health-effects of chlorinated DBP (UK), biological impact on marine environment (Malta), low-cost treatment technologies (Spain) and agronomic effects (Morocco).

The Italian team was involved in pilot and laboratory investigation of advanced disinfection methods based on ultraviolet light (UV), peracetic acid (PAA) and ozone (O_3). Major engineering and sanitary aspects of the study concerned: technical efficiency in terms of pathogen inactivation, DBP formation and costs; hydrodynamics of UV disinfection; potential migration of selected DBP in soils, crops and the marine

environment (in collaboration with Malta); epidemiology of waterborne diseases originating from municipal wastewater.

This paper summarizes the main experimental results, discussed in detail elsewhere (Liberti *et al.*, 1996, 1998, in press), of UV, PAA and O_3 disinfection achieved with a 100 m^3/h pilot plant. The latter, designed for investigating these 3 disinfectants and also equipped with multilayer filtration, has been built and operated at West Bari (Southern Italy) municipal wastewater treatment plant. Two effluents could be drawn from the plant before chlorination: one following secondary sedimentation, the other submitted also to post-precipitation with aluminum polychloride. Accordingly 3 feeds, namely *secondary* (II), *clarified* (CL) and *clarified-filtered* (F), have been alternatively treated with the 3 disinfectants in the pilot plant.

Specific objectives of this investigation were to assess:

- the effectiveness of disinfection using UV, PAA and O_3 with each feed by reference to the Italian microbial standard (2 CFU/100ml of Total Coliforms, based on the well-known 1978 California Wastewater Reclamation Criteria) as well as to 1989 WHO guideline (1,000 CFU/100ml of Faecal Coliforms) for unrestricted wastewater reuse in agriculture, paying special attention also to selected parasites and pathogens (Helminth eggs, *Giardia lamblia* cysts, *Cryptosporidium parvum* oocysts, *Pseudomonas aeruginosa*);
- the formation of disinfection by-products;
- the compliance of treated wastewater characteristics with agronomic regulations;
- disinfection costs.

UV, PAA AND O_3 MAIN FEATURES

It is well known that **UV** radiation in the range 240 to 280 nm wavelength range induces photochemical damage to RNA and DNA within the microbial cell, so that the organisms can no longer reproduce. At normal operating doses (20-200 mWs/cm^2), it is claimed that UV systems, either *submerged* or *unsubmerged* according to the water-lamp contact configuration, show biocidal action towards a wide variety of viable species with very fast kinetics and a lack of toxic DBP formation. In addition, they have relatively simple technology, of reasonable cost, and with no need for handling, storage and disposal of hazardous chemicals. The major drawbacks are: the absence of a bacteriostatic effect; the possibility of water recontamination by cell repair and photoreactivation; the unfavourable influence of water characteristics such as turbidity, suspended solids, colour, colloidal matter and dissolved organics causing shelter, scattering and absorption effects; the decline of UV output intensity due to lamp scaling and age (US-EPA, 1992; WERF, 1995).

Peracetic acid (CH_3COOOH) is a powerful antimicrobial agent recently proposed also for disinfecting drinking water, wastewater and even for municipal wastewater reuse. It is an unstable organic peracid commercialized as quaternary equilibrium mixture of acetic acid (CH_3COOH), hydrogen peroxide (H_2O_2), peracetic acid and water. It is reportedly not mutagenic or carcinogenic, and its disinfectant activity, increasing in acidic conditions where the undissociated acid prevails, is based on the release of active oxygen. At doses commonly adopted for wastewater discharge (1-5 ppm with $\leq$ 30 min contact time), PAA decomposes to harmless residuals such as acetic acid, oxygen and water, thus apparently not yielding harmful DBP. Major drawbacks associated with PAA disinfection are the increase of organic content in the treated effluents, the potential microbial regrowth due to the remaining acetic acid, the limited efficiency against viruses and parasites and the strong dependence on wastewater quality (Baldry and Fraser, 1988; Lefevre *et al.*, 1992)

Ozone is a strong disinfectant with high oxidation potential, potentially toxic and explosive requiring *on-site* generation. At doses usually reported for wastewater discharge (5-10 ppm with 5-15 min contact time) O_3 may form relatively harmful DBP (e.g., bromates) being also capable of oxidizing organic DBP precursors. Key factors affecting ozone disinfection are mass transfer efficiency, mixing, contact time and minimal short-circuiting as pursued with different ozonation systems (e.g., *diffused bubble, negative pressure or Venturi*), while it is negatively affected by soluble or suspended matter (Langlais *et al.*, 1991; Masschelein, 1991).

MATERIALS AND METHODS

West Bari municipal wastewater treatment plant treats the sewage of approx. 300,000 inhabitants (3,000 m^3/h) by primary (mechanical screening and sedimentation, including pre-precipitation with $pAlCl_3$) and secondary treatments (activated sludge with biological denitrification followed by sedimentation). A fraction of the secondary effluent (approx. 600 m^3/h) undergoes also post-precipitation (i.e., coagulation and flocculation with 30-40 mg/l of $pAlCl_3$, followed by 6 hrs sedimentation at hydraulic linear velocity of 0.9 m/hr). Final disinfection occurs by chlorination.

The pilot plant, shown in Figure 1, permits a comparison of the performance of the various disinfectants (UV, PAA, O_3) with feeds of increasing quality. Unchlorinated *secondary* (II) and *clarified* (CL) feeds were drawn directly from the West Bari plant. The *clarified-filtered* feed (F) was obtained by filtering CL on a multilayer pressure filter (MF) filled with high purity silica sand and gravel. The 5 m^3 fibre-glass vessel (RV) was used for batch disinfection with the PAA mixture *Oxymaster* (PAA 15.5 %, H_2O_2 22.8 %, Acetic acid 17 %), kindly provided by Solvay Interox (Livorno, I). UV disinfection occurred in a non-contact UV apparatus (UVA, mod. 600 L Super, maximum flow rate 140 m^3/h, kindly provided by UVT, Taranto, I), in which the water flow is split between 15 parallel, inverted, U-shaped Teflon tubes. These were surrounded externally by 62 low pressure (0.2 atm) Hg vapor lamps. Disinfection with O_3 was carried out with an industrial system (mod. NFW 410, maximum production rate 445 gO_3/h), kindly provided by Cillichemie (Milan, I) where O_3 was added to the feed through the ejector (O3E) and the hydrokinetic mixer (O3M). The feed then entered the reaction tower (O3T) consisting of a 5 m^3 fibre-glass vertical closed tank. Ozone was generated from air by high tension (max. 15 kV) electric discharge in the production unit (O3P). Wastewater samples were collected from sampling ports 1 to 7.

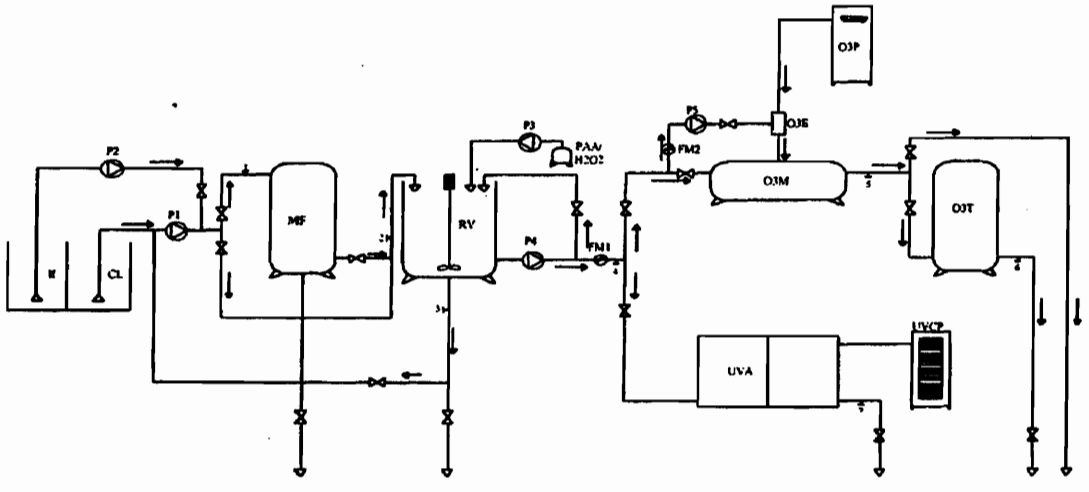


Figure 1. Flow-sheet of West Bari 100m^3/h pilot plant.

Under the experimental conditions used, maximum UV dose (D) amounted to 430 mWs/cm^2, calculated as $D = I \times t$, where I (average UV intensity inside the Teflon tubes) was 12.2, 9.9 and 8.8 mW/cm^2 for F, CL and II feeds respectively and t (exposure time in UV reactor, i.e., hydraulic retention time) was $\leq$ 50 sec. Similarly, maximum available dosages amounted to 15 ppm of applied O_3 (transferred only in part to the feed) with contact time $\leq$ 15 min and to 500 ppm PAA with contact time $\leq$ 60 min respectively.

For each disinfectant, first the best feed (i.e., clarified-filtered, F) was submitted to increasing doses until the target count of Total Coliforms was achieved (if possible). When the target count was achieved, the poorer feeds (i.e., clarified, CL and secondary, II, in that order) were then treated similarly. In particular, during PAA cycles, 3 m^3 of each feed were added batchwise in RV with the appropriate PAA dosage and mixed for different contact times. During UV cycles, 5 m^3 were conveyed to the UV apparatus at the given flow rate and exposed to the corresponding UV dose. During O_3 cycles, 5 m^3 were treated with the given O_3 dosage

through the ejector (O3E), mixed through the hydrokinetic mixer (O3M) and then held batchwise in the reaction tower (O3T) for the given contact times. For the sake of reproducibility, each run was done in triplicate so that each cycle (i.e., one feed submitted to a given dose of the given disinfectant for a given contact time) lasted approx. one full working day. Between July 1996 and January 1998, a total of 88, 76 and 68 cycles were carried out for UV, PAA and O_3 respectively, according to the planned schedule, using the appropriate configuration of the pilot plant.

Feed characteristics were analysed routinely including Temperature, pH, Conductivity, Alkalinity, Total Suspended Solids, Total Dissolved Organic Carbon, Turbidity, Transmittance at 254 nm, NH_4^+, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and Total Coliforms (before and after disinfection). Once the microbial standard was achieved, three more cycles were conducted with the same conditions in order to assess those parameters of agronomic interest in the disinfected effluent (pH, TSS, Sodium Adsorption Ratio, BOD_5, COD). In addition, selected parasites and pathogens (Helminths, *Giardia lamblia* cysts, *Cryptosporidium parvum* oocysts and *Pseudomonas aeruginosa*) were monitored before and after disinfection. Finally, the eventual formation of DBP (i.e., nitro-phenols and N-nitroso-amines for UV, total epoxides and 2/4/2,4 chloro-phenols for PAA, total aldehydes, bromates and bromoform for O_3) was investigated. Collection, storage and analytical procedures were done according to *Standard Methods*, unless specified differently (Liberti *et al.*, 1998).

SUMMARY OF RESULTS AND DISCUSSION

Table 1 reports the main characteristics of the three feeds throughout the investigation period, clearly showing the differences in quality for II, CL and F effluents, in particular for those parameters potentially affecting disinfection performance, i.e., TSS, Turbidity and Total Coliforms.

Table 1. Main characteristics of secondary (II), clarified (CL) and clarified-filtered (F) feeds

	II			CL			F		
	ave	**Min**	**max**	**ave**	**min**	**max**	**ave**	**min**	**max**
Temperature(°C)	19	17	20	21	16	27	20	16	25
pH	7.6	7.5	7.7	7.6	6.7	8.6	7.6	6.8	8.0
Conductivity (mS/cm)	1842	1560	4390	2100	1271	6300	2040	1330	6330
Turbidity (NTU)	9	5.1	29.3	5	2.3	10.4	2.1	1.2	4.0
TSS (mg/l)	20	11	39	13	6	27	5	3	10
DOC (mg/l)	13	5	28	10	3	24	10	5	24
254 nm Transmittance (%)	56	44	63	61	55	66	67	60	70
NH4 (mg/l)	28.1	21	43.4	24.5	11.5	35.5	26.3	13.8	50.5
N-NO3 (mg/l)	1.32	0.36	3.58	0.33	0.01	1.59	0.69	0.01	3.13
N-NO2 (mg/l)	0.39	0.08	0.72	0.39	0.01	1.24	0.32	0.01	1.24
Total Coliforms 1000 (CFU/100ml)	1710	8	6370	983	0.43	4550	387	0.2	1600

Disinfection effectiveness

The effectiveness of disinfection for Total Coliforms has been evaluated for each disinfectant and each feed according to the operating procedures previously described. Table 2 and Figure 2 summarize the most relevant results obtained. These data indicate that, under the experimental conditions investigated, the Italian target standard for unrestricted reuse of municipal wastewater in agriculture (2 CFU/100ml of Total Coliforms) was achieved only with clarified-filtered or clarified feeds disinfected with 100 and 160 mWs/cm^2 UV dose respectively. This is in agreement with similar investigations (Snider *et al.*, 1991; Awad *et al.*, 1993). However, the less stringent WHO guideline (1,000 CFU/100ml of Faecal Coliforms) was always achieved with all disinfectants and feeds used.

It is worth noting, as expected, the extremely fast kinetics of UV *physical* disinfection (contact time $\leq$ 30 sec) compared with O_3 or PAA *chemical* disinfection ($\geq$ 5 min). Log-inactivation values $\geq$ 5 were obtained with UV and PAA, compared with $\leq$ 4 with O_3, although this parameter may be unreliable when the colimetric content of the feed is highly variable, as in the present case.

Table 2. Summary of disinfection effectiveness of UV, PAA and O_3 towards Total Coliforms

Feed	period of Investigation	Disinfectant	Dose	no. of cycles	No			N			log (No/N)		
					ave	min	max	ave	min	max	ave	min	max
F	July-Oct 96	UV	100 mWs/cm^2	9	120	0.2	460	1	0	2	4.7	2.2	6.0
	March-June 97	PAA	400 ppm, 20 min	5	362	120	625	2	1	2	5.2	5.0	5.5
			10 ppm, 30 min	14	438	70	900	240	10	680	3.4	2.3	4.6
	Nov97-Jan98	O3	15 ppm, 10 min	8	800	270	1600	97	60	160	3.9	3.5	4.4
CL	Dec96-Jan97	UV	160 mWs/cm^2	8	694	100	2000	1	1	2	5.7	5.0	6.0
	Sept97-Jan98	O3	15 ppm, 10 min	15	1400	300	4550	1060	275	2350	3.1	2.3	3.5
II	Dec96-Feb97	UV	430 mWs/cm^2	11	908	210	3700	5	1	19	5.3	4.9	6.0

No: Total Coliforms content before disinfection (1000 CFU/100ml)
N: Total Coliforms content after disinfection (CFU/100ml)
II: secondary, CL: clarified, F: clarified-filtered feeds

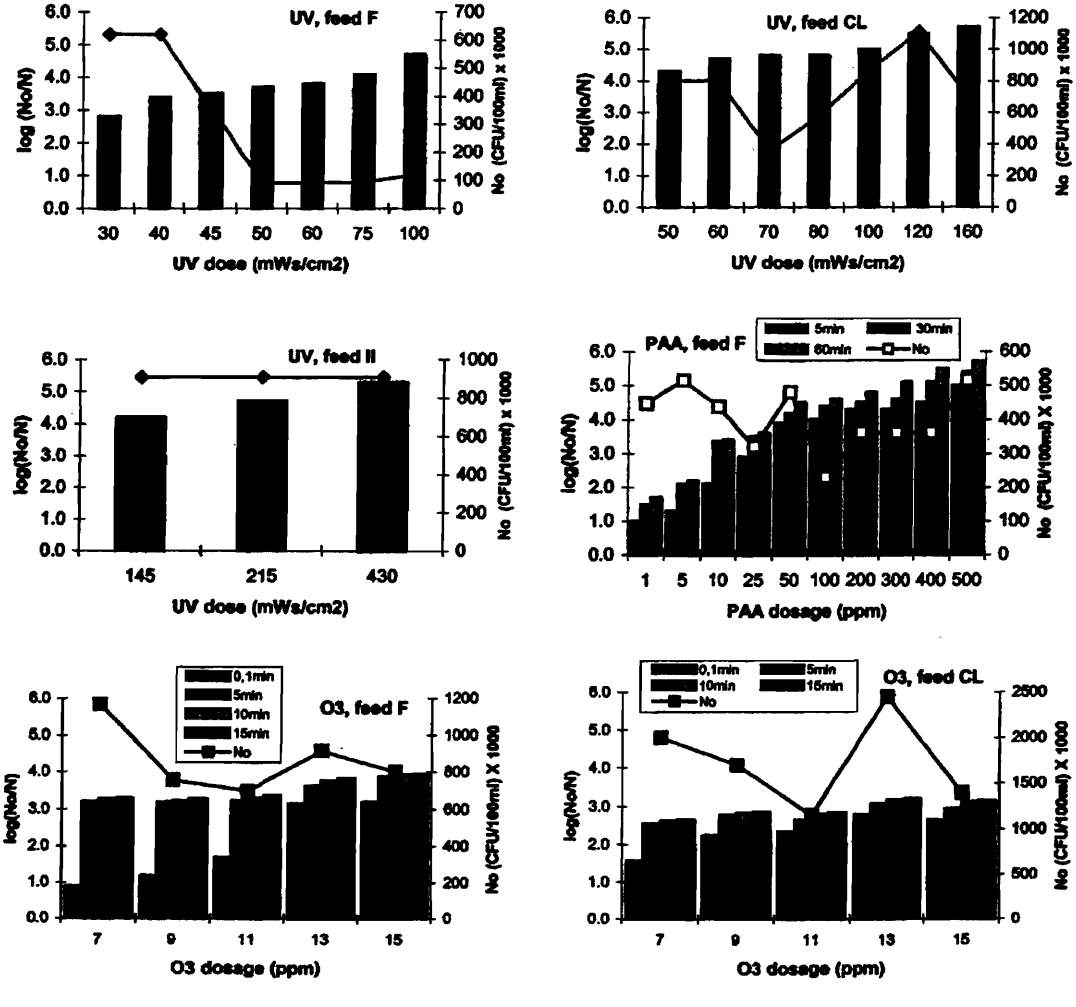


Figure 2. Total Coliforms log-inactivation with UV, PAA and O_3

Although small doses (1-5 ppm) of PAA and O_3 have been reported to be sufficient to reach the Total Coliform standard of 2 CFU/100ml during disinfection of drinking water, the same result is not achievable with municipal effluent since both powerful oxidants are rapidly consumed by organic and other oxidizable impurities. In fact, this target has been achieved with such low O_3 doses only when wastewater is previously submitted to clarification, sand-filtration and activated carbon adsorption (US-EPA, 1986). In the present investigation, the target was also achieved with PAA on the clarified-filtered feed only, using very high dosages (400-500 ppm) with a contact time of 20 min, i.e., under economically prohibitive conditions, in agreement with Mandra *et al.* (1996).

Major limiting factors for disinfection performance in the present investigation were confirmed to be O_3 gas transfer to the liquid phase and effective PAA mixing with wastewater (US-EPA, 1986). Similarly, TSS and/or Turbidity (not DOC content) were the limiting parameters for UV disinfection, reducing UV transmittance in the treated wastewater by a scattering effect (Snider *et al.*, 1991).

Effect on selected pathogens

In this study, the effectiveness of the chosen disinfectants towards selected pathogens commonly occurring in local municipal wastewater was also evaluated and the results are shown in Table 3. As indicated, Helminth eggs were never found in the feeds before disinfection, confirming that heavy and large parasites are consistently retained by clarification and filtration treatments. The data in Table 3 also indicate that all disinfectants were effective towards bacteria like *Pseudomonas aeruginosa*. Parasites like *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts were both affected by UV, O_3 was rather effective towards *Giardia* only while PAA was basically ineffective towards both parasites. These results agree only in part with previous data on O_3, reportedly effective also towards *Cryptosporidium* (Langlais *et al.*, 1991). Better agreement was found instead with PAA efficiency towards pathogens which can be ranked thus: *bacteria>viruses>bacterial spores>protozoan cysts* (Rudd and Hopkinson, 1989), as well as with UV, for which 60 to 180 mWs/cm^2 doses are claimed to yield 80 to 99% reduction of *Giardia* and *Cryptosporidium* (Campbell *et al.*, 1995). However, due to the low precision of microbiological measurements at low parasite concentration, the above conclusions should be considered speculative.

This project confirmed that the *multiple barrier concept* (i.e., filtration plus disinfection) offers the most effective approach for complete parasite removal in water and wastewater treatment (Karanis *et al.*, 1992).

Table 3. UV, PAA and O_3 disinfection effectiveness towards selected pathogens

		UV		PAA		O3	
		100 or 160 mWs/cm^2 *		10 ppm, 30 min		15 ppm, 10 min	
Pathogen	**Feed**	**In**	**Out**	**In**	**Out**	**In**	**Out**
Helminths (N/l)	F	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
	CL	0/0/0	0/0/0			0/0/0	0/0/0
Giardia lamblia (N/l)	F	80/110/150	20/60/50	20/18/12	19/17/12	41/13/46	7/10/14
	CL	411/347/278	124/188/156			125/123/390	2/37/237
Cryptosporidium parvum (N/l)	F	40/2/1	15/1/1	1/2/1	1/2/1	1/1/3	1/1/3
	CL	7/21/7	0/6/3			1/5/24	0/5/14
Pseudomonas Aeruginosa (CFU/100ml)	F	8/2×10^5/0	3/2/0	12,500/10,000/ 1,200	9/0/20	300/2,500/1,600	12/4/8
	CL	3.6×10^6/9,600/ 4.8×10^5	1/0/0			1,900/1,000/400	10/60/14

* treating feed F or CL respectively

DBP formation

It cannot be excluded, in principle, that UV irradiation of wastewater may affect the identity of the organic substances through either *direct* or *indirect* interaction and form potentially toxic by-products (von Sonntag and Schuchmann, 1992). In the former case, a molecule known as a *chromophore* may be chemically modified as a result of a direct radiation absorption. Indirect photolysis may occur when UV radiation acts on a species known as a *photosensitiser* which strongly absorbs the radiation energy and the resulting highly energetic species interacts with another molecule producing a chemical transformation. Since amino- and phenolic-derivatives (chromophores) as well as nitrate/nitrite ions and humic materials (photosensitisers) are commonly found in municipal wastewater, during the present investigation N-nitroso-amines and nitro-phenols were specifically targeted as possible DBP following UV disinfection. As summarized in Table 4, however, none of these N-derivatives was ever detected, even at the highest UV doses. It may be concluded that, under the conditions investigated, no chemical transformation occurs during UV disinfection of municipal wastewater, as found in other similar investigations (Elsinore Valley, 1994; Linden *et al.*, 1998).

Table 4. DBP formation after UV, PAA and O_3 disinfection of CL and F feeds

			DBP							
Disinfectant	**Dose**	**feed**	In	Out	In	Out	In	Out	In	Out
UV			**Nitro-phenols (ppb)**		**N-nitroso-amines (ppb)**					
	100 mWs/cm^2	F	N.D.	N.D.	N.D.	N.D.				
	160 mWs/cm^2	CL	N.D.	N.D.	N.D.	N.D.				
PAA			**Total Epoxides ^**		**2/4/2,4 Chloro-phenol (ppb)**					
	10 ppm, 30 min	F	N.D.	N.D.	N.D.	N.D.				
O3			**Total Aldehydes ***		**Bromides (ppm)**		**Bromates (ppm)**		**Bromoform (ppb)**	
	15 ppm, 10 min	F	0.12	0.47	4.13	4.21	N.D.	N.D.	0.48	0.54
	15 ppm, 10 min	CL	0.11	0.45	3.48	3.32	N.D.	N.D.	0.31	0.32

^ expressed as hydrogen peroxide (ppb); * expressed as formaldehyde (mg/l); In/Out: before/after disinfection

Even though PAA is considered to decompose to oxygen, water and acetic acid, the possibility that it could form harmful DBP cannot be completely ignored. About 10-30 ppb of aldehydes have been reported to form as transformation and/or oxidation DBP when PAA interacts with amino acids, phenols and other aromatic substances present in treated sewage (Crathorne *et al.*, 1991). Aldehydes are thought to be hepato-toxins at high dosages (ppm levels), but no toxic effects are expected at ppb concentration level. During the present investigation, indeed, about 120 ppb of aldehydes were already present in the feeds before PAA disinfection, hence their eventual further formation was ignored. Another possibility was the formation of brominated haloforms due to the presence of 3-4 ppm of bromide ion in the feeds investigated (Booth and Lester, 1995). The co-occurrence of 20-25 ppm of NH_4^+ ion, however, also prevented this possibility because the reaction rate between NH_3 and HOBr was found to be much greater ($k=8\times10^7$ M^{-1} s^{-1}) than between HOBr and organic matter ($k=20$ M^{-1} s^{-1}). It is most likely that, in the present situation, epoxides and halogenated organic compounds such as chlorinated phenols could be formed (Rudd and Hopkinson, 1989). Therefore, epoxides (as sum parameter) and chlorinated phenols (as 2-Chlorophenol, 4-Chlorophenol and 2,4 Dichlorophenol) were investigated following PAA disinfection. Table 4 demonstrates that, even for PAA, the expected formation of DBPs did not occur under the experimental conditions investigated. Indeed, epoxides are very unstable and, if formed, should behave as reaction-intermediates that immediately decompose to H_2O_2 and carbonyl-containing products. Chlorinated phenols are reported to form only under very severe conditions (Booth, 1995), not occurring in the present investigation.

The reaction mechanism of O_3 with organics involves the direct reaction of O_3 molecule or the intervention of less selective but much more reactive radical species. Both molecular and free radical ozone pathways resulting from its complex decomposition, as well as the nature of organic matter which serves as precursor material, play a role in the possible formation of harmful DBP during ozone disinfection (Minear and Amy, 1996). A number of studies, particularly in the field of drinking water treatment, have identified several ozone DBPs like mono- and dicarboxylic acids, mono- and diketones, alkanes, phthalates, organic peroxides, epoxides and aldehydes. In the last group, only simpler aldehydes (i.e., formaldehyde, acetaldehyde, glyoxal, propanal, butanal, pentanal and acetone) are likely to form in appreciable amounts

(ppb level) under common disinfection conditions (Schechter and Singer, 1995). In 1995, the European Commission included bromates and brominated THMs, potentially formed during the ozonation of Br^- containing waters, in the list of potentially toxic DBP. Accordingly, the formation of bromate (BrO_3^-), bromoform ($CHBr_3$) and aldehydes (measured as sum parameter) was assessed after O_3 disinfection during the present investigation. Only the last of these was found to form in appreciable amounts (about 350 ppb, see Table 4) during ozonation. No bromates were found probably due to the basic pH (7.6) of the feed, while bromoform, already present in the feed, showed a negligible increase in concentration, perhaps as a consequence of the relatively high NH_4^+ content of the feed (von Gunten and Hoignè, 1992).

Compliance with agronomic regulations

According to Italian regulations, apart from Total Coliforms, other parameters of agronomic interest (pH, TSS, BOD_5, COD, Sodium Adsorption Ratio and Boron) should comply for reuse of municipal wastewater in agriculture. As shown in Table 5, compliance with such regulations was always achieved, regardless of the disinfectant and the type of feed used (CL or F) for all the considered parameters (except for Total Coliforms, for which the standard was only met after UV disinfection).

Table 5. Agronomic characteristics of CL and F effluents disinfected with UV, PAA and O_3

Parameter		feed	UV effluent ^	PAA effluent ^	O_3 effluent ^	MAC°
pH		F/CL	8.1/7.8	7.5	7.8/7.7	5.5-9.5
TSS	(mg/l)	F/CL	3/8	6	6/10	80
BOD5	(mg/l)	F/CL	10/5	12	4/5	40
COD	(mg/l)	F/CL	40/57	54	51/59	160
Boron	(mg/l)	F/CL	1.3/0.9	1.1	0.8/0.9	2
Sodium Adsorption Ratio		F/CL	6/6	7	7/7	15

^ UV: 100 or 160 mWs/cm^2 (treating feed F or CL respectively); PAA: 10 ppm, 30 min; O_3: 15 ppm, 10 min

° Maximum Allowable Concentration for agriculture reuse fixed by Italian Regulations (L.319/76, DCI 4/2/77)

Furthermore, it must be emphasized that the proven occurrence of some parasites in the disinfected effluents (see Table 3) does not restrict their reuse in agriculture. In fact, according to WHO, the only parasites of concern are the intestinal nematodes (MAC < 1 egg/l), which belong to the Helminths, a group never found during this investigation.

Cost estimates

The economic feasibility of using UV, PAA and O_3 for advanced disinfection can be evaluated on the basis of the experimental results obtained. Towards this aim, estimates were made with reference to the optimal dose of each disinfectant for each feed that permitted the achievement of the 2 CFU/100ml Total Coliform standard and/or the WHO 1,000 CFU/100ml Faecal Coliform guideline with the following assumptions:

- operation & maintenance (O&M) costs only are considered;
- UV doses of 100, 160 and 430 mWs/cm^2 for disinfecting feeds F, CL and II respectively; PAA dosages of 10 and 400 ppm for feed F; O_3 dosage of 15 ppm for disinfection of feeds F and CL are considered;
- major costs account for electricity consumption and lamp replacement (UV), for chemicals consumption (PAA), for electricity consumption and generator replacement (O_3) respectively;
- maintenance requirements and miscellaneous equipment repair costs for UV and O_3 are included in UV lamp and O_3 generator replacement costs respectively, while for PAA they are negligible;
- average electricity cost is 0.065 EURO/kWhr (1 EURO = 2,000 Italian Liras);
- electricity consumption of UV and O_3 equipment is 3.1 and 15.8 kWhr respectively;
- UV lamp (45 EURO/each) and O_3 generator (400 EURO/each) replacement is based on 8,760 and 26,280 hours of use respectively;
- PAA-based Oxymaster, including the transport, costs approx. 1 EURO/kg.

A summary of the cost analysis is reported in Table 6. As indicated, the stringent 2 CFU/100 ml Total Coliform standard was steadily achieved with proper UV dose both on the clarified (CL) and the clarified-

filtered (F) feed with an O&M cost of 35 and 17.5 EURO/1000m^3 respectively. The same target was achieved with PAA on feed F only, at the excessive cost of 2,580 EURO/1000m^3 and it was never attained with O_3 under the conditions investigated.

Table 6. Cost estimates for UV, PAA and O_3 disinfection of II, CL and F feeds at West Bari pilot plant

Disinfectant	Dose	feed	Flowrate (m^3/h)	Total Coliform target achieved (CFU/100 ml)	O&M costs (EURO/1000m^3)			
					Electric power	replacement	chemicals	TOTAL
UV	100 mWs/cm^2	F	30	1	6.7	10.6		17.3
	160 mWs/cm^2	CL	15	1	13.5	21.3		34.8
	430 mWs/cm^2	II	5	5	40.3	63.7		104
PAA	10ppm,30min	F	30	240			64.5	64.5
	400ppm, 20min	F	30	2			2,580	2,580
O3	15ppm,10min	F	30	97	34.2	3.1		37.3
	15ppm, 10min	CL	30	1060	34.2	3.1		37.3

The economic conclusions are quite different if the WHO microbial guideline is considered. The data in Table 6, indeed, show that all three disinfectants can be used to achieve this aim, at proper doses, with tertiary feed (F) and the O&M cost increase, contrary to the performance achieved, follows the order *UV < O_3 < PAA*. To use O_3 to disinfect CL feed cost 37.5 EURO/1000m^3 and this substantially reached the WHO target. The cost effectiveness of UV disinfection is further confirmed by the possibility of meeting the WHO target even with the poorest quality feed (II) at a cost which is still affordable.

It should be noted, however, that the above estimates do not include capital costs (almost negligible for PAA disinfection) and can be influenced by a wide range of variables, such as feed quality, plant configuration, plant size (scale factor) and market situation.

CONCLUSIONS

The pilot (100 m^3/h) investigation carried out at West Bari (Southern Italy) municipal wastewater treatment plant between June 1996 and January 1998 permitted a comparison of the performances of three alternatives (UV rays, Peracetic Acid and Ozone) to chlorination as a method of disinfection of municipal wastewater for reuse in agriculture. On the basis of the results obtained by treating wastewater of varying strengths, namely *secondary* (II), *clarified* (CL) and *clarified-filtered* (F), with different experimental conditions, the following conclusions can be summarised:

- a clarification-filtration tertiary treatment stage was required to obtain a municipal effluent of consistently high quality to be effectively disinfected, according to Italian agronomic regulations;
- the stringent microbial standard for unrestricted reuse of municipal wastewater in agriculture (2 CFU/100ml for Total Coliforms), requiring log-inactivation value ≥ 5, was achieved with UV disinfection of either CL or F feeds with a dose of 160 and 100 mWs/cm^2 respectively. Similar results required very high doses (400 ppm and 20 min) of peracetic acid on F feed only and were never achieved with ozone;
- the corresponding WHO guideline (1,000 CFU/100ml for Faecal Coliforms) was easily achieved with all three disinfectants on feeds CL and F;
- all three disinfectants were very effective against bacteria like *Pseudomonas aeruginosa*. Parasites like *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts were affected by UV radiation, while O_3 was rather effective only towards *Giardia* and PAA showed poor action towards such resistant pathogens;
- harmful disinfection by-products were not found to form after UV or PAA disinfection, while limited formation of aldehydes occurred during O_3 disinfection;
- O&M costs ranged from 17.5 up to 2,600 EURO/1000m^3 for UV and PAA disinfection respectively (2 CFU/100ml for Total Coliforms) and 37.5 EURO/1000m^3 for O_3 (1,000 CFU/100ml for Fecal Coliforms).

Further investigations are planned on the following major aspects:

- application of the alternative disinfection methods to full tertiary municipal wastewater;
- possible synergy and/or catalytic effects of mixed disinfectants;
- more extensive search for possible DBP formation;
- full scale cost evaluation of the whole process (advanced wastewater treatment and disinfection).

ACKNOWLEDGEMENTS

This work was partly supported by the European Commission under the Avicenne 1994 Initiative with the contract No. AVI-CT94-0010 "Advanced disinfection and health-care aspects of wastewater reclamation and reuse in agriculture in Mediterranean regions".

REFERENCES

Awad J., Gerba C. and Magnuson G. (1993). Ultraviolet disinfection for water reuse. In: *Proc. of WEF Specialty Conference Series: Planning, Design and Operation of Effluent Disinfection Systems*, Water Environ. Fed. (ed.), Alexandria, pp. 1-12.

Baldry M.G.C. and Fraser J.A.L. (1988). Disinfection with peroxygens. In: *Critical Reports on Applied Chemistry*, K.R. Payne (ed.), vol. 23, John Wiley & Sons, Chichester, pp. 91-116.

Booth R.A. and Lester J.N. (1995). The potential formation of halogenated by-products during peracetic acid treatment of final sewage effluent. *Wat. Res.*, 29(7), 1793-1801.

Campbell A.T., Robertson L.J., Snowball M.R. and Smith H.V. (1995). Inactivation of oocysts of Cryptosporidium Parvum by ultraviolet irradiation. *Wat. Res.*, 29(11), 2583-2586.

Crathorne B., Fawell J., Irving T., Harris N., Denny S., Whitmore T., Horth H., Roddie B., Smith D.J. and Taylor L. (1991). *Sewage disinfection: by-product formation, ecotoxicology and microbiological efficacy*, Report NR 2727, Water Research Centre, Medmenham, UK.

Elsinore Valley Municipal Water District and National Water Research Institute (1994). *A comparative study of UV and chlorine disinfection for wastewater reclamation*, Executive Summary, Montgomery, Pasadena, USA.

IWSA (1997). *The practice of chlorination: application, efficacy, problems and alternatives*. International Water Supply Association Blue Pages, August 1997.

Karanis P., Maier W. and Seitz H. (1992). UV sensitivity of protozoan parasites. *J. Water SRT-Aqua*, 41(2), 95-100.

Langlais B., Reckhow D. and Brink D. (1991). *Ozone in water treatment - Application and engineering*. Lewis publishers, Chelsea.

Lefevre F., Audic J.M. and Ferrand F. (1992). Peracetic acid disinfection of secondary effluents discharged off coastal seawater. *Wat. Sci. Tech.*, 25(12), 155-164.

Liberti L., Notarnicola M., Zicari S., Carnimeo D., Arena R. and Portincasa F. (1996). Advanced disinfection for municipal wastewater reuse in agriculture: first results with UV treatment of clariflocculated and filtered secondary effluent. In: *Proc. of International Workshop on sewage treatment and reuse for small communities: mediterranean and european experience*, R.Choukr-Allah (ed.), Agadir, Morocco, pp. 425-436.

Liberti L., Lopez A. and Notarnicola M. (1998). UV disinfection of clarified secondary effluent for municipal wastewater reuse in agriculture. In: *Proc. of Disinfection '98 - WEF Specialty Conference*, Water Environ. Fed. (ed.), Alexandria, pp. 47-57.

Liberti L., Lopez A. and Notarnicola M. (in press). Disinfection with peracetic acid for municipal wastewater reuse in agriculture. *J. Instn. Wat. & Envir. Mangt.*

Linden K.G., Soriano G.S. and Darby J.L. (1998). Investigation of disinfection by-product formation following low and medium pressure UV irradiation of wastewater. In: *Proc. of Disinfection '98 - WEF Specialty Conference*, Water Environ. Fed. (ed.), Alexandria, pp. 137-147.

Mandra V., Lazarova V., Dumoutier N. and Audic J.M. (1996). Etude comparative de la desinfection des eaux residuaires urbaines par l'acide peracetique, l'irradiation UV et l'ozone. In: *Proc. of JIE96, Conf. du traitement des Eaux et des nuisances de poitiers*.

Masschelein W.J. (1991). *Ozone et ozonation des eaux*. IO$_3$A Comitè Europeen, Lavoisier-Tec&Doc, Paris.

Minear R.A. and Amy G.L. (1996). *Disinfection by-products in water treatment*. CRC Press, New York.

Rudd T. and Hopkinson L.M. (1989). Comparison of disinfection techniques for sewage and sewage effluents. *J. Instn. Wat. & Envir. Mangt.*, 3, 612-618.

Schechter D. and Singer P. (1995). Formation of aldehydes during ozonation. *Ozone Sci. & Eng.*, 17(1), 53-69.

Snider K.E., Darby J.L. and Tchobanoglous G. (1991). *Evaluation of ultraviolet disinfection for wastewater reuse applications in California*, Report of Dept. of Civil Engineering, University of California, Davis, USA.

US-EPA (1986). *Municipal wastewater disinfection-Design manual*, Report EPA/625/1-86/021, United States Environmental Protection Agency, Washington, USA.

US-EPA (1992). *Ultraviolet Disinfection Technology Assessment*, Report EPA 832-R-92-004, United States Environmental Protection Agency, Washington, USA.

von Gunten U. and Hoignè J. (1992). Factors controlling the formation of bromate ion during ozonation of bromide-containing waters. *J. Water SRT - Aqua*, 41(5), 299-304.

von Sonntag C. and Schuchmann H.P. (1992). UV disinfection of drinking water and by-product formation - some basic considerations. *J. Water SRT - Aqua*, 41(2), 67-74.

WEF (1996). *Disinfecting Wastewater for Discharge & Reuse. Proceedings of WEF Specialty Conference*, Water Environment Federation, Alexandria, USA.

WERF (1995). *Comparison of UV irradiation to Chlorination: guidance for achieving optimal UV performance*, Project Report 91-WWD-1, Water Environment Research Foundation, Alexandria, USA.

J. Amer. Soc. Hort. Sci. 119(3):563–567. 1994.

Effect of Ozone and Storage Temperature on Postharvest Diseases and Physiology of Carrots (*Daucus carota* L.)

Chiam L. Liew[1] and Robert K. Prange[2]

Research Station, Agriculture Canada, Kentville, N.S. B4N 1J5, Canada

Additional index words. Daucus carota, Botrytis cinerea, Sclerotinia sclerotiorum, respiration, electrolyte leakage, color

Abstract. **Effects of ozone and storage temperature on carrots and two postharvest pathogens—*Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* de Bary—were investigated. Pathogen-inoculated and uninoculated whole carrots were exposed to an ozone concentration of 0 (control), 7.5, 15, 30, or 60 μl·liter^{-1}. Treatment chambers were flushed with a total flow rate of 0.5 liters·min^{-1} (air and ozone) for 8 h daily for 28 days. The experiment was repeated twice at storage temperatures of 2, 8, and 16C. The residual ozone concentration (ozone supplied–exhausted and reacted ozone) increased with ozone supply concentration but was less at higher storage temperatures. A 50% reduction of daily growth rates of both fungi at the highest ozone concentration indicated that ozone was fungistatic. Carrot respiration rate, electrolyte leakage, and total color differences increased with ozone concentration. Ozone-treated carrots were lighter (higher L* values) and less intense (lower chroma values) in color than control carrots.**

Watery soft rot (*Sclerotinia sclerotiorum*) and gray mold (*Botrytis cinerea*) are common postharvest diseases of carrots. Until recently, growers used the fungicide benomyl to control these storage rots. The manufacturer's withdrawal of benomyl as a postharvest fungicidal dip on carrots has necessitated a search for alternatives; ozone is one potential candidate.

Ozone is the tri-atomic form of oxygen that is unstable and decomposes either spontaneously or after it comes in contact with oxidizable surfaces. Ozone's high oxidation potential (Lide, 1991) makes it an effective disinfectant for poultry hatcheries, poultry chill water, water treatment plants, soft-drink bottling plants, and ...ria water (Guinvarch, 1959; Rosenthal and Wilson, 1987; ...eldon and Brown, 1986; Torricelli, 1959; Whistler and Sheldon, 1989; Yang and Chen, 1979). Since ozone can be easily and economically generated on site, transportation and storage costs are not incurred. Unlike CO_2 and N, which are two gases commonly used in storage, ozone has a characteristic odor. For this reason, harmful levels of ozone can be instantly detected and avoided by workers.

Ozone can be applied as a gas or as ozonated water. Reported differences on the effectiveness of ozone as a storage disinfectant may be due to differing application methods or measurements of ozone concentration, treatment period, and pathogen and product sensitivity to ozone. Gibson et al. (1960) and Rice et al. (1983) concluded that ozone is an effective agent for controlling microbial and fungal pathogens in stored produce such as cheese, strawberries, raspberries, currants, bananas, and potatoes. Continuous exposure to 0.05 ppm ozone (gas) effectively killed *Escherichia coli* after 3 days and *Staphylococcus aureus* in vitro after 15 days (Kashiwagi et al., 1987). Ogawa et al. (1990) demonstrated that spores of *B. cinerea* on the surface of tomatoes were inactivated by exposure to 3.8 g of ozone/ml of water in 10 min. Other reports, however, have suggested that ozone is ineffective in controlling rots on apples, muskmelons, peaches, strawberries, blueberries, and green beans. Ozonated apples, cantaloupes, cranberries, corn kernels, and cereal grains demonstrated more decay or damage than those not ozonated (Barger et al., 1948; Brooks and Csallany, 1978; Naito, 1989; Norton, 1968; Schomer and McCulloch, 1948; Spalding, 1968). The objectives of this study were 1) to determine the residual concentration of ozone, 2) to determine the effect of ozone on the two major storage pathogens of carrots, and 3) to observe ozone-induced changes in carrot physiology and quality during storage.

Received for publication 24 Mar. 1993. Accepted for publication 20 Sept. 1993. This study was supported in part by a fellowship from Acadia Univ., Wolfville, N.S. We thank K.B. McRae (statistician) for advice on experimental planning, statistical analysis, presentation of results, and critical reviews of the manuscript in draft form and P.D. Hildebrand (plant pathologist) for advice and assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Graduate student.

[2]Postharvest physiologist.

Methods and Materials

'Vitabrite' carrots, obtained from a local grower (Berwick, N.S.), were hand-washed and stored at 0C until use. Crown diameters of the carrots were from 3 to 4 cm.

An ozone generator (Tri-Ox, Swindon, England) was set to produce 76.5 μl·liter^{-1} of ozone in air. Air containing ozone at flow rates of 0 (control), 0.05, 0.1, 0.2, or 0.4 liters·min^{-1} were blended with compressed air to produce ozone concentrations of 0, 7.5, 15, 30, or 60 μl·liter^{-1}, in a total flow of 0.5 liters·min^{-1} for each treatment. Ozone and compressed air flows were controlled with needle valves (Nupro Co., Willoughby, Ohio). Treatment chambers consisted of air-tight 64-liter polyvinylchloride containers (IPL, St. Damien, Que.) placed in storage rooms set at 2, 8, or 16C. The chambers were flushed continuously for 8 h daily for 28 days.

Ozone concentrations in the chambers were monitored during the treatment period with an ultraviolet-based detector with a measurement range of 0 to 100 μl·liter^{-1} at 253.7 nm (ozone analyzer model IN-2000-5; In-USA, Newtonville, Mass.). Values presented in Table 1 represent stable concentrations after 4 h of treatment.

Disease. Isolates of *S. sclerotiorum* and *B. cinerea* were obtained from infected carrots in local storage. Fungal stock cultures and inoculum were maintained on potato dextrose agar (PDA) (Becton-Dickinson, Cockeysville, Md.).

A 1.0-cm-diameter mycelial plug, obtained near the margin of a 4- to 5-day-old fungal culture, was placed in a wound of each carrot 1.0 ± 0.5 cm from the crown. The wound was a 1.0-cm-diameter × 0.5-cm-deep depression created with a 1.0-cm-diam-

eter corkborer. Fungal growth along the length of the carrot was measured once every 7 days for 28 days. A sample of 10 carrots was inoculated separately with each fungus for the 15 combinations of ozone concentration and temperature. The entire experiment was repeated twice.

Physiology. Fifteen carrots from each treatment were monitored for weight loss and color changes over the 28-day test period (initial and final measurements were recorded). Carrot moisture loss was measured as percentage weight loss [(initial weight – final weight)/initial weight × 100]. Initial and final carrot color readings, using Commision International d'Eclairage (CIE) L*, a*, b* color space coordinates, were obtained with a tristimulus colorimeter. The Chroma Meter CR-200 (Minolta, Ramsey, N.J.) has an 8-mm-diameter measuring port that uses diffuse illumination and a 0° viewing angle. The meter was calibrated with a white standard calibration plate. Colorimetric values for hue (color) and chroma (intensity) were calculated as chroma = $\sqrt{(a^2 + b^2)}$ and hue = $\cos^{-1}[a/\sqrt{(a^2 + b^2)}]$ (Little, 1975). Total color difference was calculated as $(\Delta E) = \sqrt{[(L_{fin} - L_{ini})^2 + (a_{fin} - a_{ini})^2 + (b_{fin} - b_{ini})^2]}$ (Gnanasekharan et al., 1992). Color measurements were taken between 1 to 2 cm from the carrot crown for initial and final readings.

After 28 days, five preweighed carrots were selected from each ozone treatment for respiration measurements at 9C. The carrots were placed in Nalgene jars and continuously flushed with 25 to 35 ml·min^{-1} of CO_2-free air. Carbon dioxide evolution rates were measured daily for 5 days with a gas chromatograph (model 3400; Varian Instruments, Mississauga, Ont.) equipped with a CRT-1 column (Alltech Assn., Guelph, Ont.) and a thermal conductivity detector.

A conductivity meter (Radiometer Conductivity Meter, Copenhagen) was used to measure electrolyte leakage of 5 carrots after the experimental period. Initial electrolyte leakage was determined after a 24-h incubation of 1 g of carrot peel from each carrot in 20 ml of distilled water at 5C. The water contained 1 µl·liter^{-1} Tween 20 to reduce surface tension (Prange and Lidster, 1991). The peels and bathing solution were then frozen at –18C and held for another 24 h before total conductivity was measured on the thawed solution. Percent total solute leakage was calculated as initial conductivity/final conductivity × 100 (Beckerson and Hofstra, 1980).

The two factors, temperature and ozone concentration, were arranged in a split-plot design and replicated twice. Temperatures were assigned to the storage rooms (main plot), and the ozone concentrations (split plot) were randomized to chambers within each storage room. The results were analyzed using the analysis of variance directive in Genstat 5 (Payne et al., 1988).

A logarithmic ($\log_{10}$) transformation was used to stabilize variance of fungal growth rates. Mean log values were back-transformed and are presented in parentheses in Table 2.

Results

Ozone. The quantitative factor of ozone concentration can be expressed as either supplied or residual ozone concentration. Supplied ozone concentration refers to the ozone concentration provided to the chambers, whereas residual concentration was the ozone concentration measured in a chamber containing experimental carrots (residual concentration = supplied concentration – exhausted and reacted ozone) (Table 1).

In this study, residual ozone concentration seemed to be influenced by temperature and supplied ozone concentration (Table 1). It seems that the rate of ozone depletion increased with temperature since higher temperatures produced lower residual ozone concentrations.

To help identify the influence of ozone concentration at each of the temperatures, regression coefficients of the variate means at the three temperatures have been provided in Tables 2–4. Significant differences in regression coefficients among the treatment temperatures would indicate a different linear effect of ozone concentration for each temperature.

Disease. The fungal inoculum was applied to wounded carrots to ensure disease development. Fungal surface growth included spores, sclerotia and mycelia of *B. cinerea,* and only sclerotia and mycelia of *S. sclerotiorum.* Both fungi developed in the ozonated environment but exhibited varied mycelial growth rates and surface structure (sclerotia and spores) development on the carrots. At 2C there were fewer surface structures present with increasing ozone concentration (data not shown). At the highest ozone concentration (60 µl·liter^{-1}), water-soaked lesions (cellular necrosis) were the only indicators of fungal growth; however, mycelia developed on the carrots after removal from the ozonated atmospheres.

Growth rates of *B. cinerea* and *S. sclerotiorum* increased with temperature, with most of the increase occurring between 2 and 8C (Table 2). A trend of decreased growth rate with increased ozone concentration was observed within each storage temperature. The growth rate for *B. cinerea* at 2, 8, and 16C at 60 µl·liter^{-1} ozone was 43% to 58% of the respective control treatment.

Although growth rate of *S. sclerotiorum* increased with storage temperature, the magnitude of the increase was reduced with higher ozone concentration (Table 2). The growth rate of *S. sclerotiorum* at 2, 8, and 16C was reduced to between 44% to 63% at 60 µl·liter^{-1} compared with the respective control treatments. The lower linear and quadratic regression coefficients at 2C for *S. sclerotiorum* than at 8 and 16C suggests that the higher residual ozone concentration at 2C (Table 1) could have caused a greater reduction in fungal growth rate than those observed at 8 and 16C. This effect was not observed for *B. cinerea.*

Physiology. Ozone did not affect carrot weight loss (data not presented). Carrot respiration rate at 9C generally increased with ozone concentration, with a mean ranging from 18.2 mg CO_2/kg per h in the control to 20.7 mg CO_2/kg per h at 60 µl·liter^{-1} ozone (Table 3). Even though the respiration rate was higher in carrots treated with ozone, carrot leaves, which appeared at 8 and 16C, were fewer, shorter, and necrotic in the treatments receiving ozone (data not shown). The presence of carrot leaves decreased with increasing ozone concentration.

Ozone treatment increased electrolyte leakage from 37.4% in the control to 48.8% at 60 µl·liter^{-1} ozone. The highest electrolyte leakages occurred at 2 and 8C. The linear regression coefficient at 16C was lower than at 2 and 8C and could be caused by the existence of higher residual ozone concentrations at the two lower temperatures (Table 1).

Visual observations of carrots agreed with the results of electrolyte leakage. Carrot surfaces in ozone treatments were pitted with dry white blotches, which intensified with increasing ozone concentration. Visible injury in the form of brown water-soaked lesions also appeared on carrot leaves treated with

Table 1. The effect of temperature and ozone supply on residual concentration.

Temp	Ozone supply (µl·liter^{-1})				
(°C)	0	7.5	15	30	60
2	0	1.0	3.1	10	22
8	0	1.0	3.0	6.7	20
16	0	0.6	1.8	3.0	18

Table 2. Daily growth rate of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on carrots at different temperatures and ozone concentrations in storage.

Storage temp (°C)	Ozone concn (µl·liter^{-1}) 0	7.5	15	30	60	Regression coefficient ($\times 10^{-3}$) Linear	Quadratic[z]
			Botrytis cinerea (log_{10} mm·day^{-1})				
2	−0.302	−0.396	−0.459	−0.629	−0.667	−6.0	0.14
	(0.499)[y]	(0.401)	(0.347)	(0.237)	(0.215)		
8	0.306	0.250	0.130	0.090	0.071	−6.3	0.12
	(2.02)	(1.78)	(1.35)	(1.23)	(1.18)		
16	0.379	0.323	0.117	0.044	0.036	−6.8	0.17
	(2.39)	(2.10)	(1.31)	(1.11)	(1.09)		
SEM[x] (n = 20, df = 12): 0.0417					SE (n = 2, df = 3):	0.620	0.037
Significant effects ($P < 0.05$): $T,O_{l,q}$[w]							
			Sclerotinia sclerotiorum (log_{10} mm·day^{-1})				
2	−0.275	0.331	−0.395	−0.498	−0.631	−5.9	0.05
	(0.531)	(0.466)	(0.403)	(0.318)	(0.234)		
8	0.472	0.389	0.315	0.345	0.271	−2.7	0.06
	(2.96)	(2.49)	(2.07)	(2.21)	(1.87)		
16	0.607	0.514	0.487	0.406	0.382	−3.4	0.10
	(4.05)	(3.27)	(3.06)	(2.55)	(2.41)		
SEM (n = 20, df = 12): 0.0497					SE (n = 2, df = 3):	0.65	0.038
Significant effects ($P < 0.05$): $T,O_{l,q}$, $T \times O_l$							

[z]Orthogonalized coefficient.
[y]Back-transformed mean (mm·day^{-1}).
[x]To compare effect of temperature within ozone supply concentration.
[w]T = temperature, O_l = linear effect for ozone, O_q = quadratic effect for ozone.

Table 3. Respiration rate and electrolyte leakage of carrots stored for 28 days at different temperatures and ozone concentrations.

Storage temp (°C)	Ozone supply concn (µl·liter^{-1}) 0	7.5	15	30	60	Regression coefficient ($\times 10^{-2}$) Linear
			Respiration rate (mg CO_2/kg per h)[z]			
2	11.4	11.4	13.4	16.8	15.3	0.75
8	19.7	17.3	18.2	17.8	22.8	0.65
16	23.4	23.5	20.4	21.6	24.2	0.16
SEM[y] (n = 2, df = 12): 2.21					SE (n = 2, df = 3):	0.344
Significant effects ($P < 0.05$): O_l[x]						
			Electrolyte leakage (%)			
2	39.3	44.2	41.3	43.2	53.2	20.8
8	36.3	42.6	41.4	43.6	53.1	24.6
16	36.6	36.9	36.3	40.8	40.0	7.1
SEM (n = 10, df = 12): 3.38					SE (n = 2, df = 3):	2.7
Significant effects ($P < 0.05$): O_l, $T \times O_l$						

[z]Respiration rate is the mean of five daily measurements at 9C.
[y]To compare effect of temperature within ozone supply concentration.
[x]T = temperature, O_l = linear effect for ozone.

the highest ozone concentration at 2C.

The normal orange–red surface color of carrots appeared to be bleached on ozone-treated carrots. This was reflected in mean L* values, which increased from 49.6 in the control to 52 at an ozone concentration of 60 µl·liter^{-1} (Table 4). Ozone also affected the intensity of carrot color. Mean chroma values of 30.2 in the control decreased to 28.7 at 60 µl·liter^{-1} ozone (Table 4). Although not significant, the decrease in mean chroma was greatest at 2C, followed by 8 and 16C. Although hue was unaffected by the ozone treatments, higher hue values were obtained at a storage temperature of 8C (Table 4). Mean total color differences in the control increased from 4.2 to 6.6 when treated with an ozone concentration of 60 µl·liter^{-1} (Table 4).

Discussion

Although some storage studies have reported ozone concentrations as residual ozone (Schomer and McCulloch, 1948; Spalding, 1968), experiments using residual ozone concentration may not be appropriate since residual ozone concentration may be affected by temperature and reactivity of exposed materials. Ewell (1933) reported that applying 1 ppm ozone to produce such as eggs, apples, and beef (with similar amounts of surface area) resulted in different residual ozone concentrations. This study indicated that the residual concentration was influenced by temperature. Increasing the storage temperature would increase the amount of ozone required to maintain a specific residual concentration. Therefore,

Table 4. Lightness, chroma, hue and total color difference of carrots stored for 28 days at different temperatures and ozone concentrations.

Storage temp	Ozone concn ($\mu l \cdot liter^{-1}$)					Regression coefficient ($\times 10^{-2}$)	
(°C)	0	7.5	15	30	60	Linear	Quadratic
			Lightness (L)*				
2	49.5	50.2	51.2	51.4	51.5	2.9	–0.17
8	49.3	49.8	50.4	51.2	52.1	4.6	–0.059
16	49.9	50.7	50.9	51.2	52.3	3.5	–0.019
SEM[y] (n = 30, df = 12): 0.558					SE (n = 2, df = 3)	0.686	–0.0408
Significant effects ($P < 0.05$): $O_{l,q}$[x]							
			Chroma				
2	30.2	29.4	28.4	27.1	26.6	–5.8	
8	29.7	31.1	31.4	30.3	28.8	–2.8	
16	30.6	31.0	30.9	30.1	30.6	–0.6	
SEM (n = 30, df = 12): 1.21					SE (n = 2, df=3):	1.46	
Significant effects ($P < 0.05$): O_l							
			Hue				
2	49.0	47.2	47.6	46.7	47.1	–2.2	
8	49.1	50.6	48.8	49.1	48.8	–1.4	
16	48.3	48.3	49.3	48.8	48.2	–0.29	
SEM (n = 30, df = 12): 0.694					SE (n = 2, df = 3):	1.58	
Significant effects ($P < 0.05$): T[x]							
			Total color difference				
2	3.3	3.9	3.8	4.3	5.8	3.4	
8	4.4	2.8	3.9	3.8	7.3	5.8	
16	4.9	3.5	4.9	4.9	6.6	3.7	
SEM (n = 30, df = 12): 1.07					SE (n = 2, df = 3):	1.94	
Significant effects ($P < 0.05$): O_l							

[z]Orthogonalized coefficient.
[y]To compare effect of temperature within ozone supply concentration.
[x]T = temperature, O_l= linear effect for ozone, O_q= quadratic effect for ozone.

maintaining a constant residual ozone concentration over a storage period will require adjustments in the amount of ozone supplied. These adjustments could be made with an automated gas control system to maintain gas concentrations, such as the ozone application and measurement systems frequently used by environmental and pollution researchers (Hale-Marie et al., 1991).

Our results suggest that the efficacy of ozone as a disinfectant must be individually assessed for each commodity at its ideal storage temperature. Furthermore, the quantity of produce in storage and the system available for dispensing and measuring ozone must be considered.

The effect of ozone on *B. cinerea* and *S. sclerotiorum* was fungistatic and not fungicidal. Although some inhibitory effects were observed with ozone residual concentrations of 10 to 22 $\mu l \cdot liter^{-1}$ at 2C, these concentrations seemed to cause physical and physiological damage to carrots. Symptoms of physiological disruptions included increased respiration rates, electrolyte leakage, and color changes. Higher respiration rates with increasing ozone concentrations are likely an expression of abnormal metabolism or injury caused by ozone to the carrots. Further, alterations in appearance of ozone-treated carrots due to color changes and surface pitting may affect consumer appeal.

The results of this study agree with other studies that have examined the effects of ozone on plants (Beckerson and Hofstra, 1980; Frederick and Heath, 1975; Hewitt et al., 1990; Sakaki et al., 1983; Tomlinson and Rich, 1970). These studies demonstrated that plants exposed to ozone have higher electrolyte leakage compared with control plants (no ozone) and are subjected to pigment destruction (chlorophyll a and carotenoids). Some researchers have speculated that ozone causes some form of lipid peroxidation in the plant cells, a result suggesting that membrane lipids are susceptible to ozone damage. In addition, physiological disruptions to plant tissue caused by ozone treatment may also result in losses of organic and inorganic nutrients.

If ozone is to be used for carrot storage, a balance must be found between preserving carrot quality and effective disease control. This study has demonstrated that reducing temperature from 16 to 2C significantly reduced fungal growth of nonozonated carrots. Immediate ozonation may be unnecessary if carrots are properly harvested, washed, hydro-cooled, and cold-stored at the appropriate temperature. Although commercial carrot storages have air temperatures between 0 and 1C, carrots stored in large bins may build up pockets of heat in the middle of the bins during long storage periods. These heat pockets are prime areas for fungal growth and development. It is often the combination of reduced resistance to infection by the carrots and poor heat removal that encourages pathogen proliferation. With proper air circulation and temperature control, ozonation may be introduced in the latter part of the storage period, when carrots are less resistant to fungal attacks (Goodliffe and Heale, 1978; Harding and Heale, 1980). Even at low temperatures, diseases can spread by mycelial growth and spore production. Since carrot diseases often spread from one root to the next from a focus of infection or from contaminated storage bins, reducing surface fungal growth with ozone may limit the spread of pathogens. Based on our results, an ozone supply of 15 $\mu l \cdot liter^{-1}$ for 8 h a day at 2C could provide some disease protection with a minimum of physical and physiological damage.

Literature Cited

Barger, W.R., J.S. Wiant, W.T. Pentzer, A.L. Ryall, and D.H. Dewey. 1948. A comparison of fungicidal treatments for the control of decay in California cantaloupes. Phytopathology 38:1019–1024.

Beckerson, D.W. and G. Hofstra. 1980. Effects of sulphur and ozone, singly or in combination, on membrane permeability. Can. J. Bot. 58:451–457.

Brooks, R.I. and A.S. Csallany. 1978. Effects of air, ozone, and nitrogen dioxide exposure on the oxidation of corn and soybean lipids. J. Agr. Food Chem. 26:1203–1209.

Ewell, A.W. 1933. Decomposition of ozone. Refrigeration Eng. 26:68–69.

Frederick, P.E. and R.L. Heath. 1975. Ozone induced fatty acid viability changes in *Chlorella*. Plant Physiol. 55:15–19.

Gibson, C.A., J.A. Elliot, and D.C. Beckett. 1960. Ozone for controlling mold on cheddar cheese. Can. Dairy and Ice Cream J. (Dec.):24–28.

Gnanasekharan, V., R.L. Shewfelt, and M.S. Chinnan. 1992. Detection of color changes in green vegetables. J. Food Sci. 57:149–154.

Goodliffe, J.P. and J.B. Heale. 1978. The role of 6-methoxymellein in the resistance and susceptibility of carrot root tissue to the cold storage pathogen *Botrytis cinerea*. Physiol. Plant Pathol. 12:27–43.

Guinvarch, P. 1959. Ozone chemistry and technology. Adv. Chem. Ser. 21. Amer. Chem. Soc., Wash., D.C. p. 416–430.

Hale-Marie, B., R.G. Dutton, O.B. Allen, D.P. Omrod, S.N. Goodyear, and L.G. Pyear. 1991. Design and verification of a programmable gas dispensing system for exposing plants to dynamic concentrations of air pollutants. J. Air Waste Mgt. Assn. 41:460–463.

Harding, V. and J.B. Heale. 1980. Isolation and identification of the antifungal compounds accumulating in the induced resistance response of carrot root slices to *Botrytis cinerea*. Physiol. Plant Pathol. 17:277–289.

Hewitt, C.N., G.L. Kok, and R. Fall. 1990. Hydroxyperoxides exposed to ozone mediate air pollution damage to alkene emitters. Nature 344:56–58.

Kashiwagi, Y., T. Ueta, Y. Tsuchiya, H. Ichikawa, and M. Kazama. 1987. Studies on sterilization with ozone gas. Annu. Rpt. Tokyo Metropolitan Res. Lab. of Public Health 38:22–27.

Lide, D.R. (ed.). 1991. CRC Handbook of chemistry and physics. 2nd ed. CRC Press, Boca Raton, Fla. Section 16, p. 16.

Little, A.C. 1975. Off on a tangent. J. Food Sci. 40:410–411.

Naito, S. 1989. The influence of ozone treatment on lipids contained in cereal grains, cereal grain powders, peas, beans and pulse products. Nippon Shokuhin Kogyo Gakkaishi 36:878. (Abstr.)

Norton, J.S., A.J. Charig, and I.E. Demoranville. 1968. The effect of ozone on storage of cranberries. Proc. Amer. Soc. Hort. Sci. 93:792–796.

Ogawa, J.M., A.J. Feliciano, and B.T. Manji. 1990. Evaluation of ozone as a disinfectant in postharvest dumptank treatments for tomato. Phytopathology 80:1020. (Abstr.)

Payne, R., P. Lane, A. Ainsley, K. Bicknell, P. Digby, S. Harding, P. Leech, H. Simpson, A. Todd, P. Verrier, R. White, J. Gower, G. Wilson, and L. Paterson. 1988. Genstat 5 reference manual. Oxford Univ. Press, New York.

Prange, R.K. and P.D. Lidster. 1991. Controlled atmosphere and lighting effects on storage of winter cabbage. Can. J. Plant Sci. 71:263–268.

Rice, R.G., J.W. Farquhar, and J.L. Bollyky. 1983. Review of the applications of ozone for increasing storage times of perishable goods. Proc. 6th Ozone World Congr. Intl. Ozone Assn. p. 41–44.

Rosenthal, H. and J.S. Wilson. 1987. An up-dated bibliography (1845–1986) on ozone, its biological effects and technical applications. Dept. of Fisheries and Oceans. Can. Tech. Rpt. Fisheries and Aquatic Sci. 1542.

Sakaki, T, N. Kondo, and K. Sugahara. 1983. Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation: Role of active oxygens. Physiol. Plant. 59:28–34.

Schomer, H.A. and L.P. McCulloch. 1948. Ozone in relation to storage of apples. U.S. Dept. of Agr. Circ. 765.

Sheldon, B.W. and A.L. Brown. 1986. Efficacy of ozone as a disinfectant for poultry carcasses and chill water. J. Food Sci. 51:305–309.

Spalding, D.H. 1968. Effects of ozone atmospheres on spoilage of fruits and vegetables after harvest. U.S. Dept. of Agr. Mktg. Res. Rpt. 801.

Tomlinson, H. and S. Rich. 1970. Lipid peroxidation. A result of injury in bean leaves exposed to ozone. Phytopathology 60:1531–1532.

Torricelli, A. 1959. Ozone chemistry and technology. Adv. Chem. Ser. 21. Amer. Chem. Soc., Wash. D.C. p. 375–380.

Whistler, P.L. and B.W. Sheldon. 1989. Bactericidal activity, eggshell conductance and hatchability effects of ozone versus formaldehyde disinfection. Poultry Sci. 68:1074–1077.

Yang, P.P.W. and T.C. Chen. 1979. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. J. Food Sci. 44:501–504.

OZONE SCIENCE & ENGINEERING
Vol. 23, pp. 41-51
Printed in the U.S.A.

0191-9512/01 $3.00 + .00
International Ozone Association

The Effect of Ozonation of Humic Acids on the Removal Efficiency of Humic Acid-Copper Complexes via Filtration

Mingta Lin[1] and Douglas G. Klarup[2] *

[1]No. 15, Ln 16, Fiuchung 2nd St.
Taichung, Taiwan R. O. C.

[2]Department of Chemistry, Eastern Illinois University
Charleston, Illinois 61920

* Author to whom correspondence should be addressed

Received for Review: 15 October 1998
Accepted for Publication: 7 March 2000

Abstract

The effect of ozonation on the ability of humic acid to complex/coagulate with copper in aqueous solution was explored. Solutions of commercial humic acid were ozonized for various time periods and changes in total organic carbon, aromatic organic carbon vs. total organic carbon, total acidity, and copper complexation capacity of the humic acid were measured. The results showed that the ability of humic acid to complex copper decreased with exposure to ozone.

Keywords

Ozone; Humic Acid; Copper Removal; Complexation; Drinking Water Treatment;

Introduction

Humic substances (HS) consist of the operationally defined fractions humin (insoluble), humic acid (insoluble in acidic solution), and fulvic acid (soluble). A significant fraction of dissolved organic carbon (DOC) in natural water is comprised of humic and fulvic acids (HA and FA) which play an important role in the transport and speciation of both organic and inorganic pollutants. Neutral organic molecules often are associated with HS due to its hydrophobic nature (Josephson, 1982; Stevenson, 1982; Manahan, 1989) and inorganic metal cations (heavy metals are of particular concern and interest) complex with functional groups contained in HS. As much as 60-70 % of a particular metal in certain natural waters may be associated with HS (Hiraide, et al., 1990).

Ozone may be used to both disinfect and remove dissolved organic carbon (DOC) from drinking water supplies (Arai, et al., 1986; Nakamuro, et al., 1990). The first step in the overall process is a rapid coagulation and filtration of solids, followed by ozone application to degrade the remaining DOC to species which can then be adsorbed on activated carbon filters (Kruithof, et al., 1989). The role of ozone in DOC removal is to remove the color (turbidity) and break up the DOC (Lefebvre, et al., 1990), although success is dependent on the type of HS present.

The purpose of the study reported here was to investigate the effect that ozonation of humic material has on its metal complexation/coagulation capacity. The specific system studied was a

commercial humic acid/copper system, although the results of the study can be generalized to other transition metal/humic substance systems.

Background

The oxidation of humic substances with ozone significantly alters it in many ways (Staehelin and Hoigné, 1985; Xiong, et al., 1992). In the 1960s, a series of studies conducted by Bernatek and co-workers confirmed that ozonation of phenols produces smaller fragments of carboxylic acids and secondary byproducts such as CO, CO_2, and H_2O_2 (Bernatek, et al., 1961; Bernatek and Frengen, 1961; Bernatek and Frengen, 1962; Bernatek and Vincze, 1965). These studies showed that for one phenolic group, ozonation can generate 1 to 4 carboxylic acid groups. Reactions of ozone with carbon-carbon double bonds, including aliphatic and aromatic carbons, were reported to produce aldehydes and hydrogen peroxide (Staehelin and Hoigné, 1985; Gilli, et al., 1990; Nakamuro, et al., 1990). The study by Kruithof, et al. (1989), indicated that, at a maximum ozone dosage of about 2.0 mg O_3/mg DOC, 80% of the UV absorbance was eliminated, and less than 20% of DOC was removed.

Clem and Hodgson (1978) reported that carboxyl groups seemed to be an end product of the ozonation of humic acids and further attack on this group appeared to proceed very slowly. In addition, phenols were more reactive with ozone than were other aromatics. Aliphatic carboxylic compounds were reported to be important end products of HA ozonation (Dogut, et al., 1989; Ueno, et al., 1989; Gilli, et. al, 1990; Takahashi, et al., 1995; Gracia, et al., 1996). In the study of Ueno, et al. (1989), approximately 0.6 mg O_3/mg total organic carbon (TOC) was used to ozonize humic acids, and the products were identified as formic acid, oxalic acid, glyoxylic acid, and mesoxelic acid. In other studies, minor products such as aldehydes, CO_2, and H_2O_2 also were found when ozone dosages were similar or greater than in Ueno's study (Clem and Hodgson, 1978; Dogut, et al., 1989; Ueno, et al., 1989; Gilli, et al., 1990; Nakamuro, et al., 1990).

Besides altering the chemical characteristics of HS, ozonation converts HS molecules into molecules with smaller molecular weights. The study performed by Anderson, et al. (1986), provided a clear illustration showing the effect of fulvic acid (FA) ozonation on different molecular-size portions of the FA. There was a loss of organic carbon from all molecular size fractions at all ozone dosages, and carbon loss was relatively greater from the large size fractions. At low doses (0.4 mg O_3/g TOC) intermediate size fractions (10,000>M.W.>1,000) increased in relative abundance (Anderson, et al., 1986). Similar trends were observed for HA by Takahashi, et. al. (1995).

The factors responsible for the stability and quantity of metal-HA complex formation include pH, ionic strength, metal and HA concentrations, metal species, molecular weight and functional group content of HA (Kerndorff and Schnitzer, 1980; Truitt and Weber, 1981; Ephralm, et. al., 1986; Khalili, 1989; Koul, et. al., 1989; Nor and Cheng, 1989; Chairidchai and Ritchie, 1990). Several studies have suggested that the stability of metal complexes with humic and fulvic acids is strongly related to the metal/HA (or FA) ratio (Bresnahan, et al., 1978; Stevenson, 1982; Nor and Cheng, 1989). Metal-HS bonding occurs via complexation (Stevenson, 1982; MacCarthy and Suffet, 1989) with carboxylic, phenolic, and carbonyl groups and minor involvement of amino, and imino groups (Bresnahan, et al., 1978; Gamble, et al., 1980; Snoeyink and Jenkins, 1980; Stevenson, 1982; Manahan, 1984). At least two types of chelating sites of HA have been identified. They are salicylic-type chelation, which involves one carboxylic and one phenolic group coordinating with a cation; and phthalic-type chelation, in which a cation complexes with two carboxylic groups. The bonding sites are rarely identical in geometry and complex capacity. The chelation could occur on sites 1) on the same aromatic ring, 2) on the same polymer molecule, but not on the same ring, or 3) between two polymer molecules (Gamble, et al., 1980). In the third case. the cation bridge between two macromolecules could result in coagulation of a humate-metal complex (Gamble, et al., 1980; Ruch, 1980). Accordingly, the structure of a metal-HA complex is not limited to bidenate complexes (Khalili, 1989).

Given the number of possible metal-humic substance complexes, and the myriad of effects of ozonation on humic substances, it is difficult to predict exactly how the ozonation of humic acid will affect its metal complexing ability. On the one hand, oxidation of the humic substances may produce more complexing functional groups, such as carboxylic acid groups. On the other hand, the formation of smaller

molecular weight fractions due to ozonation will make the amount of complex formed, particularly as defined by the complexes able to undergo coagulation/filtration, much less. These experiments determined which of these two processes dominates.

Experimental

The experimental procedure included (1) preparation of a humic acid stock solution, (2) ozonizing portions of this solution for varying time periods, and (3) comparing the total acidity, total organic carbon, relative aromatic carbon fractions, and the copper complexation capacity of the modified humic acid solutions.

Preparation of Humic Acid (HA) Stock Solution

Stock solutions of humic acid were prepared by placing 7.0 g of solid humic acid (Aldrich Chemical Company) into a 1 L beaker with about 750 ml of distilled water. The beaker was then covered and the headspace flushed with N_2 gas to protect HA from air oxidation. A pH electrode was lowered into the solution and stirred for 24 hrs under N_2. Due to the pH-dependent solubility of HA, the pH value of HA solution was maintained at 7.0 ± 0.1 with small additions of 0.1 N sodium hydroxide. The addition of sodium hydroxide was as minimal as possible to avoid alkaline degradation of HA which may occur when the pH is greater than 8 (Stevenson, 1982). Once the pH of the HA solution had stabilized within 0.1 - 0.2 unit after 24 hr of stirring, the solution was centrifuged and filtered with vacuum suction (Whatman filter paper No.54, 20-25 μm particles retained). The residual remaining on the filter was repeatedly rinsed with distilled water. The solution was diluted to 1000 ml, stored in a 1 L polyacetyl bottle, and placed in a refrigerator.

To quantify the amount of dissolved HA, the used filter papers with filtered residue were collected, oven-dried at 70°C overnight and placed in a desiccator at room temperature for several hours. The amount of dissolved HA was the initial weight of solid HA plus filter papers minus the dried filter paper with HA residue. Working solutions were prepared from the stock solution.

Ozone Flow Calibration

Ozone was produced by a homemade high-voltage ozone generator. Since the field of the generator could destroy as well as produce ozone, the outlet gas was a mixture of O_3 and O_2 gases, and the ozone concentration in the mixture was low (Clem and Hodgson, 1978).

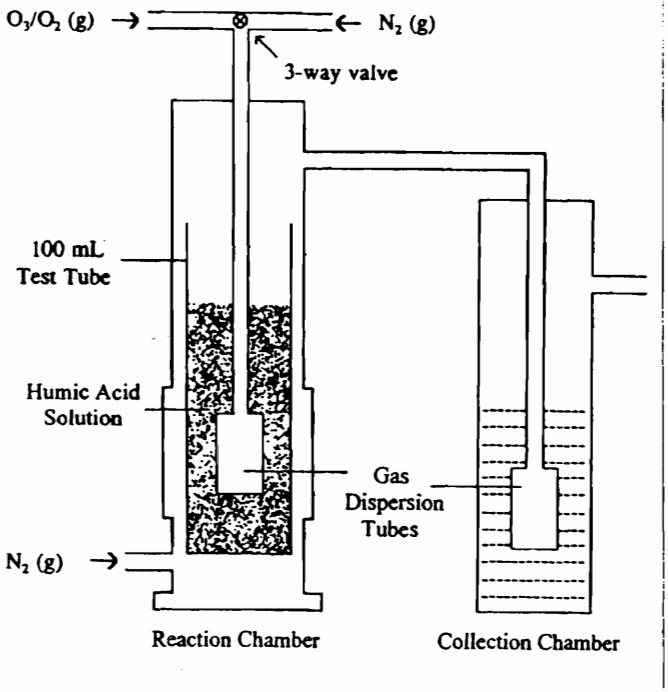


Figure 1. Schematic of the ozonation reaction and collection chambers.

A 30 mL blank (the same amount as the HA solution about to be used in the subsequent experiments) of distilled water was placed in a 100-mL test tube and set up in the ozonation reaction chamber (Figure 1). The O_3/O_2 gas was applied through a gas dispersion tube into the sample at a flow rate of approximately 40 mL/s. A 20 g/L KI solution was prepared and 40 mL of this solution placed in the collection chamber to react with the ozone which runs through and out of the reaction chamber. The O_3/I^- reaction is:

$$O_3 + 2H^+ + 2I^- \leftrightarrows O_2 + H_2O + I_2 \qquad [1]$$

Nitrogen gas was applied from the bottom of the reaction chamber to carry the unreacted ozone and product gasses from the reaction chamber to the KI absorbent solution during ozonation (see Fig. 1). Once the ozonation time was complete, nitrogen gas was applied from both the gas dispersion tube and the bottom of the reaction chamber for 3 minutes to expel the dissolved/residual ozone and possible gas products into the absorbent. The oxidized KI

solution (I_2) was then titrated with standardized sodium thiosulfate solution to quantify the absorbed ozone (Iodometric Method, American Public Health Association, 1986). The sodium thiosulfate was standardized against a $K_2Cr_2O_7$ standard.

Ozonation periods ranging from 2 to 8 minutes were used in these experiments. Each run was repeated three times. The mg of ozone reacting in the collection chamber was (48.0 * N * V)/2, where 48.0 is the molecular weight of ozone, N is the normality of sodium thiosulfate solution, and V is the volume (mL) of $Na_2S_2O_3$ solution used in the titration.

Humic Acid (HA) Ozonation

A 30.0 mL sample of a stock HA solution (ranging from 600 to 1000 mg/L) was placed in a 100-mL test tube, positioned in the reaction chamber and the ozonation process was carried out as described in the "Ozone Flow Calibration" section above. An ozone production rate of 8.1 mg/min was used for all experiments. After flushing the reaction test tube with N_2, it was capped and refrigerated. The blanks were run right after the corresponding sample following the procedures in the "Ozone Flow Calibration" section above. The amount of ozone consumed by HA ozonation was the amount of ozone collected in the blank sample ozonation minus that in HA sample ozonation.

Acidity Titration

The method used to determine the total acidity of HA samples was adopted from the Baryta adsorption method (Stevenson, 1982). The acidity determined with this method accounts for the carboxylic acid groups plus phenolic and/or enolic-OH groups of the humic acids. Because of the slight acidity distinction between samples, the concentrations of the $Ba(OH)_2$ and HCl solutions were about 0.04 N, whereas in the Baryta adsorption method the $Ba(OH)_2$ solution used is saturated and the HCl solution is 0.1 N.

The oxidized HA sample was added to 10.00 mL of 0.04 N $Ba(OH)_2$ solution and the headspace was flushed with $N_{2(g)}$. Three blanks also were prepared by adding 5.0 mL of the same $Ba(OH)_2$ solution to 30 mL of distilled water. The treated samples were shaken overnight, and then filtered through 0.1 μm filter membrane into a 150 mL beaker. The residue was rinsed thoroughly with CO_2 free distilled water. The filtrate was titrated with standardized 0.04 N HCl solution to pH 8.4.

The acidity of the humic acid can be expressed as:

$$Q = (A + B - N * D)/P \qquad [2]$$

where Q is the total acidity of sample (meq/g), A is the initial amount of NaOH added per 30.0 ml of HA stock solution (meq), B is the amount of $Ba(OH)_{2(aq)}$ added in 30.0 ml of sample (meq), N is the normality of HCl (meq/ml), D is the volume of HCl titrated to bring the pH to 8.4 (ml), and P is the amount of HA dissolved in 30.0 ml of HA solution (g).

Copper Complexation Capacity of Humic Acid Samples

Large volumes of different HA samples (ozonized for 2,4, and 6 minutes) were required for these experiments. To achieve this, multiple 30 mL batches of each sample type were first ozonized then combined and used as soon as possible. A HA solution which had not been subjected to ozonation was used for comparative purposes.

A 400.5 ppm copper stock solution quantified by Inductively Coupled Plasma Spectroscopy was prepared by dissolving 1.35 g of $Cu(NO_3)_2.2H_2O$ in 1 L of deionized distilled water, and then stored in the refrigerator for further use.

The copper complexation capacity of the HA was measured by first placing 30.0 ml of the prepared HA solution in a 100-ml test tube and adding 1 ml of the 400.5 ppm copper solution. The total volume was then adjusted to 50 ml. The pH value of the solution was adjusted to pH 5.0 ± 0.1 with a drop of saturated sodium acetate. The main reason that this step requires pH 5 is to avoid the formation of copper hydroxides. This complicates data interpretation somewhat because of possible copper acetate complex formation. Since this procedure was followed for all of the samples, however, the trends in the data are still correct.

The prepared Cu-HA solution was equilibrated for 48 hours. After equilibration, the Cu-HA complex was allowed to settle, and the whole solution filtered through a 0.1 μm filter. The filtrate was then analyzed with Inductively Coupled Plasma Spectroscopy.

This process was repeated using different volumes of Cu solution with the HA samples. Each type of HA solution was treated in this way. The amount of copper removed with humic acid complex was considered to be the initial amount of Cu added to the solution minus the amount of Cu in the filtrate.

Total Organic Carbon

The total organic carbon (TOC) of the original and oxidized humic acid solutions was measured by a carbon dioxide coulometer (Coulometric Inc., model 5011), in combination with a sealed ampoule oxidation apparatus (Coulometric, Inc., model 5040).

A 1.0 ml sample of HA solution (stock and treated) was placed in a clean ampoule, which had been heated at 500°C overnight to remove any organic materials. Distilled water (3.0 mL) and 12% H_3PO_4 (0.2 mL) were added to bring the pH below 2 and remove the inorganic carbon in the sample. The sample was purged with purified oxygen for 10 minutes at a flow rate of approximately 50 cc/min per ampoule. A scoop of potassium persulfate (about 0.2-0.3 g) was then added to each ampoule as oxidant and the ampoules were immediately flame-sealed. The sealed ampoules were placed in a rack and heated in an autoclave at 121°C and 20 psi for 18 hours. The cooled ampoule was opened in the sealed beaker allowing purified air (passed through a 45% KOH solution) carrying the CO_2 gas through stannous chloride and silver scrubbers (to remove potential interferences) into the coulometer, where the coulometric titration took place.

The standard used in this analysis was glycine with a concentration of 25.3 µg/ml. The standard was prepared by placing 4.0 ml of standard solution in an ampoule, and repeating the steps for sample preparation. Each sample (standard and HAs) was duplicated. The main advantage of this method is that large samples (containing 100-300 µg organic carbon) can be used providing low detection limits.

Aromatic Carbon Determination

The study by Traina, et al. (1990) showed that UV Spectroscopy is an effective method to estimate the aromatic organic carbon (AOC) content of humic acids. Hence this method was used to determine the relative aromatic carbon content in the various humic acid samples.

The humic acid solutions were prepared for UV absorptivity measurements by taking 2.0 ml of treated HA solution, adding a drop of 0.05 M NaCl solution, and diluting to 25.0 ml. The solution was then adjusted to pH 7.0 ± 0.1 by small additions of 0.1 M NaOH with a micropipet. Triplicate samples were prepared and allowed to equilibrate for 24 hours for UV spectroscopic analysis.

A Beckman model DU-6, UV-VIS spectrophotometer, equipped with a 1.0 cm path length cell, was used to measure the absorbance of each sample at a wavelength of 272 nm. The wavelength of 272 nm is in the region of overlapping $\pi \rightarrow \pi^*$ transitions for phenolic arenes, benzoic acid, polyenes, and polycyclic aromatic hydrocarbons with ring numbers of two or more (Traina, et al., 1990). The detected absorptivity of each sample was compared with the corresponding TOC value to determine the relative amount of aromatic carbon in the humic acid samples.

Results and Discussion

Ozone Flow Calibration

The purpose of this analysis was to determine how much ozone was collected in the collection chamber when only distilled water was used in the reaction chamber. The amount of ozone collected reflects the amount of ozone generated as well as the amount which decomposes in water alone. Ozone does exhibit some instability in water (Anderson, et. al., 1986).

Table I shows the amounts of ozone collected as a function of ozonation time. The slope of the line generated from this data, which is the ozone production rate, is 8.1 mg O_3 /min (R^2 = 1.0). The standard errors of the mean of ozone collected after various ozonation times (S_m) varied from 0.37 (0.8 %) to 1.37 mg (4.5 %). This may reflect the re-installation of the reaction chamber between samples. During re-installation, the ozone flow was directed, via a two-way switch, to a polyvinyl bottle with approximately 300 mL of saturated KI solution. The pressure difference between the sample solution (30 mL) and the saturated KI solution when the ozone flow was first directed back to the reaction chamber may have affected ozone flow for a short while. The instability would have been more significant at the shorter ozonation times.

Table I. Amount of O_3 collected within various periods

Ozonation Period (min)	Ozone Collected (mg)
2	14.84 ± 0.42
4	30.14 ± 1.37
6	46.64 ± 0.37
8	63.28 ± 0.52

Coulibaly and Jensen's study (1991) indicated that the consumption of ozone by distilled water increased almost proportionally with the application periods at a rate of approximately 0.25 mg O_3/L-min. For 30 ml of distilled water (the volume of each sample in this study), the ozone consumption would have been approximately 0.0075 mg/min., which is within the errors of measurements of this study. Therefore, the quantity of ozone consumed by water was neglected and the data in Table I were used as measures of the amount of ozone which was collected in the absence of any humic acid in the reaction chamber, i.e., total ozone produced.

Estimation of Ozone Consumption

To determine the ozone consumption by HA solutions we posed two questions: 1) how much ozone introduced into the solution was consumed by water, and 2) how much reacted with the humic acid. The ozone consumption was considered to be the difference between the amount of ozone collected after reacting with the HA solution for the designated period and the corresponding value of the blank curve previously obtained. The results are shown in Table II and plotted in Figure 2.

Table II. Ozone consumption during HA ozonation

Ozonation Period (min)	Ozone Consumption (mg/mg HA)
2	0.104 (± 15.2%)
4	0.149 (± 9.1%)
6	0.219 (± 6.2%)
8	0.255 (± 5.4%)

As presented in Figure 2, no obvious cut-off of the curve was observed. The ozone consumption of the same amount of HA increased gradually with ozonation period, and a little larger consumption rate was found in the early stages of the ozonation. This observation correlated with that of other researchers' (Anderson, 1986; Xiong, et al., 1992). The amount of ozone consumed by HA solution within 2 minutes of ozonation is approximately 0.1 mg O_3/mg HA, where it is 0.26 mg O_3/mg HA for samples ozonized for 8 minutes.

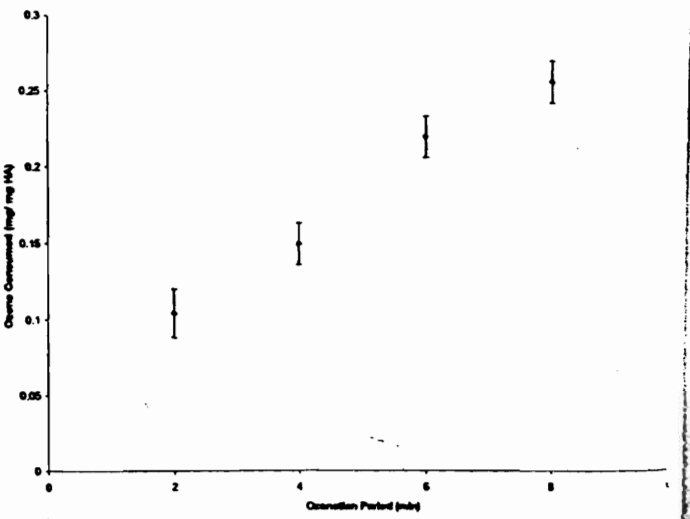


Figure 2. Ozone consumption by humic acid as a function of ozonation time.

A notable phenomenon observed while ozonizing the humic acid samples was that the color of HA solution gradually faded from the original dark brown to a pale straw color as the ozonation periods increased. This implies alteration of the chemical characteristics of the HA solution. Foams on top of the HA solution also were observed within seconds after ozone was applied.

Total Organic Carbon and Aromatic Carbon

As discussed above, two major pathways lead to the degradation of organic carbon by ozonation of HA solutions: 1) ozone reacts with carboxyl groups on smaller molecules and converts them into carbon dioxide; and 2) ozone attacks phenolic groups and produces carboxylic acids and carbon dioxide. As our results show (see Table III and Figure 3), the reaction seemed to proceed more rapidly in the early stages of ozonation. This observation agrees with those reported by others (Anderson, et al., 1986; Takahashi, et al., 1995). In this study, approximately 6% of TOC was removed when HA was ozonized for 1 minute, and only 13.5% was removed after 6 minutes of ozonation.

Ozonation degrades the aromatic organic compounds (AOC) of HA via two major pathways: 1) ozone attacks C=C double bonds to form aldehydes and hydrogen peroxide; and 2) ozone reacts with phenolic groups forming carboxylic acids and carbon dioxide. Following the methodology of Traina (Traina, et al., 1990), a UV wavelength of 272 nm was used in this study to determine the relative content of aromatic compounds in the samples.

Table III. Degradation of TOC and AOC

Ozonation Period (min)	TOC (mg/L)	Absorbance at 272 nm	(Absorbance at 272 nm) / TOC
0	180.0	0.659	0.00366
1	169.2	0.555	0.00328
2	159.2	0.485	0.00303
3	150.4	0.412	0.00274
4	148.4	0.402	0.00271
5	146.1	0.386	0.00264
6	145.7	0.335	0.00230

Our results indicate that about 16% of aromatic compounds were removed after 1 minute of ozonation, and approximately 50% were removed by ozonizing the HA for 6 minutes. A relatively larger removal efficiency at the early stages of ozonation was also observed, which is in agreement with the results reported by other investigators (Anderson, et al., 1986; Kruithof, et al., 1989; Lefebvre, et al., 1990). A difference of our observation from others' (see Figure 4) is that there is an increase of the removal rate at 6 minutes, which may imply that further effective reduction of aromatic compounds would occur if ozonation were carried on for longer period of time.

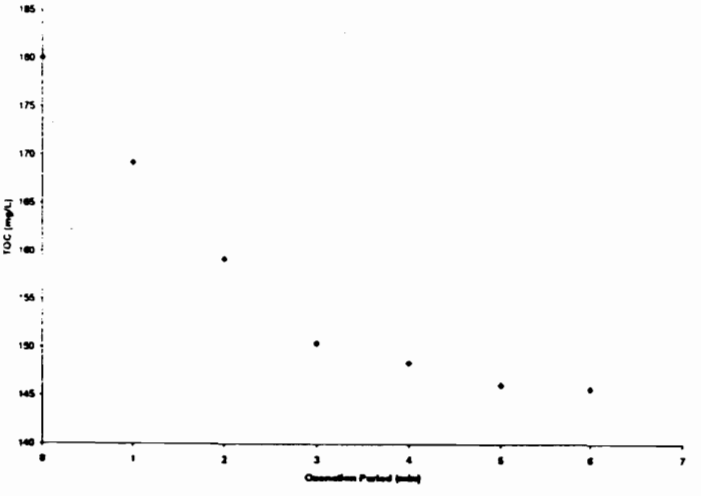


Figure 3. Total organic carbon remaining in the humic acid solutions as a function of ozonation time.

By comparing the results of the TOC and AOC analyses, it is apparent that the (272 nm absorbance)/TOC ratio decreased as ozonation continued (see Figure 5). Based on the pathways discussed above, this result suggests the pathway which converts C=C double bonds into aldehydes and hydrogen peroxide was significant in the HA ozonation. It shows that this pathway competes with, and may even dominate, the oxidation of phenolic groups into carboxylic acids.

Total Acidity

The total acidity of the humic acid used in this study was determined as 7.3 meq/g HA, which fell into the range reported in other studies (Manka, et al., 1974; Stevenson, 1982; Jekel, 1986; Khalili, 1989; Xiong, et al., 1992). The total acidity of the ozonized HA did not significantly increase with the amount of ozone applied. With two minutes of ozonation at an ozone supply rate similar to that of this experiment, the pH of distilled water was reduced to 6.5. With a maximum of 8 minutes of ozonation, the pH was reduced to 4.5, and no further significant shifting of pH occurred as ozonation continued. A similar result was reported by Coulibaly and Jenson (1991). A solution with a pH of 4.5 could account for only about 0.03 meq/L of acidity. This amount is negligible compared to the acidity attributable to the HA. Our results indicate that the acidity of HA does not decrease while the humic acid is degraded by ozonation. The result is shown in Table IV.

Table IV .Total Acidity of HA Samples with Different Levels of Ozonation

Ozonation Period (min)	Total Acidity (meq/g HA)
0	7.3
2	7.3
4	7.4
6	7.4
8	7.5

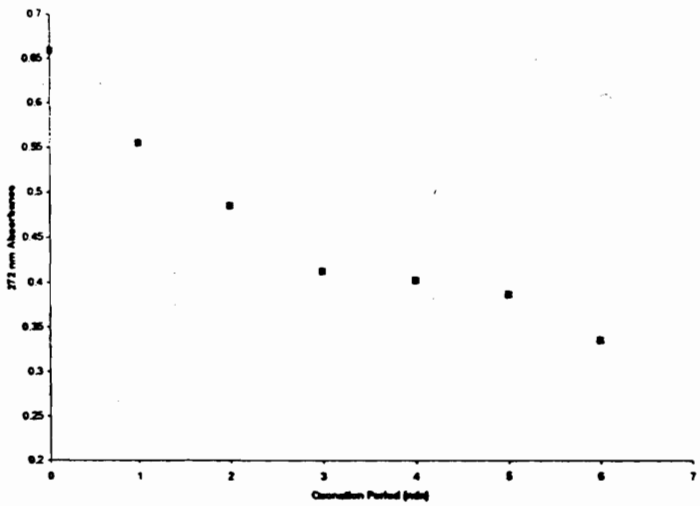


Figure 4. Solution absorbance at 272 nm as a function of ozonation time.

One of the major pathways by which ozonation of HA could generate carboxylic acids is the reaction of ozone with phenolic groups. With one phenolic group, ozonation could produce 1-4 carboxylic acids, which means for every phenolic group, possibly 0-3 more acidic groups are generated undergoing this pathway. Comparing the result of the TOC analysis to that of total acidity, the higher TOC degradation rate in the early stages of ozonation is not associated with a higher total acidity. As a consequence, we may presume that in these stages, the phenols → carboxylic acids reactions, which produce less carboxylic acids (1 or 2) dominate over the reactions which produce more carboxylic acids (3 or 4). In addition, considering the relative content of phenolic groups in HAs (20-30% of total acidity), and the ozone dosages applied in this study, the observation of insignificant increase in total acidity through ozonation suggests that the phenols → aldehydes reaction is important or may even dominant in the ozonation of HA.

Referring to the literature and the previous discussion, we conclude that ozonation of HA has the following effects on humic acid chemistry: 1) as evidenced by the loss of TOC, some carboxylic acid groups, probably from the lower molecular weight fraction, are converted to carbon dioxide; 2) the (272 nm absorbance)/TOC result suggests there is an increase in the relative abundance of aliphatic compounds in the system; 3) there is no overall increase of acid functional groups, indicating that the ozonation of a single phenolic groups leads either to a single carboxylic acid group or aldehyde formation.

Copper Removal Efficiency

It has been widely accepted that materials passing through a 0.45 μm filter in an aqueous system are functionally defined as "dissolved". In reality, some materials matching this definition do exist in aqueous systems in colloid form rather than truly dissolved substances. Therefore, we chose 0.1 μm as the threshold, defining that particles, which fail to pass through a membrane with this pore size, are not dissolved.

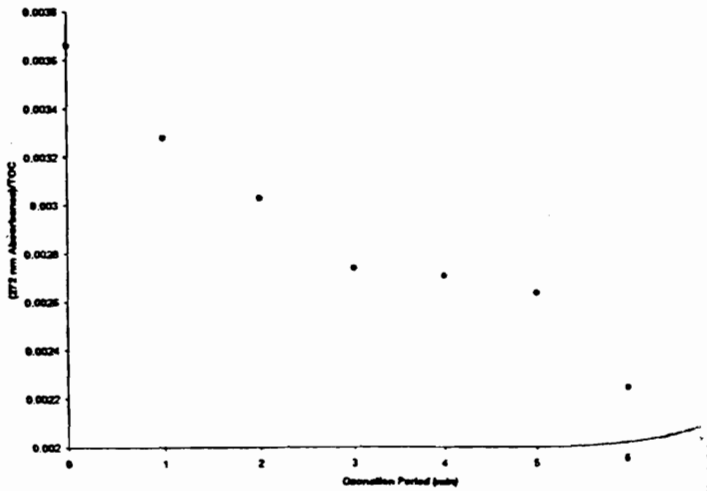


Figure 5. The ratio of solution absorbance at 272 nm to total organic carbon as a function of ozonation time. The decrease suggests that aromatic organic carbon is preferentially degraded.

In our results (see Table V and Figure 6), given the same amount of humic acid and copper concentration, the quantity of copper removed decreased as the ozone doses increased. With the

separation method applied in this study (filtration), our result shows that even under slight ozone doses, the amount of Cu-HA complex molecules captured by filtration still decreased, i.e., the HA molecular size degrades too much to be captured by a 0.1 μm filter. For the HA samples ozonized for 6 and 8 minutes, virtually no copper is removed via filtration of the Cu-HA complexes. Clearly the breaking up of the HA molecules prevents efficient complexation with the copper. Lefebvre (1990) also attributed the decline of metal-HA removal after the ozonation of HA to the reduction of molecular size of the HA by ozonation and copper-containing dyes show similar behavior in that when ozonized, they release previously bound copper (Adams, et al., 1995).

TABLE V
Amount Of Copper Removed By HA Complexation

Initial Cu Initial Cu^{2+} Concentration (mg/L)	Unozonized HA Sample Concentration Decrease (mg/L)	2 min Ozonized HA Sample Concentration Decrease (mg/L)	4 min Ozonized HA Sample Concentration Decrease (mg/L)
8.0	4.7	0.1	0.0
16.0	7.7	2.9	1.2
24.1	8.5	5.4	1.8
32.1	9.0	6.1	1.9
40.1	9.9	7.3	3.4
48.1	9.5	7.6	3.6
56.1	9.7	8.1	3.9
64.2	10.6	8.6	4.7
72.2	10.8	8.9	5.0

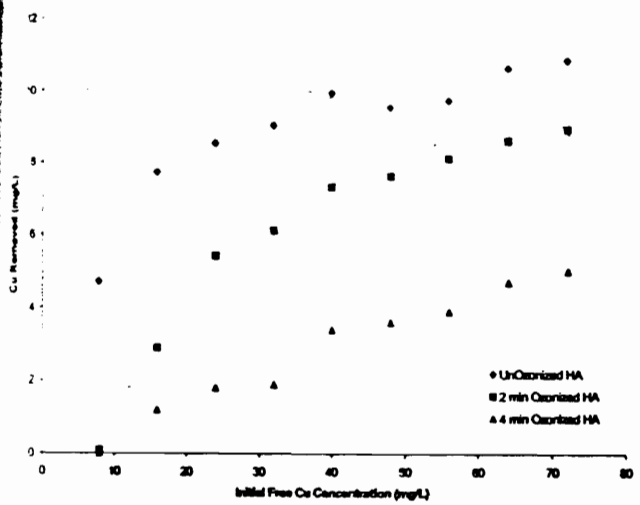


Figure 6. The amount of copper removed by complexation with humic acid as a function of both the length of time the humic acid was ozonized and the initial copper concentration in the complexation solution.

In all cases (untreated, 2-, and 4-minute-ozonized HA solutions), with the approximate initial HA concentration of 1.00 g/L, the amount of copper removed reached a maximum when the initial Cu^{+2} concentration was 72.2 mg/L. For the untreated HA, the maximum removal is 10.8 mg Cu/L, while for the HA treated with 2 and 4 minute ozonation, a maximum of 8.9 and 5.0 mg/L of copper is removed, respectively.

For all the HA samples, the removal of Cu-HA complex increases with the concentration of free copper. This agrees with the concept mentioned by Stevenson (1982) that the formation of insoluble metal-HA complexes depends upon the degree of saturation of both Cu^{+2} and HA. Since the increment of the amount of copper removed is minimal when the initial Cu^{+2} concentration approaches 72.2 mg/L, the complex sites are assumed to be saturated with Cu at this point.

The buffer (sodium acetate) added for the preparation of HA samples may cause competitive complexation between HA ligands and acetate groups. The stability constant of acetate-Cu

complexation (K_1) is $10^{2.16}$, where that of HA-Cu is estimated to be 10^3-10^4. However, the amount of added sodium acetate is consistent for each sample, so the effect of acetate-Cu complexation is not considered crucial to our results.

Conclusions

Due to the complicated nature of the humic acid-copper system, a complete description of how ozonation affects all the relevant chemical interactions is not possible-- there are too many factors influencing the system. In this study, only a slight increase of acidity with increasing ozone dosage was observed, and the amount of removable HA-Cu complex declined substantially within the same range of ozone dosage. Apparently the disintegration of the large HA molecules, rather than any increase in acidic functional groups, controls the HA-Cu removal efficiency. Ozonation of humic substances breaks up the molecules into smaller fragments that either don't complex metal or form only relatively mobile complexes. This may enhance the bioavailability and mobility of both organic carbon and metals, which is of interest for water treatment systems that utilize ozone as a disinfectant.

Literature Cited

Adams, C.D., Fusco, W. and Kanzelmeyer, T "Ozone, Hydrogen Peroxide/Ozone and UV/-Ozone Treatment of Chromium- and Copper-Complex Dyes: Decolorization and Metal Release", Ozone: Sci. Eng. 17(2): 149-160 (1995).

American Public Health Association. Standard Method for Examination of Water and Wastewater, (Washington, D.C. 1986).

Anderson, L.J.,. Johnson, J.D and Christman, R.F "Extent of ozone's reaction with isolated aquatic fulvic acids", Environ. Sci. Technol. 20:739-742 (1986).

Arai, H., Arai, M. and. Sakumoto, A "Exhausive degradation of humic acid in water by simultaneous application of radiation and ozone", Water Research 20(7):885-891 (1986).

Bernatek, E., Moskeland, J. and Valen, K. "Ozonolysis of phenols", Acta. Chem. Scand. 15(7):1454-1460 (1961).

Bernatek, E. and Frengen, C. "Ozonolysis of phenols", Acta. Chem. Scand. 15(3):471-476 (1961).

Bernatek, E. and Frengen, C., "Ozonolysis of phenols", Acta. Chem. Scand. 16(10):2421-2428 (1962).

Bernatek, E. and Vincze, A. "Ozonolysis of phenols", Acta. Chem. Scand. 19(8):2007-2008 (1965).

Bresnahan, W.T., Grant, C.L. and Weber, J.H. "Stability constants for the complexation of copper(II) ions with water and soil fulvic acids measuresd by an ion selective electrode", Anal. Chem. 50(12):1675-1679 (1978).

Chairidchai, P. and Ritchie, G.S.P. "Division S-2 soil chemistry", Soil Sci. Soc. Am. J. 54:1242-1248 (1990).

Clem, R.G. and Hodgson, A.T. "Ozone oxidation of organic sequestering agents in water prior to the determination of trace metals by anodic stripping voltammetry", Anal. Chem. 50(1):102-110 (1978).

Coulibaly, T., and Jensen, J.N. "A mixed order model for ozone demand in natural waters", Presentation at the 201st national meeting. Atlanta, Ga. Am. Chem. Soc. (1991).

Dogut, J.P., Anselme, C., Mazounie, P. and Mallevialle, J. "Application of combined ozone-hydrogen peroxide for the removal of aromatic compounds from a groundrwater", Water Supply 7(4), IWSA Spec. Conf. Org. Micropollut):281-294 (1989).

Ephralm, J., Alegret, S., Mathuthu, A., Bickin, M., Malcolm, R.L. and. Marinsky, J.A "A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids)", Environ. Sci.Technol. 20(4): 354-366 (1986).

Gamble, D.S., Undedown, A.W., and Langford, C.H. "Copper(II) titration of fulvic acids ligand sites with theoretical, potentiometric, and spectrophotometric analysis", Anal. Chem. 52:1901-1908 (1980).

Gilli, G., Scursatone, E., Palin, L., Bono, R., Carraro, E. and Meucci, L. "Water disinfection: A relationship between ozone and aldehyde production", Ozone: Sci. Eng., 12(3):231-241 (1990).

Gracia, R., Aragiles, J.L.and Ovelleiro, J.L. "Study of the Catalytic Ozonation of Humic Substances in Water and Their Ozonation Byproducts", Ozone: Sci. Eng. 18(3):195-208 (1996).

Hiraide, M., Hommi, H. and Kawaguchi, H. "Dissociation of copper(II) -Humic complexes in acidified water", Analytic Sciences 6:479-483 (1990).

Jekel, M.R. "Interactions of humic acids and aluminum salts in the flocculation process", Water Res. 20(12):1535-1542 (1986).

Josephson, J. "Humic substances", Environ. Sci. Technol. 16(1):20A-24A (1982).

Kerndorff, and Schnitzer, M. "Sorption of metals on humic acid", Geochimica et Cosmochimica Acta. 44: 1701-1708 (1980).

Khalili, F. "Preparation and characterization of selected

metal-humate complexees", Soil Science 150(3):565-570 (1989).

Koul, V.K., Zutshi, D.P. and Dubey, K.P. "Physico-chemical studies of the complexes of transition metal ions with humic and fulvic acids of lake waters of Kashmir", Proc. Nat. Acad. Sci. India 59:221-226 (1989).

Kruithof, J.C., Van der Gaag, M.S. and D. Van der Kooy, D. "Effect of ozonation and chlorination on humic substances in water", in I. H. Suffet; MacCarthy, P., (ED). *Aquatic Humic Substances*. (Am. Chem. Soc. Washington, D.C.,1989).

Lefebvre, E., Paillard, H. and Legube, B. "The effect of ozonation on the removal of organics by Coagulation-flocculation", Ozone: Sci. Eng. 12(3):295-313 (1990).

MacCarthy. P. and Suffet, I.H. "Introduction", in I.H. SUFFET; P. MACCARTHY, (ED). Aquatic Humic Substances. (Am. Chem. Soc. Washington, D.C., 1989), p.xvii-xxx.

Manahan, S.E. *Environmental Chemistry*, (PWS. Boston, 1984).

Manahan, S.E. "Interactions of hazardous-waste chemicals with humic substances", in I.H. Suffet; P. MacCarthy, (ED). *Aquatic Humic Substances*, (Am. Chem. Soc., Washington, D.C., 1989).

Manka, J., Rebhun, M., Mandelbaum, A. and Borting, A. "Characterization of organics in secondary effluents", Technion-Israel Institute of Technology 8(20):1017 1020 (1974).

Nakamuro, K., Ueno, H., Nakao, M. and Sayato, Y. "Formation of hydrogen peroxide by aqueous ozonation of humic acid and aromatic hydrocarbons", Chemosphere 20(5):525-531 (1990).

Nor, Y.M., and Cheng, H.H. "Characterization of H^+ and Cu^{+2} binding to humic and fulvic acids", Chem. Speciation. Bioavailability 1(3):93-101 (1989).

Ruch, R. *Stabilization of Colloidal Dispersions by Polymer Adsorption*, (Marcel Dekker. New York, 1980).

Snoeyink, V.L.and Jenkins, D. *Water Chemistry*, (John Wiley & Sons, New York, 1980)

Staehelin, J. and Hoigné, J. "Decomposition of ozone in water in the presence of organic solute acting as promoters and inhibitors of radical chain reactions" Environ. Sci. Technol. 19:1206-1213 (1985).

Stevenson, F.J. *Humus Chemistry: Genesis, composition, and reactions*, (John Wiley & Sons, New York, 1982).

Takahashi, M., Nakai, T. AND Katoh, Y. "Ozonolysis of Humic Acid and Its Effect on Decoloration and Biodegradability", Ozone: Sci. Eng. 17(5): 511-525 (1995).

Traina, S.J., Novak, J. and Smeck, N.E. "An ultraviolet absorbance method of estimating the percent aromatic carbon content of humic acids", J. Environ. Qual. 19:151-153 (1990).

Truitt, R.E. and Weber, J.H. "Determination of complexing capacity of fulvic acid for copper (II) and cadmium (II) by dialysis titration", Anal. Chem. 53:337-342 (1981).

Ueno, H., Segawa, T., Nakamuro, K., Sayato, Y. and Okoda, S. "Mutagenicity and identification of products formed by aqueous ozonation of humic acids of different origins", Chemosphere 19(12):1843-1852 (1989).

Xiong, F., Croue, J.P., and Legube, B "Long-term ozone consumption by aquatic fulvic acids acting as precursors of radical chain reactions", Environ. Sci. Technol. 26:1059-1064 (1992).

Nursery & Landscape Production & Management
Basic Green | Department of Horticulture and Crop Science
Ohio State University

Home
About Us
Out & About:
- Container Production
- Weed Control
- Diseases
- Nutrition
- Trends

Newsletters
Noticieros
Presentations
Feedback
Links
Events
E-mail Hannah

Weed Control

Out- and-About with Hannah

Root Rots, Recirculated Water and Disinfectants. Part 2

Dr. Hannah Mathers

March, 2000

Fig. 1. Nursery and recirculation pond.

It takes a lot of knowledge to produce quality nursery stock. Since 1991 there has been a tremendous increase in intensive culture. The major sectors showing growth in the Oregon industry have been container and greenhouse production. Container production has increased by 77 percent between 1991 to 1998 and greenhouse production has increased by 82 percent in the same period, see Figure 1.

With the rise in intensive culture comes a need for more technical skills, information and skilled labor. In part one of this series, we discussed the increase in need for pathology skills with the increased incidence of root rots with container production and recirculation ponds. In this article, we will discuss the four main irrigation water characteristics and other factors to consider when choosing a disinfectant for your nursery.

Disinfection is the process by which pathogenic microorganisms are destroyed. Not all disinfectants are the same and none work instantly. There are several different ways nursery and greenhouse growers use

disinfectants. There are disinfectant programs for production surfaces such as cement, gravel, sand beds or capillary mats. There are disinfectant programs for propagation materials and tools such as cuttings, bulbs, divisions and pruners. Growers have disinfectant programs for their working surfaces such as pots, trays, equipment, tools and benches. This article, however, deals with choosing a disinfection program for irrigation water.

Growers use disinfectants to kill bacteria, fungi and in some situations nematodes. The main disinfectants used in irrigation water are chlorine, bromine and ozone. Slow sand-filtration units have also been used in some parts of the world with success. The four main water characteristics that determine the choice of an irrigation disinfectant are turbidity, organic and inorganic content, pH and temperature. Three other important factors to consider are water source, form of disinfectant and contact time. The oxygen content of the water may also be an important consideration. We will now discuss each one separately.

First, turbidity often interferes with the effectiveness of disinfectant. The suspended solids tend to surround and protect the microorganisms from the disinfectant. Chlorine, bromine and ozone are all effected by turbidity. A pretreatment of the irrigation water to filter out suspended solids may be necessary if the irrigation water is excessively cloudy.

Organic content in the water can reduce or eliminate the effectiveness of the disinfectant. Generally the greater the organic content of the irrigation water the greater the amount of disinfectant required. Chlorine and bromine are susceptible to combining with organic matter. Chlorine can be rendered inert. Ozone can destroy organic matter present in the water acting as an oxidizer and is not effected by organic content. Inorganic materials such as Fe, ammonia, hydrogen sulfide, Mn, and cyanides can also reduce or eliminate a disinfectant's potency. The effect of inorganic load is greater on chlorine and thus water with inorganic loads would require

far less bromine compared to chlorine to achieve the same disinfectant results. Ozone, again, is very effective in oxidizing inorganic loads.

Generally, the lower the pH of the water the more effective the disinfectant. Bromine is more effective over a broader range of pH than chlorine. However, ozone is effective regardless of water pH.

Physical chemistry teaches us that the higher the temperature the faster the chemical reaction rate. Therefore, the degree of success of a disinfectant is indirectly related to temperature. Because the temperature of the pond will change over the growing season, the disinfectant rate may need to be adjusted at different times. Low oxygen content may result in higher anaerobic bacteria activity and decreased aerobic flora and fauna. In this case, aeration may be necessary.

Many nursery and greenhouse growers use chlorine to reduce innoculum buildup of pathogens in their irrigation ponds. However, reduction in growth and quality of crops is associated with tissue levels of 0.5-2% Cl for sensitive crops and 4% or more in the dry matter of tolerant plant species (Reisenauer et al. 1973). If using chlorine as a disinfectant, keep in mind that as little as 7-mg/L free chlorine can damage some plants. Frequent monitoring for chlorine levels and vigilantly checking plant health are essential. Cl toxicity symptoms include burning of leaf tips or margins, bronzing, premature yellowing and abscission of leaves (Eaton 1966). Bromine may be a better disinfectant choice as bromine is less phytotoxic than chlorine. In fact, as much as 100 mg/L may not cause phytotoxic effects.

Cost is dependent on the dose, which ultimately depends on the quality of the water supply. In comparisons that I have seen, ozone may be the most cost effective, if the cost of the ozone generator is discounted. It is also highly effective.

Don't forget that pesticides and fertilizer salts have been found in nursery irrigation run-off. A direct correlation exist between the amount applied and the amount recovered in the recirculation pond.

Environmental and Experimental Botany, Vol. 30, No. 4, pp. 443–449, 1990
Printed in Great Britain.

0098-8472/90 $3.00 + 0.00
Pergamon Press plc

RESPONSE OF CORN TO COMBINATIONS OF ATRAZINE, PROPYL GALLATE AND OZONE

WONDIMAGEGNEHU MERSIE,* TADESSE MEBRAHTU and MUDDAPPA RANGAPPA

College of Agriculture and Applied Sciences, Virginia State University, Petersburg, VA 23803, U.S.A.

(*Received* 31 *October* 1989; *accepted in revised form* 26 *February* 1990)

MERSIE W., MEBRAHTU T. and RANGAPPA M. *Response of corn to combinations of atrazine, propyl gallate and ozone.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **30,** 443–449, 1990.—Growth chamber experiments were conducted to determine the influence of the air pollutant ozone (O_3) [0.2 and 0.3 ppm (v/v)] on the growth of corn (*Zea mays* L.) treated with the herbicide atrazine (2.5 and 3.5 kg/ha) and the antioxidant propyl gallate (5 and 8 kg/ha). At both concentrations O_3 at 0.3 ppm alone reduced dry weights of corn and chlorophyll. Chlorophyll *a* was more affected than chlorophyll *b* by O_3 at 0.3 ppm alone and in combination with atrazine. Propyl gallate at both rates protected corn against O_3 injury. The interaction between atrazine and O_3 at 0.2 ppm was additive whereas at 0.3 ppm it was antagonistic. But combinations of atrazine and propyl gallate did not protect corn from O_3 injury.

INTRODUCTION

ENVIRONMENTAL factors modify herbicidal efficacy even in those instances in which the major basis for selectivity is thought to reside in differential metabolism. Numerous research efforts have revealed interactive effects of environment and agronomic practices that can modify the activities of herbicides.[5]

These environmental factors which could influence the response of plants to herbicides include air pollutants. Ozone (O_3), produced from photochemical activity induced by sunlight on nitrogen oxides and reactive hydrocarbons, is generally considered the air pollutant most injurious to plants[8] and was reported to interact with chemical weed control.[17] Studies have shown that O_3 may interact with selected herbicides on certain plant species, thereby modifying either the overall plant response to herbicides[2–4,7,17] or the metabolism of herbicides in fumigated plants.[9–12] For example, pebulate and chloramben can interact synergistically with O_3 on selected cultivars of tobacco (*Nicotiana tabacum* L.).[2] The herbicides benefin and diphenamid interacted anatagonistically with O_3 in selected tobacco cultivars.[16,23]

MERSIE *et al.*[15] also investigated the potential interactive effects of metolachlor and O_3 on the growth of corn (*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.). Shoot dry weight of corn was reduced by O_3 plus metolachlor more than expected, thus indicating synergism. But the interaction between O_3 and metolachlor on bean and soybean was antagonistic.

These studies not only showed the potential for interaction between O_3 and herbicides but also that the interaction could vary with herbicides and crop. Thus it is necessary to evaluate each herbicide and crop with O_3 in order to predict the influence of this air pollutant on the selectivity and efficacy of major herbicides. One of these

* To whom all correspondence should be addressed.

herbicides is atrazine which is widely used in corn to control annual grasses and broadleaf weeds.[25] To date there are no published reports on the possible effect of O_3 on corn treated with atrazine.

The objective of the present study was to evaluate any potential interactions between the herbicide atrazine and the air pollutant ozone (O_3) on corn treated with or without the antioxidant propyl gallate.

MATERIALS AND METHODS

Plant culture

Corn variety Golden Acres was grown from seed in potting soil (Michigan Peat Co., P.O. Box 980129 Houston, TX 77098) in 1 l plastic pots. Atrazine at 0, 2.5 and 3.5 kg/ha or *n*-propyl gallate at 0, 5.0 and 8.0 kg/ha were applied to the surface after planting three seeds in each pot. In a separate experiment, potting soil was treated similarly with atrazine at 3.5 kg/ha and/or propyl gallate at 8.0 kg/ha. Thus there were three experiments; (1) atrazine, (2) propyl gallate and (3) atrazine + propyl gallate with ozone. After planting, pots were placed in growth chambers with a 25/22°C day/night temperature, 65 ± 3% relative humidity and 350 μE m^{-2} sec^{-2} photosynthetic photon flux density for 14 hr. After emergence seedlings were thinned to two plants per pot.

Fumigation of corn seedlings with O_3

Five days after emergence, corn seedlings grown in pots whose soil had been treated or not treated with atrazine or propyl gallate or atrazine + propyl gallate were fumigated with O_3. Exposure conditions in the growth chamber were 25 ± 2°C, 65 ± 3% relative humidity and 350 μE m^{-2} sec^{-2} photosynthetic photon flux density provided by mixed incandescent and fluorescent lighting. Mean wind velocity in the cabinet was 0.19 m/sec. Exposure to O_3 was initiated after plants were pre-equilibrated in the chamber for 1 hr. At this stage corn seedlings were 8–10 cm (1½ leaf). Seedlings in one set were fumigated with O_3 at 0.2 ppm for 6 hr between 9 a.m. and 3 p.m.; another set was treated at 0.3 ppm for 6 hr the next day during the same period. Plants were exposed to O_3 twice a week for 3 weeks. Exposure conditions in the growth chamber were 25 ± 2°C, 65 ± 3% relative humidity and 350 μE m^{-2} sec^{-2} photosynthetic photon flux density provided by mixed incandescent and fluorescent lighting. Exposure to O_3 was initiated after plants were pre-equilibrated in the chamber for 1 hr. Untreated seedlings (0 ppm (v/v) of O_3) were left in the growth chambers. O_3 was generated by passing dry air through an O_3 generator (Griffin Model GTC-1A generator, Griffin Technics Corp., P.O. Box 330, 178 Route 46, Lodi, NJ 07644). O_3 concentrations at plant height were monitored with a Dasibi AH 1003 Ozone Monitor (Dasibi Environ. Corp., 515 W. Colorado St., Glendale, CA 91204-1101) calibrated every month with the Dasibi model 1003-PC. After each fumigation with O_3 (0.2 or 0.3 ppm) seedlings were returned to the growth chamber.

Assessment

Plants were periodically examined for general vigor, occurrence of foliar lesions and degree of foliar injury. Three weeks after the first fumigation, chlorophyll concentrations were determined by ethanol extraction[14] on one whole shoot of corn. The absorbences of the extracts were measured at 649 and 665 nm using a scanning and recording spectrophotometer. Chlorophyll concentrations were calculated from the spectrophotometric data using the formula of WINTERMANS and DE MOTS.[26] The other plant in each pot was dried in a forced-air oven at 50°C for 48 hr, and weighed.

Statistical methods

Data presented are the means of two experiments with three replications in each experiment and are expressed as the average chlorophyll content or dry weight per plant. Data were analyzed by the General Linear Models Procedure of SAS (Statistical Analysis System, Carey, NC 27511) statistical package. Chlorophylls *a*, *b* and their sum were analyzed separately and standard errors were calculated. The shoot dry weights were further analyzed to determine statistically significant interactions for each treatment combination and expected responses were calculated as described by HATZIOS and PENNER.[6] Briefly shoot dry weight data were analyzed for variance as a four-by-three (herbicide or antioxidant by O_3) factorial experiment in a randomized com-

plete block design. The standard errors of the observed mean responses were calculated.

The expected responses from each treatment combination were calculated assuming no interactions (additive model) by subtracting the observed response of the untreated from the sum of the observed response of the separate level of each herbicide or antioxidant and O_3. In Table 4, the expected response of corn seedling to the combined treatment of propyl gallate at 5 kg/ha and O_3 at 0.2 ppm was calculated as follows: $(1825 + 1605) - 2283 = 1147$. These expected responses for each treatment combination were compared to the observed value; if it is less than the expected value, synergism is indicated; when the observed value exceeds the expected value, antagonism is indicated. Finally, when observed and expected value are similar, the interactive effect is additive.

RESULTS

Visual symptoms

No visible foliar symptoms were detected on corn treated with propyl gallate and atrazine at either rate. Visual symptoms of injury were evident as tip burning of lower leaves from O_3 at 0.3 ppm. These symptoms were observed 2 days after fumigation and new growth was not affected.

Chlorophyll content

The chlorophyll concentration of corn was not affected by O_3 alone at 0.2 ppm as compared to the untreated (Tables 1, 2 and 3). Atrazine and propyl gallate alone at both rates also did not significantly reduce total chlorophyll. However, O_3 at 0.3 ppm reduced the content of total chlorophyll in all experiments. The above reductions of total chlorophyll were largely because of the degradation of chlorophyll *a* by some treatments. The lowest chlorophyll *a* levels from all experiments were observed from corn fumigated with O_3 at 0.3 ppm alone or in combinations with atrazine. The antioxidant propyl gallate at 8 kg/ha protected against chlorophyll destruction from O_3 at 0.3 ppm (Tables 1 and 3), but this protection was not evident from the combination of propyl gallate + atrazine + O_3 at 0.3 ppm (Table 3). Also the lower rate of propyl gallate did not reduce the effect of O_3 at 0.3 ppm on chlorophyll *a*.

Table 1. Chlorophyll concentrations of corn treated with combinations of propyl gallate and ozone

Propyl gallate (kg/ha)	O_3 (ppm)	Chlorophyll concentration*		
		a	*b*	Total
		(mg/g leaf dry weight)		
0	0	12.1 a	5.2 b	17.2 ab
5	0	10.6 b	5.2 b	16.5 b
8	0	12.4 a	5.9 b	18.3 ab
0	0.2	12.5 a	5.8 b	18.2 ab
0	0.3	9.9 b	5.5 b	15.4 b
5	0.2	11.3 a	5.5 b	16.8 a
8	0.2	13.8 a	7.1 a	20.9 a
5	0.3	7.8 b	3.1 c	10.9 d
8	0.3	12.1 a	6.2 ab	18.3 ab

* Means within a column followed by a common letter are not significantly different at the 5% level of probability according to Duncan's multiple range test.

The response of chlorophyll *b* to O_3 and/or atrazine or propyl gallate was different from that of chlorophyll *a*. Unlike chlorophyll *a*, chlorophyll *b* was not affected in all experiments. Propyl gallate at 8 kg/ha plus O_3 at 0.2 ppm had significantly higher chlorophyll *b* than the untreated (Table 1). Also as shown in Table 2, the chlorophyll

Table 2. Chlorophyll concentrations of corn treated with combinations of atrazine and ozone

Atrazine (kg/ha)	O_3 (ppm)	Chlorophyll concentration*		
		a	*b*	Total
		(mg/g leaf dry weight)		
0	0	11.1 b	4.9 ab	16.0 b
2.5	0	11.0 b	4.1 ab	15.1 b
3.5	0	14.3 a	5.3 ab	19.6 a
0	0.2	8.3 c	3.8 b	12.1 bc
0	0.3	6.8 c	4.0 b	10.8 c
2.5	0.2	11.0 b	3.8 b	14.8 b
3.5	0.2	11.4 b	3.8 b	14.2 b
2.5	0.3	7.4 c	6.1 a	13.5 b
3.5	0.3	6.2 c	3.6 b	9.8 c

* Means within a column followed by a common letter are not significantly different at the 5% level of probability according to Duncan's multiple range test.

Table 3. Chlorophyll concentrations of corn treated with combinations of propyl gallate, atrazine and ozone

Atrazine (kg/ha)	Propyl gallate (kg/ha)	O_3 (ppm)	Chlorophyll concentration*		
			a	*b*	Total
			(mg/g leaf dry weight)		
0	0	0	9.7 b	3.8 b	13.5 bc
0	8	0	13.4 a	5.0 a	18.4 a
3.5	0	0	12.5 a	4.9 b	17.4 ab
0	0	0.2	12.1 a	4.9 b	17.0 ab
0	0	0.3	3.8 c	4.3 b	8.1 c
3.5	8	0	12.1 a	4.4 b	16.5 b
0	8	0.2	7.2 b	3.6 b	10.8 c
0	8	0.3	6.8 bc	3.1 b	9.9 c
3.5	0	0.2	9.4 b	3.8 b	13.2 bc
3.5	8	0.2	8.6 b	3.4 b	12.0 c
3.5	0	0.3	4.2 c	4.6 b	8.8 cd
3.5	8	0.3	5.4 c	3.2 b	4.2 d

* Means within a column followed by a common letter are not significantly different at the 5% level of probability according to Duncan's multiple range test.

b content of corn treated with atrazine at 2.5 kg/ha + O_3 at 0.3 ppm was higher than that of the untreated whereas the corresponding chlorophyll *a* was lower than that of the untreated.

Dry weight

O_3 alone at both concentrations significantly reduced the dry weight of corn in all three experiments (Tables 4, 5 and 6), but atrazine or propyl gallate alone at both rates did not have a significant effect on dry matter accumulation in corn.

The antioxidant propyl gallate and O_3 interacted antagonistically as indicated by the observed and expected values from each of these combinations and the *F*-tests (Table 4). There is an especially strong interaction between propyl gallate at 8 kg/ha and O_3 at both concentrations. In all combinations the expected values are significantly lower than those observed. This indicates that propyl gallate can protect corn from O_3 injury.

The interaction between atrazine and O_3 at 0.2 ppm was additive (Table 5). The *F*-test of the two combinations was not significant. However, the combinations between atrazine and O_3 at 0.3 ppm were antagonistic. The expected values were significantly lower than the respective observed values for both rates of atrazine.

Because significant interactions were observed at the higher rates of atrazine (3.5 kg/ha) and propyl gallate (8 kg/ha) they were applied in combination to corn. The combination between the herbicide and the antioxidant alone was not significantly different from the untreated (Table 6). Again propyl gallate alone protected corn from O_3 at 0.3 ppm. No significant interaction was observed at the lower concentration of O_3. Atrazine interacted antagonistically with both concentrations of O_3 at 0.3 ppm. The combination of atrazine plus propyl gallate did not significantly interact with both concentrations of O_3.

DISCUSSION

Propyl gallate applied alone at 5 and 8 kg/ha had no injurious effect on chlorophyll content or dry weight of corn. HATZIOS[4] also did not observe any injury on sorghum (*Sorghum bicolor* L.) from propyl gallate applied as high as 9 kg/ha. The effect of atrazine on corn was also not significant. This result is expected as corn is tolerant to atrazine.[20] The primary factor for atrazine selectivity

Table 4. Shoot dry weights of corn treated with combinations of propyl gallate and ozone

Propyl gallate (kg/ha)	Type of comparison	Ozone concentration (ppm) 0	0.2 (mg)	0.3
0	Observed	2283 ± 403	1605 ± 397	1462 ± 303
5	Observed	1825 ± 302	1835** ± 302	1588* ± 253
	Expected		(1147)	(1044)
8	Observed	2055 ± 391	1946** ± 392	1697** ± 253
	Expected		(1377)	(1234)

Mean weight values are from three replications ± standard error of each mean. Expected values in parentheses were calculated by multiplicative survival model (see "Materials and Methods"). Asterisks indicate significant interactions at 5% (*) and 1% (**) levels of probability as determined by *F*-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of propyl gallate and O_3 involved.

Table 5. Shoot dry weights of corn treated with combinations of the herbicide atrazine and ozone

Atrazine (kg/ha)	Type of comparison	Ozone concentration (ppm) 0	0.2 (mg)	0.3
0	Observed	2423 ± 228	1982 ± 413	1402 ± 237
5	Observed	2351 ± 508	1976 ± 487	1687* ± 351
	Expected		(1910)	(1330)
8	Observed	2052 ± 660	1728 ± 493	1486** ± 665
	Expected		(1611)	(1031)

Mean weight values are from three replications ± standard error of each mean. Expected values in parentheses were calculated by multiplicative survival model (see "Materials and Methods"). Asterisks indicate significant interactions at 5% (*) and 1% (**) levels of probability as determined by *F*-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of propyl gallate and O_3 involved.

in corn is the activity of the enzyme glutathione *S*-transferase which detoxifies atrazine by catalyzing the formation of an atrazine–glutathione conjugate.[19,21]

O_3 at 0.3 ppm significantly reduced the concentration of chlorophyll in corn. All combinations which had O_3 at 0.3 ppm reduced chlorophyll concentration. Chlorophyll *a* was reduced more than chlorophyll *b*. There were no significant differences among chlorophyll *b* means in all experiments except for the chlorophyll *b* content for atrazine at 2.5 + O_3 at 0.3 ppm, which was higher than that of the untreated (0 atrazine). KNUDSON *et al.*[14] also reported a similar higher

Table 6. Shoot dry weights of corn treated with combinations of propyl gallate, atrazine and ozone

Propyl gallate (kg/ha)	Atrazine (kg/ha)	Type of comparison	Ozone concentration (ppm) 0	0.2 (mg)	0.3
0	0	Observed	2287±410	1793±170	1537±187
0	8	Observed	2189±273	1537**±460	1654**±295
		Expected		(1326)	(1070)
3.5	0	Observed	1820±421	2005**±308	1751*±305
		Expected		(1695)	(1439)
3.5	8	Observed	2118±689	1408±188	1409*±233
		Expected		(1628)	(1370

Mean weight values are from three replications ± standard error of each mean. Expected values in parentheses were calculated by multiplicative survival model (see "Materials and Methods"). Asterisks indicate significant interactions at 5% (*) and 1% (**) levels of probability as determined by *F*-values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate levels of propyl gallate and O_3 involved.

sensitivity of chlorophyll *a* than chlorophyll *b* to O_3. They suggested that chlorophyll *a* may be more readily degraded by O_3 than chlorophyll *b* or that O_3 may affect the synthesis of new chlorophyll so that the synthesis of chlorophyll *a* is reduced or the synthesis of chlorophyll *b* is relatively increased. Another air pollutant, SO_2, also reduced chlorophyll *a* more than *b*.[22]

The dry weight data showed that O_3 at 0.3 ppm was injurious to corn. This injury was partially alleviated by the antioxidant propyl gallate at 8 kg/ha. Protection from O_3 injury by other antioxidants has also been reported previously.[1,13,18] The biological mechanism for the lack of protection from ozone injury by atrazine plus propyl gallate is not yet known.

Atrazine interacted with O_3 antagonistically. Such antagonistic interactions are also difficult to explain. Other investigators have reported that selected herbicides can protect plants such as tobacco against O_3 injury.[16,23] However, the exact mechanism by which these herbicides neutralize the effects of O_3 on tobacco and other plants is unknown. Detailed studies at the cellular level are necessary to elucidate why O_3 interacts with some herbicides antagonistically in plants.

Ambient diurnal summer time concentrations of O_3 in the eastern United States often range from 0.06 to 0.08 ppm with occasional peak concentrations exceeding the National Ambient Air Quality Standards (NAAS) of 0.12 ppm. In 1988 several Virginia localities have exceeded this concentration many times.[24] Thus O_3 concentrations at the levels used in this study could or do occur in the eastern United States. Based on the results of the present investigation propyl gallate afforded at least some degree of protection from O_3 injury and the herbicide atrazine interacted antagonistically with O_3.

Acknowledgement—Contribution of Virginia State University Agriculture Experiment Station Journal Article No. 166.

REFERENCES

1. Carnahan J. E., Jenner E. L. and Wat E. K. W. (1976) Prevention of ozone injury to plants by a new protectant chemical. *Pl. Pathol.* **68,** 1224–1229.
2. Carney A. A., Stephenson G. R., Ormood D. P. and Ashton C. G. (1973) Ozone–herbicide interactions in crop plants. *Weed Sci.* **21,** 508–511.
3. Hatzios K. K. (1983) Interactions of the herbicide

EPTC plus R-25788 with ozone and antioxidants in corn. *J. Agric. Food Chem.* **31,** 1187–1191.
4. Hatzios K. K. (1983) Effects of CGA-43089 on response of sorghum (*Sorghum bicolor*) to metolachlor combined with ozone or antioxidants. *Weed Sci.* **31,** 280–284.
5. Hatzios K. K. and Penner D. (1984) Pages 97–99 *in Metabolism of herbicides in higher plants.* Burgess Publishing, Minneapolis.
6. Hatzios K. K. and Penner D. (1985) Interaction of herbicides with other agrochemicals in higher plants. *Rev. Weed Sci.* **1,** 1–63.
7. Hatzios K. K. and Yang Y. S. (1983) Ozone–herbicide interactions on sorghum (*Sorghum bicolor*) and velvetleaf (*Abutilon theophrasti*) seedlings. *Weed Sci.* **31,** 857–861.
8. Heath R. L. (1980) Initial events in injury to plants by air pollutants. *A. Rev. Pl. Physiol.* **31,** 395–431.
9. Hodgson R. H., Dusbabek K. E. and Hoffer B. L. (1974) Diphenamid metabolism in tomato: time course of an ozone fumigation effect. *Weed Sci.* **28,** 205–210.
10. Hodgson R. H., Frear H. R., Swanson H. R. and Regan L. A. (1973) Alteration of diphenamid metabolism in tomato by ozone. *Weed Sci.* **21,** 542–549.
11. Hodgson R. H. and Hoffer B. L. (1977) Diphenamid metabolism in pepper. II. Herbicide metabolite characterization. *Weed Sci.* **25,** 331–337.
12. Hodgson R. H. and Hoffer B. L. (1980) Diphenamid metabolism in pepper and an ozone fumigation effect. I. Absorption, translocation and the extent of metabolism. *Weed Sci.* **25,** 324–330.
13. Hofstra G., Littlejohns D. A. and Wukasch R. T. (1978) The efficacy of the antioxidant ethylenediurea (EDU) compared to carboxin and benomyl in reducing yield losses from ozone in navy bean. *Pl. Dis. Reptr* **62,** 350–352.
14. Knudson L. L., Tibbits T. W. and Edwards G. F. (1977) Measurement of ozone injury by determination of leaf chlorophyll concentration. *Pl. Physiol.* **60,** 606–608.
15. Mersie W., Mebrahtu T. and Rangappa M. (1989) Ozone interactions on corn (*Zea mays*), bean (*Phaseolus vulgaris*), and soybean (*Glycine max*). *Weed Tech.* **3,** 650–653.
16. Reilly J. J. and Moore L. D. (1982) Influence of selected herbicides on ozone injury in tobacco (*Nicotiana tabacum*). *Weed Sci.* **30,** 260–263.
17. Rich S. (1975) Interactions of air pollution and agricultural practices. Pages 335–360 *in* J. B. Mudd and T. T. Kozlawski, eds *Responses of plants to air pollution.* Academic Press, New York.
18. Roberts B. R., Jensen K. F. and Cathey H. M. (1985) Modification of ozone sensitivity in seedlings by ethylenediurea: soil application vs stem injection. *J. Am. Soc. Hort. Sci.* **110,** 178–180.
19. Shimabukuro R. H., Frear D. S., Swanson H. R. and Walsh W. C. (1971) Glutathione conjugation: an enzymatic basis for atrazine resistance in corn. *Pl. Physiol.* **47,** 10–14.
20. Shimabukuro R. H. and Swanson H. R. (1969) Atrazine metabolism, selectivity, and mode of action. *J. Agric. Food Chem.* **14,** 392–395.
21. Shimabukuro R. H., Swanson H. R. and Walsh W. C. (1970) Glutathione conjugation: atrazine detoxification mechanism in corn. *Pl. Physicol.* **46,** 103–107.
22. Shimazaki K., Sakaki T., Kondo N. and Sugahara K. (1980) Active oxygen participation in chlorophyll destruction and lipid peroxidation in SO_2-fumigated leaves of spinach. *Pl. Cell Physiol.* **21,** 1193–1204.
23. Sung S. S. and Moore L. D. (1979) Influence of three herbicides on the sensitivity of greenhouse-grown flue-cured tobacco (*Nicotiana tabacum*) plants to ozone. *Weed Sci.* **27,** 167–173.
24. Virginia Air Control Board (1988) *Virginia ambient air quality data report* 1988. Richmond, VA.
25. Weed Science Society of America (1989) *Herbicide handbook,* Sixth edition. Champaign, IL.
26. Wintermans J. F. G. M. and de Mots A. (1965) Spectrophotometric characteristics of chlorophylls *a* and *b* and their pheophytins in ethanol. *Biochim. biophys. Acta* **109,** 448–453.

The *Nursery* Papers

ESSENTIAL INFORMATION FOR AUSTRALIAN PROFESSIONAL NURSERY OPERATORS

AN INITIATIVE OF THE NATIONAL NIDO PROJECT. ISSN 1326-1495 ISSUE NO:1997#008

Water disinfestation – Chloro-bromination and ozone systems get the thumbs up !

Chloro-bromination has been used successfully for some time in Queensland nurseries and this project proved they work well even in poor quality water. They're relatively inexpensive to install but have a continuing cost for chemicals, similar to chlorination. Ozone systems also work well and though expensive, are expected to get cheaper. Ozonation of water has the added benefit of reducing pesticide and herbicide residues.

Table 1 Doses required to control fungal pathogens.

Deciding on water disinfection systems

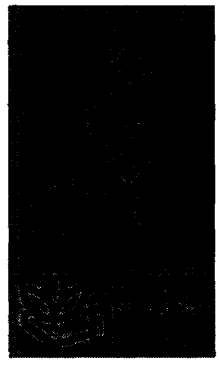
Water from many sources is often contaminated with fungal pathogens which will severely affect nursery crops unless controlled by disinfestation. Bacteria are relatively easy to kill using chloro-bromination or ozone and effective doses are generally much lower than quoted for fungal pathogens. There are several water disinfection systems available for the disinfection of irrigation water.

Ultra-violet light and chlorine dioxide systems were discussed in *The Nursery Papers* 1996 #005.

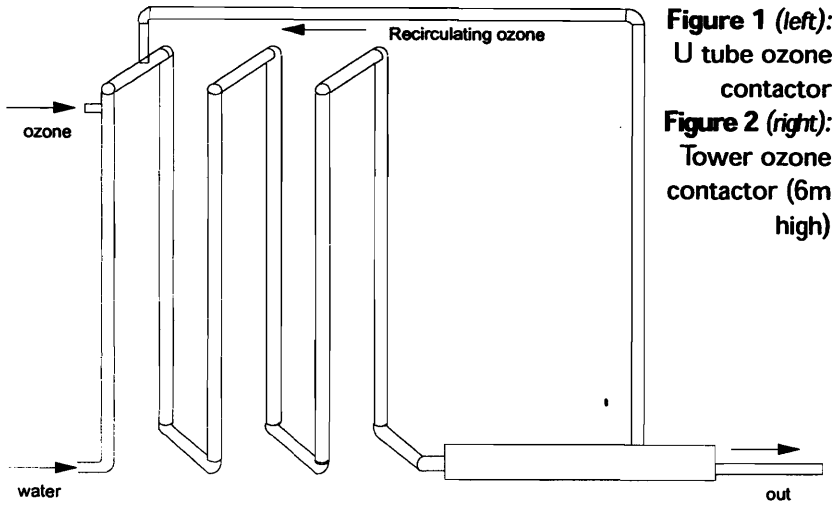
Chloro-bromination cheap and effective

Chloro-bromination is similar to chlorination with sodium or calcium hypochlorite except that sodium bromide is also added to the water. In chlorination systems the hypochlorite ion reacts with water to form hypochlorous acid, the main form of disinfectant. In chloro-bromination systems, hypochlorous acid reacts with the added bromide to form hypobromous acid, a useful disinfectant which is more stable than hypochlorous acid at higher pH. Chloro-bromination is therefore recommended for the disinfection of water with pH values above 7.

The cost of chloro-bromination dosing systems vary but basic dosing pumps and materials cost less than $2,000. Chlorine and bromine levels are easily monitored using various DPD reagent kits. Most are inexpensive as similar kits are used for domestic swimming pools. DPD tests do not distinguish between the two acids so a reading denotes the combined concentration of the two acids.



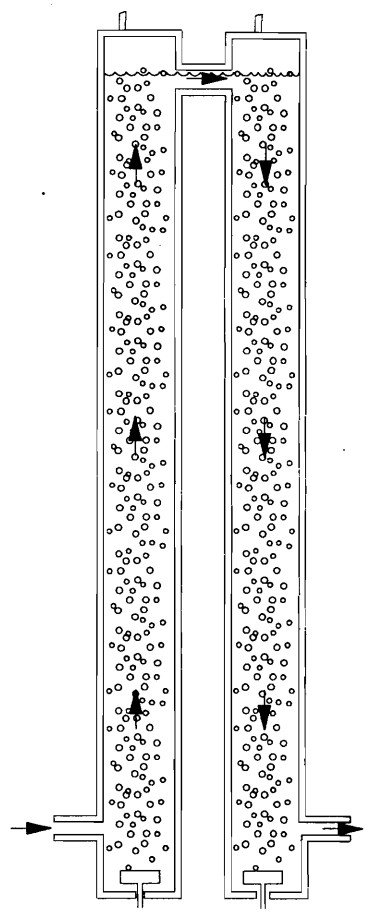
Levels of total chlorine/bromine in water which are safe for plants will vary between species and stage of growth. However, it is advisable to ensure that total chlorine/bromine in water is below 1 ppm prior to irrigation to avoid possible phytotoxic effects.



Patent holder - BOC Gases

Figure 1 *(left):* U tube ozone contactor

Figure 2 *(right):* Tower ozone contactor (6m high)



Ozone works within limits

Ozone is destroyed quickly in water with a pH above 7.0 or in water high in manganese, iron, nitrite, bicarbonate ions or dissolved organic carbon. Ozone disinfection systems are therefore best with neutral to acidic water which is also low in organic matter, acidification of water may be required. Excessive ozone levels in air and water are phytotoxic to plants therefore residual ozone in water should be under 1 ppm before irrigation.

For a medium sized nursery a high ozone input, approximately 20-80g/hr is required, depending on the volume of water to be treated. Capital cost of generator is typically $12,000 - $35,000. Running cost: if generating purified oxygen on site, 600W power. If the ozone generator relies on a bottled oxygen feed, running costs will be high. The average dose of ozone over time will be difficult to measure, especially in an in-line injection system. In-line ozone monitors which detect up to 20 ppm ozone are approximately $5,000.

Getting the ozone to dissolve

High efficiency of the dissolution of ozone into water is the most important consideration of an ozone disinfection system and will determine the capacity of the ozone generator required. Tower and U tube designs are examples of efficient systems for nursery situations *(Figures 1 and 2)*. The U tube design is the most efficient contact system tested. Contact BOC Gases for design specifications for the U tube design as this is a patented system. Ozone generators require dry, high oxygen content air for maximum ozone output. There are many methods of dissolution, including contact tower and U tube designs.

Corrosion risk

Chlorobromine and ozone solutions are corrosive so that some metals fittings may corrode when exposed to high concentrations of the disinfectants. Ozone corrodes brass fittings, rubber and many synthetic plastics. Where ozone levels are high, use stainless steel fittings and viton or silicone gaskets and O rings.

Further reading

Monitoring and managing recycled water quality in nurseries, The Nursery Papers 1996#004.

Nursery Industry Water Management – Best Practice Guidelines – Australia 1997, (book), available from all State Nursery Industry Associations.

Using ultra violet radiation and chlorine dioxide to control fungal plant pathogens in water, The Nursery Papers 1996#005.

Note: Past issues of '*The Nursery Papers*' are available at the NIAA Web Site (www.niaa.org.au)

Acknowledgements

This project was funded by the nursery industry product levy, Flowers Victoria, HRDC and AgVic. Special thanks to ProMinent and Fluid Controls Pty Ltd for assistance with ozone equipment and BOC Gases for the U-tube contactor design.

1999

Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions

Sponsored by
Methyl Bromide Alternatives Outreach

In cooperation with
Crop Protection Coalition,
U S Environmental Protection Agency
And
U S Department of Agriculture

1999 International Logistical & Technical Support Provided by the UNEP TIE

Office: Methyl Bromide Alternatives Outreach

Gary L. Obenauf
144 W. Peace River Drive
Fresno, CA. 93711-6953
Tel: 559 447 2127
Fax: 559 436 0692
e-mail:

Ms. Anna Williams
144 W. Peace River Drive
Fresno, CA. 93711-6953
Tel: 559 447 2127
Fax: 559 436 0692
e-mail:

RESULTS OF 2 YEARS OF FIELD TRIALS USING OZONE GAS AS A SOIL TREATMENT

Alan Pryor, SoilZone, Inc.

Introduction – Ozone (O_3) is a light, colorless gas which has a characteristic pungent odor. Naturally, it is produced from diatomic oxygen in the atmosphere by ultraviolet light and electrical discharges (lightning). These phenomena produce the protective "ozone layer" in the upper stratosphere that is at risk , in part, due to methyl bromide. Paradoxically, ozone itself may be an effective tool for controlling soil borne plant pathogens and improving soil microflora. Due to its reactivity, ozone is a very effective biocide and its use has been approved in a number of agricultural and food processing applications. These include post harvest fumigation for storage of certain fruits and non-perishable commodities, wash waste treatment for fruits infested with surface pests, and as a structural treatment for food storage areas.

Ozone is unstable and rapidly breaks within minutes when dissolved in water or within hours in the gaseous state. Thus, ozone cannot be stored and transported and must be produced onsite for immediate use. For commercial purposes, ozone is produced from oxygen in ambient air through an electrical discharge process with relatively simple pieces of equipment known as ozone generators. Because the byproduct of ozone reaction or decomposition is simple diatomic oxygen, ozone is increasingly viewed as a possible environmentally benign alternative to more persistent and/or toxic fumigants in agricultural applications. Other potential benefits of ozone treatment of soil include:

1. No Transportation, Storage, or Discharge of Hazardous or Toxic Chemicals
2. No Environmentally-Persistent Chemicals Left in Soil
3. No Reentry, Permitting, or Use Restrictions
4. Minimum Human Acute and Chronic Toxicity
5. No Human Carcinogenicity or Teratogenicity
6. No Broad Spectrum Environmental Toxicity

SoilZone has been broadly investigating the use of ozone gas as a soil treatment agent in field trials for 3 years. This study reports the results of several of these field trials performed in carrots, tomatoes, and strawberries. These specific trials were chosen to report because they have been functionally repeated for 2 years in a row in the same field thus lending more validity to the results.

TOMATO FIELD TRIAL RESULTS

1997 Tomato Field Trials - These trials were performed in a field heavily infested with root knot nematodes at the University of California South Coast Extension Field Station in Irvine, California. The research was conducted in conjunction with Dr. Becky Westerdahl of the University of California at Davis Department of Nematology. Ozone

was injected in early July with and without pre- and post-treatment irrigation at the rate of 250 lb./acre through underground drip tubing buried 2.5 – 3.5 inches deep in the center of 32" furrows. Tomato seedlings were planted 3 weeks later and the total yield and number of root galls were compiled at the end of the September harvest. Each treatment consisted of 6 replicated 20 ft. plots in randomized blocks. The increase or decrease in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 1).

Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Marketable Crop Yield Compared to Untreated Control
250 lb. O_3	Yes	No	+ 79.5 %
250 lb. O_3	No	No	+ 14.5 %
250 lb. O_3	No	No	- 1.0 %
250 lb. O_3	Yes	Yes	- 1.9 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Marketable Crop Yield Compared to Alternative Fumigant
250 lb. O_3	Yes	Yes	+ 12.5 % vs. Telone II - 19.8 % vs. Vapam

Statistically, the extent of nematode root galling was no lower in the higher yielding ozone treated plots than in the untreated control plots despite the improved yield in the ozone treated plots (see Figure 2). This indicates that other biological factors (possibly increased nutrient availability) in addition to the biocidal aspects of ozone treatment may also be important in plant yield.

1998 Tomato Field Trials - These field experiments were again performed at the UC South Coast Field Station with Dr. Becky Westerdahl. The methods of application were functionally identical to those used in the experiments performed the previous year. In the 1999 experiments the effects of varying dosages were tested as well as the effect of coinjecting carbon dioxide to increase soil penetration by the ozone. All plots were irrigated prior to ozonation to about 10% moisture level unless otherwise indicated. None of the plots received post-treatment irrigation as did some plots the prior year. The increase in yield resulting from each ozone treatment compared to the untreated control is shown below (also see Figure 3).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
250 lb. O_3	+ 44.2 %
250 lb. O_3 w/o Preirrigation	+ 35.1 %
50 lb. O_3 w/ 100 lb. CO_2	+ 30.0 %
250 lb. O_3 in O_2	+ 22.1 %
50 lb. O_3,	+ 17.6 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatment (lbs. O_3/acre)	Ozone Marketable Crop Yield Compared to Alternative Fumigant

250 lb. O_3	+ 17.1 % vs. Telone II - 1.4 % vs. Vapam

CARROT FIELD TRIAL RESULTS

1997 Carrot Field Trials - These experiments were also performed at the UC South Coast Field Station with Dr. Westerdahl. Ozone was injected into plots in the same manner as the tomato trials followed by carrot seed planting 3 weeks later. The increase in marketable yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 4).

Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Marketable Crop Yield Compared to Untreated Control
250 lb. O_3	Yes	Yes	+ 64.8 %
250 lb. O_3	Yes	No	+ 60.1 %
250 lb. O_3	No	Yes	+ 46.3 %
250 lb. O_3	No	No	- 25.5 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatments (lbs. O_3/acre)	Pretreatment Irrigation	Post-treatment Irrigation	Ozone Marketable Crop Yield Compared to Alternative Fumigant
250 lb. O_3	Yes	Yes	- 39.4 % vs. Telone II - 17.8 % vs. Vapam

1998 Carrot Field Trials – These trials were performed in a manner similar to the prior year with only minor dosage variations. The increase in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 5).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
50 lb. O_3	+ 92.2 %
250 lb. O_3	+ 92.0 %
250 lb. O_3 in O_2	+ 53.6 %
50 lb. O_3 w/ 100 lb. CO_2	+ 45.4 %
250 lb. O_3 w/o Preirrigation	- 9.1 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below. The total yield (including nematode damaged produce) was greatest in the 250 and 50 lb./acre ozonated plots possibly indicating increased nutrient uptake and growth in the ozonated plots (see Figure 6).

Best Ozone Treatment (lbs. O_3/acre)	Ozone Marketable Crop Yield Compared to Alternative Fumigant

50 lb. O_3	- 20.8 % vs. Telone EC
	- 19.2 % vs. Vapam

STRAWBERRY FIELD TRIAL RESULTS

1997-98 Strawberry Field Trials - This experiment was performed at a research site maintained by the California Strawberry Commission in Watsonville, CA in conjunction with Dr. John Duniway of the UC Davis Department of Plant Pathology. The soils were heavily infested with *Verticillium* sp. fungi. In November of 1997, ozone was injected at the rate of 400 lb. per acre through two drip tubes buried about 3.5 - 4.0" deep approximately 5" off center in 42" beds. Ozonation applications were made with and without pre-inoculation with Bioworks T-22 *Trichoderma* fungi granules at the rate of 100 lbs./acre. Transplant planting followed 5 days later. In early June 1998 an additional midseason application of 15 lb./acre was made to those ozonated plots that had been previously inoculated with the *Trichoderma sp.* fungi. The increase in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 7).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
400 lb. O_3 w/100 lb. *Trichoderma* T-22	+ 96.9 % (w/ 1x 15 lb./acre O3 midseason)
400 lb. O_3	+ 51.1 %
100 lb. *Trichoderma* T-22	+ 35.2 %

The increase or decrease in yield resulting from the best ozone treatment at this site compared to the alternative fumigants tested is shown below.

Best Ozone Treatment (lbs. O_3/acre)	Ozone Marketable Crop Yield Compared to Alternative Fumigant
400 lb. O_3 w/100 lb. *Trichoderma* T-22	- 5.0 % vs. Methyl Bromide

1998-99 Strawberry Field Trials – This experiments were performed at the same site and in nearly the same manner as above. The differences included burying the drip tube at 6" depth vs. 3.5-4.0" the previous year and additional treatments as described below.. The increase in yield resulting from each ozone treatment at this site compared to the untreated control is shown below (also see Figure 8).

Ozone Treatments (lbs. O_3/acre)	Marketable Crop Yield Compared to Untreated Control
400 lb. O_3 w/100 lb. *Trichoderma* T-22	+ 11.3 %
100 lb. O_3 w/100 lb. *Trichoderma* T-22	+ 9.7 % (w/ 3x 5-15 lb./acre O3 midseason)
400 lb. O_3	+ 6.1 %
400 lb. O_3 w/100 lb. *Trichoderma* T-22	- 6.0 % (w/ 3x 5-15 lb./acre O3 midseason)
100 lb. O_3	- 6.5 % (w/ 3x 5-15 lb./acre O3 midseason)

DISCUSSION AND CONCLUSIONS

Ozone treatments in both tomatoes and carrots in 1997 and 1998 showed the highest yield with pretreatment irrigation. Almost all of the ozone treated plots that received a

pretreatment irrigation (to about half of field capacity) showed numerical increases in marketable yield compared to untreated controls. All 1997 ozone treatments in carrots and tomatoes were at a rate of 250 lb./acre. In 1998, several treatments of 50 lb./acre were also tried. When ozone was applied at a 50 lb./acre basis on a standalone basis in the carrot trials, it was almost as effective as a 250 lb./acre treatment in increasing yield. When ozone was injected in tomatoes at 50 lb./acre in conjunction with CO_2, it was slightly more effective in increasing yield than a 250 lb./acre treatment.

Overall, the effects of mixing carbon dioxide with ozone gas were mixed. In the case of the tomato trials, coextensive use of carbon dioxide with ozone resulted in increased yield. The opposite effect was seen in the carrot trials.

Both ozone treatments (with and without pre-inoculated *Trichoderma* sp. fungi) in the 1997-98 growing season showed substantial increases in yield compared to untreated controls. The average yield from the best ozone treatment was only slightly less than that of the methyl bromide treated control. (Note that both the untreated and methyl bromide treated controls in this trial were physically separate from the ozone treated plots by several hundred feet). The best yielding ozone treatment in 1997-98 (i.e. 400 lb./acre ozone w/ *Trichoderma* sp. fungi) also showed an accelerated yield increase immediately after receiving a single midseason ozone dosage of 15 lb./acre. This was in spite of showing modest levels of phytotoxicity in the form of lower leaf burn in a number of plants receiving the midseason application.

In 1998-99, an average 10% increase in yield was seen in the ozone treated plots with the exception of two of the three treatments receiving midseason applications of ozone. These 2 plots receiving 3 midseason ozone treatments resulted in average yield decline of about 5-7%. It is believed that this yield reduction is directly correlated with the amount of phytotoxicity suffered by these plants upon midseason ozone application. It is further believed that the escaping ozone was due to slight leaks in the type of push-on compression fitting used to connect the tubing in the various plots. A screw type compression fitting with an inert O-ring has been subsequently used in other trials which has seemingly eliminated all leaks. Further work needs to be performed to properly defined the correct dosage levels that yield the maximum growth response without phytotoxicity. The reduced increases in yield in the other ozone treated plots compared to the untreated control in 1998-99 were due to a number of factors. In the 1998-99 trials, the ozone injection tubing was buried at 5.5-6" beneath the surface as compared to 3-3.5" in 1997-98. Based on other trials in other crops, the author now believes that a more shallow drip tube depth provides greater control in the all important top few inches of soil into which new transplants are placed. Further, in 1998-98, the trial was conducted in ground that had been fumigated with methyl bromide the previous year. In the prior year's trial, the soil had not been recently fumigated. Combined with the substantially reduced temperatures in the 1998-99 growing season, this contributed to greatly reduced soil pathogen pressures that reduced the apparent differences between the untreated and ozonated plots. As an example, the untreated controls in 1998-98 produced over 2,000 g of strawberries per plant whereas in 1997-98 the untreated control plots had produced an average of less than 600 g of berries per

plant.

In summary, the results of these field trials generally demonstrate the potential effectiveness of ozone treatment of soil in increasing plant yield in these crops. Much additional work is necessary to be able to accurately predict the specific growth response achieved by ozonation in different crops grown in different soil types with different pathogens and different climatic conditions.

Figure 1 - 1997 TOMATO YIELD

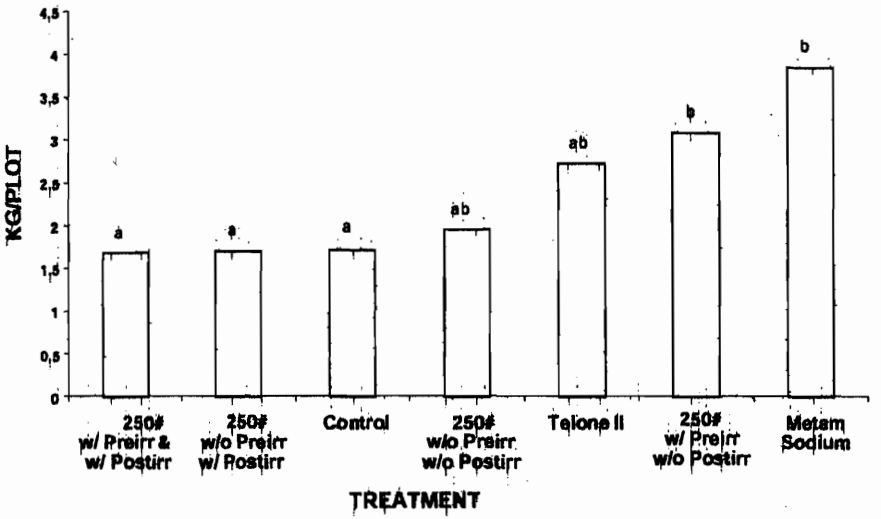


Figure 2 - 1997 TOMATO ROOT GALLING

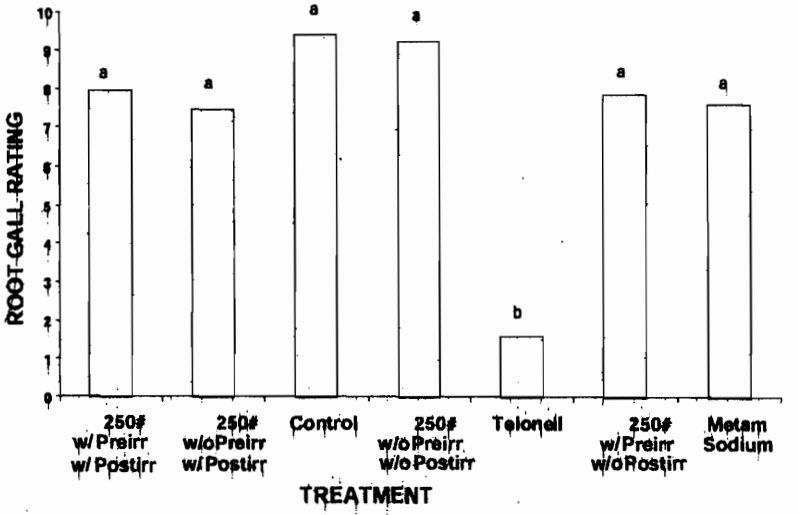


Figure 3 - 1998 TOMATO YIELD

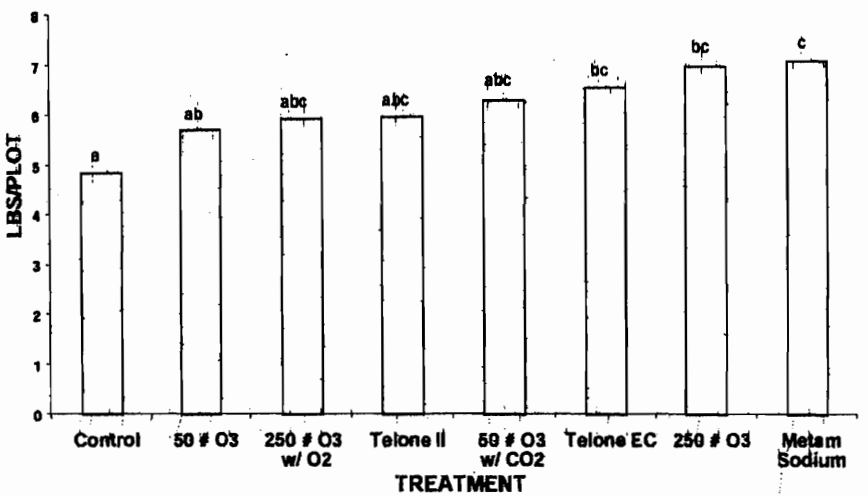


Figure 4 - 1997 CARROT MARKETABLE YIELD

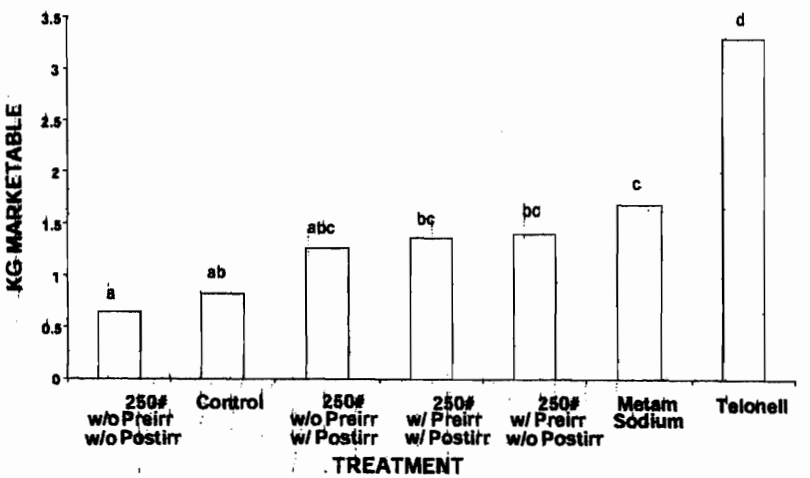


Figure 5 - 1998 CARROT MARKETABLE YIELD

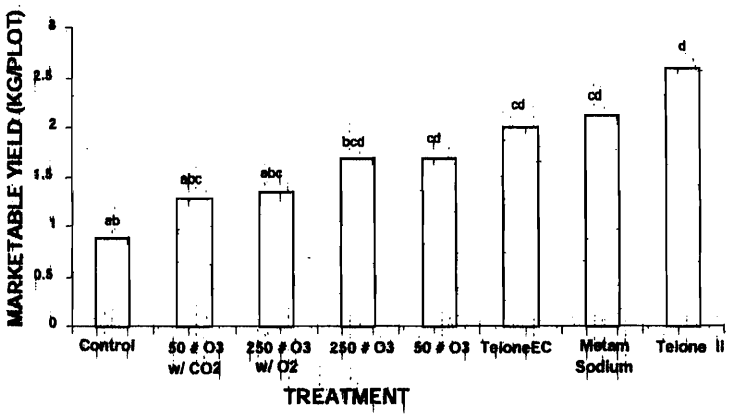


Figure 6 - 1998 CARROT TOTAL YIELD

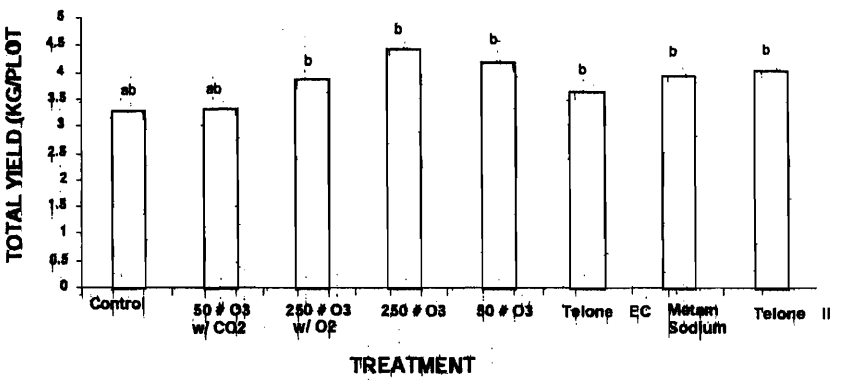


Figure 7 - 1998 STRAWBERRY MARKETABLE YIELD

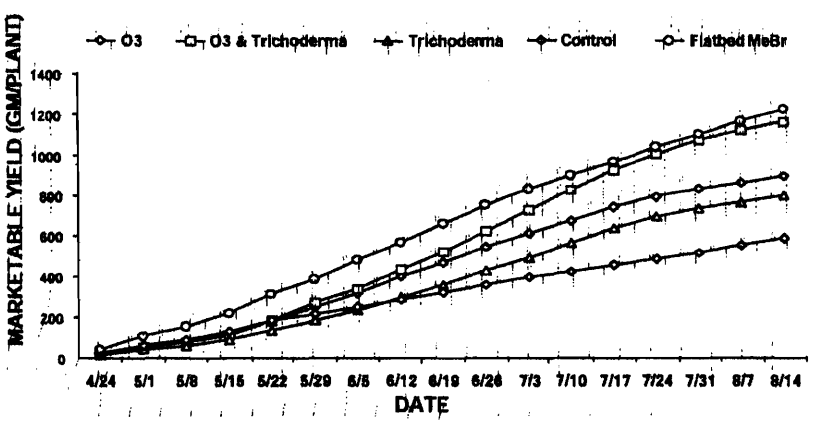
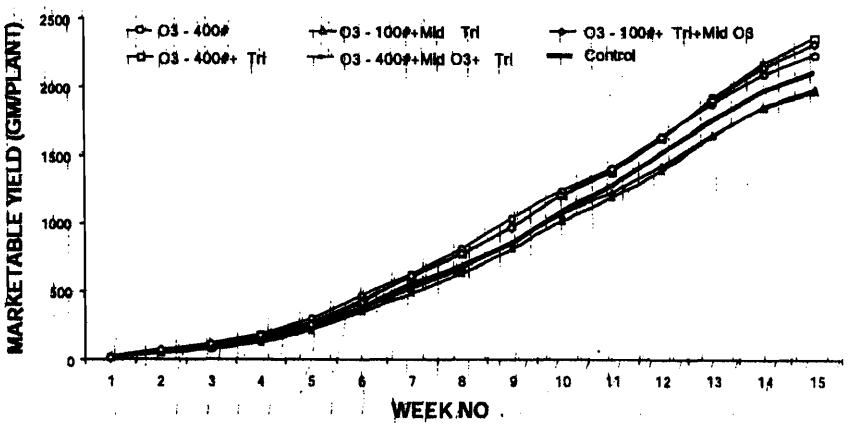


Figure 8 - 1999 STRAWBERRY MARKETABLE YIELD



Bioherbicides as Alternatives to Methyl Bromide for Weed Control in Tomato

C. D. Boyette, and H. K. Abbas USDA-ARS, SWSRU, Stoneville, MS, and H.L. Walker, Louisiana Tech University, Ruston, LA

Common purslane (*Portulaca oleracea* L.), horse purslane (*P. portulacastrum* L.), ground spurge (*Euphorbia prostrata* L.) and spotted spurge (*Euphorbia maculata* L.) are serious weed pests in commercial tomato [*Lycopersicon esculentum* (Mill.) Swingle] fields in the southeastern U.S. Methyl bromide has been used to control these weeds, but restrictions on usage and eventual EPA banning have resulted in searches for effective alternatives. The bioherbicide *Myrothecium verrucaria* (Alb.& Schwein.) Ditmar: Fr. (*MV*) has shown promise as a bioherbicide for several weeds, such as sicklepod [*Senna obtusifolia* (L.) and kudzu [*Pueraria lobata* (Willd.) Ohwi] Irwin & Barneby). In field tests. Spores of MV were applied postemerggence to natural infestations of purslane and spurge in replicated 1 m^2 test plots located at the Jamie Whitten Delta States Research Center, Stoneville, Mississippi. Treatments consisted of: *MV* only, *MV* in 0.2% Silwet L-77 surfactant, surfactant only, and untreated controls. The treatments were replicated 3 times. Inoculum density of *MV* was approximately 2×10^7 spores/ml applied at a spray rate of 500 L/ha. The fungus was highly virulent on all four weed species. After 7 days, the fungus had killed 90-95% of both purslane spp., and 85-95% of spurges. After 14 days, tomatoes (Beefsteak cv.) were transplanted into plots treated with *M. verrucaria*. No visible effects were observed on either of these crops.

In a separate experiment, microconidia, macroconida and chlamydospres of the fungus *Fusarium solani* were applied postemergence to spurge and purslane in field test plots as described previously. Inoculum rates were approximately 2×10^7 fungal propagules/ml applied in distilled water at a rate 500 L/ha. After 7 days, the fungus had killed approximately 80% of both purslane species and 75% of common spurge. These results suggest that these pathogens have potential for use as an alternative to methyl bromide for controlling purslane and spurge in tomatoes.

Efficacy of Methyl Bromide Alternatives in Tomato and Double-cropped Cucumber

J. P. Gilreath[1], J. W. Noling[2], S. J. Locascio[3] and D. O. Chellemi[4]
IFAS, University of Florida, Bradenton, FL 34203[1],
Lake Alfred, FL 33850[2], and Gainesville, FL 32611[3],
and USDA, ARS, Ft. Pierce, FL 34945[4]

Previous research has demonstrated the efficacy of 1,3-D + chloropicrin when combined with pebulate as an alternative package for soil fumigation in tomato production. In most cases, this combination has resulted in tomato yields similar to those achieved with methyl bromide. Soil solarization has been proposed as an alternative to methyl bromide. Most research conducted to date has focused on the effects of alternatives on a first crop, mostly tomato, and little work has addressed the residual effects on a double-crop, such as cucumber. The purpose of this research was to compare standard methyl bromide soil fumigation to fumigation with the best chemical alternative, a mixture of 1,3-dichloroporopene (1,3-D) and chloropicrin used in combination with pebulate, and the best nonchemical alternative, soil solarization, for soilborne pest control and crop response in both fall tomatoes and spring double-cropped cucumbers.

The experiment was conducted at the Gulf Coast Research and Education Center in Bradenton, FL on an EauGallie fine sand soil during the fall of 1998 and the spring of 1999. Treatments were assigned to 3 bed plots 210 ft in length arranged in a randomized complete block design and replicated 6 times. Fall treatments consisted of a nontreated control, methyl bromide and chloropicrin (67/33 %, respectively) at 350 lbs./acre, 35 gal/acre of a mixture of 1,3-D and chloropicrin (83/17%, respectively) with pebulate herbicide (4 lb./acre) applied prior to fumigant application), and 8 weeks of soil solarization from 28 July to 15 Sept 1998. Pebulate was applied to the soil surface pre-bed and incorporated 6 to 8 inches deep in one pass with a s-tine harrow (field cultivator) on 3 Aug 1998. Beds were formed on the 5 Aug 1998 and methyl bromide and 1,3-D/chloropicrin were applied that day with 3 chisels per bed spaced 12 inches apart. Seven days preplant, all solarization and nontreated control plots were sprayed with paraquat (0.5 lb./acre) to dessicate existing weed cover (primarily yellow and purple nutsedge) so it would not interfere with early plant growth. Methyl bromide and 1,3-D treated plots were not sprayed because there was no nutsedge emerged. Six week-old "Solamar" tomato plants were transplanted 2 ft apart into the beds on 17 and 18 Sept 1998. Tomato plants and weeds were sprayed with paraquat after the last tomato harvest in the fall and a second application was made 2 weeks prior to planting the spring cucumbers on 18 Feb 1999.

Tomato plants were more vigorous in soil treated with methyl bromide and 1,3-D + chloropicrin + pebulate than in soil which received no chemical treatment. Fumigation with methyl bromide was superior to soil solarization at both evaluation dates. The combination of 1,3-D + chloropicrin + pebulate improved tomato plant vigor over what was observed with soil solarization at the second evaluation in November. Plants were no more vigorous with soil solarization than with no treatment. Prior to planting the tomatoes, nutsedge had begun to emerge and penetrate the mulch in all of the plots, but there were more plants in the nontreated and solarization plots than in the fumigant plots, necessitating an application of paraquat to

desicate the foliage. Both fumigants and soil solarization reduced nutsedge compared to the nontreated throughout the season and there was no statistically significant differences in the number of nutsedge plants between either fumigant or between the fumigants and soil solarization, due in large part to the early desication of nutsedge in solarization plots. Pigweed control was good with all treatments relative to the nontreated, but only the fumigant treatments reduced crabgrass. The soil in the test area had a low population of root knot nematodes at the beginning of the experiment. After the final tomato harvest, the most severe galling of roots was observed with soil solarization. The extent of galling was not severe in the test but was much higher with solarization than occurred with any other treatment, including the nontreated control. Methyl bromide resulted in no gall formation while galling on plants grown in soil treated with 1,3-D was intermediate. Soil fumigation with either methyl bromide or 1,3-D resulted in few tomato plants with symptoms of Fusarium wilt and both fumigants were superior to either soil solarization or the nontreated control in reduction of the incidence of Fusarium wilt of tomato. Soil solarization reduced the incidence compared with no treatment but the level of infestation was over 20% which would be unacceptable commercially.

The most extra large and total marketable fruit were produced with methyl bromide and 1,3-D + chloropicrin + pebulate in the first two harvests and for the season total. In the third harvest, more extra large fruit were produced with methyl bromide and 1,3-D than with solarization or the nontreated and more marketable fruit were produced compared to the nontreated plots.
Production of medium size fruit was less in the first harvest with the two fumigants, but it was greater in the second and third harvests and for the season as a total. There was no difference in the number of large fruit in the first harvest, but fumigation increased production in the second and third harvests and for the season total. Generally, soil fumigation increased cull production, indicating that overall fruit production was greater with methyl bromide and 1,3-D compared to no treatment or soil solarization. There was no difference in fruit production in any size category or marketable or cull yields between methyl bromide and 1,3-D + chloropicrin + pebulate.
Yields of all sizes and grades were highest with the fumigant treatments, intermediate with soil solarization and lowest where no soil treatment was applied.

Even though all of the nutsedge was desicated with paraquat after the final tomato harvest and again shortly before seeding cucumbers, by Apr., nutsedge was once again present in all plots but with no difference in numbers emerged through the plastic with either fumigant treatment or with solarization. Significantly more crabgrass was present in the beds of solarization plots than in fumigated plots, but there were no differences in populations of pigweed or eclipta.
The extent of root galling by rootknot nematodes was assessed visually after the last harvest and there was considerable galling on the cucumber roots. Methyl bromide was the only treatment to significantly reduce gall formation relative to the nontreated control treatment. The degree of galling with soil solarization was the same as that with no fumigant, whereas 1,3-D was intermediate between methyl bromide and solarization.
Cucumber yields following fall application of methyl bromide were greater than with solarization or no soil treatment. Cucumber production in solarized plots was equal to the nontreated. The production of slicer cucumbers in soil treated in the fall with 1,3-D + chloropicrin + pebulate was not significantly different from that observed with methyl bromide or solarization.

Conventional and organic alternatives to methyl bromide on California strawberries.

Frank V. Sances and Elaine R. Ingham

Ag Alliance/Pacific Ag Research, San Luis Obispo, CA and Oregon State University, Corvalis, OR.

Fifth year field experimentation with alternative soil fumigants and various non-chemical soil amendments for strawberry production on the Central California Coast have yielded repeated trends with alternative plug plants and fumigants common to studies in 1995, 1996, 1997 and 1998. These previous results have been reported at MBAO annual meetings, and have been published in the literature or are in current manuscript form by these authors. 1999 season results are discussed as follows:

1. Site description: Current experimentation is being conducted at two Central California locations with differing soil types and planted to the same cultivars. They are designated as Carpenter Canyon and Edna Valley. The Carpenter Canyon site is a loamy sand previously in pasture for 20 years, which was planted to Camerosa plug and bare root plants in the fall of 1998 for certified organic production in the spring of 1999. The Edna Valley site has clay soil and was planted to cover crops the winter and spring of 1999, followed by lettuce according to a Coastal Cropping Systems project with organic, biorational and conventional production methods. It is planted in strawberries for fall 1999 in comparisons of plug and bare root transplants.

2. Alternative chemical fumigation. In previous years, several chemical fumigants were tested in comparison to methyl bromide/chloropicrin for performance under field conditions. Following fumigation, the highly disease susceptible UC cultivar Camerosa was planted and growth and yield parameters recorded. Across all years, the best performing fumigant was Telone/Chloropicrin at 30-35% chloropicrin. These results were consistent for both loam and clay soils among the years. In addition to Telone/chloropicrin, data for metham sodium in clay indicated relatively good control of soil diseases, but not in sandy loam. By contrast, performance of Telone/chloropicrin was not impacted by soil type and yields were only 5-8% less than those of methyl bromide/chloropicrin. Current soil fumigation1999 studies have included, 1) ozone and combination ozone + *Trichoderma* soil treatments in cooperation with Soilzone, Inc., to control soil diseases and weeds in strawberry and cut flowers, 2) In-season treatments of chemigated Telone for control of grape phylloxera and nematodes in grapes, and 3) Pre-plant drip chemigation of C-35 for control of nematodes and soil diseases of pepper.

3. Organic soil amendments. In 1999, as in all previous year's studies, efforts were made to build the soil foodweb to the extent that the rhizosphere was suppressive to plant pathogenic microorganisms. In earlier years, these organic soil treatments also included *Brassica* residues (shredded broccoli plants), alone and with high rates of compost, and

VAM (Vesicular-arbuscular mycorrhizae) inoculation. Our results with *Brassica* residues in light and heavier soil types show that while pathogenicity of soils are somewhat reduced, a single preplant soil application of shredded *Brassica* even at high rates is not sufficient to maintain roots pathogen free season-long. In plantings so treated, yield was significantly reduced over conventional fumigation with methyl bromide, and usually only slightly better than untreated soil without fumigation or organic amendments. Yield results from utilization of compost for building microbial diversity and a pathogen suppressive soil environment varied depending on the history of the soil prior to planting. In the first year compost studies (1996), soil had no history of conditioning but instead was fallowed three years prior to planting. Here, compost application at high rates with VAM inoculation had beneficial effects on fruit production. Second year results (1997) at this same site, had one year of conditioning prior to planting. These replicated plots were composted and VAM inoculated. Yields in this preconditioned soil differed only 28 flats of fruit per acre from the high yielding methyl bromide standard. Compared to untreated soil, this difference also favored VAM and compost with an average 216 additional flats of fruit per acre. In 1998 however, following two years of preconditioning prior to experimentation, smaller differences occurred from the addition of compost, and almost no benefit on either bare root or plug plants was demonstrated from inoculation with VAM.

Current soil amendment experimentation. A six acre farm was certified organic for the purpose of concentrating on soil amendment regimes and utilization of four seasons of information on alternative strawberry production methods. Plug plants and VAM inoculation were compared to bare root and non inoculated plants on amended soil for two varieties. Camerosa was significantly more affected by soil pathogens and yielded much less than the Catalina cultivar under these non-fumigated conditions. By the end of the season in August, Camerosa was showing foliar symptoms of root infection with visibly reddened and desiccated foliage, and *Vertilicillium* and *Collitrichium* were easily isolated from decomposing roots. By contrast, the Catalina variety had no visible signs of root disease and roots were white and healthy. Plant size was greater and harvest continued for 6 weeks longer with the Catalina variety compared to Camerosa.

4. Alternative plug plants. The 1999 production season at the Carpenter Canyon ranch involved first year organic production with two primary cultivars, Camarosa and Catalina (proprietary) in both plug and conventional bare root plantings. These researchers produced the plug plants at our research farm in San Luis Obispo, and the first year results from this production were not as good as in 1996-1998 when plug plants were purchased from commercial suppliers. Nevertheless, while yields were only slightly higher than bare root plants of either variety across the entire season, production was significantly earlier than bare rooted plants. If the season had terminated in June as is normal, the plug plants would have clearly out-performed bare rooted types. In this year, the market price differential in early season fruit was significant enough to increase profits well above bare root plants, even after allowance for the markedly higher plant costs associated with strawberry plugs.

5. 1999-2000 season trials. Current season experimentation involves studies at both sites on the central California coast. At the Carpenter Canyon site, the entire planting is comprised of comparison trials of soil amendments and various organic pest controls for foliar diseases and invertebrate pests. The Edna Valley site has a three year CAL-DPR sponsored Coastal Cropping Project that compares conventional, biorational and organic production methods for vegetables and strawberries in a rotational system within the same replicated field areas for three years. Soil food web and crop production methods and yields are being monitored through each successive season.

6. Data are presented from current season's (1998-99) results with alternative strawberry plug plants and various soil amendment treatments.

STRAWBERRY PRODUCTION WITH ALTERNATIVES TO METHYL BROMIDE FUMIGATION

S.J. Locascio[1,3*], S.M. Olson[1,4], C.A. Chase[2,3], T. R. Sinclair[2,3], D.W. Dickson[1,3], D. J. Mitchell[1,3], and D. O. Chellemi[2,5] University of Florida[1], and USDA-ARS[2], Gainesville, FL 32611-0690[3], Quincy, FL 32351-9500[4], and Fort Pierce, FL 34945-3138[5]

Strawberries were transplanted in the fall of 1997 to polyethylene mulched beds in Gainesville on a Millhopper fine sand and in Quincy on an Orangeburg fine sandy loam to evaluate the effects of soil fumigants and solarization on fruit production and reported (Locascio, et al., 1999). Fumigant and solarization treatments were applied on Aug. 21 and 19, 1997, respectively. On selected plots, napropamide was applied at 4.8 kg/ha for weed control and fumigant metam-Na at 300 L/ha were rototilled into the bed. Soil fumigants were injected with two chisels per bed spaced 30 cm apart at a depth of 20 to 25 cm at 390 kg/ha 67 % MBr + 33 % Pic, 340 kg/ha Pic, 327 L/ha a C-17, and C-35, and 440 kg/ha dazomet. Drip tubing was placed on the beds before application of 38.1µm (1.5 mil) black polyethylene mulch or 102 µm (4 mil) clear thermal infrared-absorbing film (AT Plastics, Inc., Edmonton, Canada). Soil temperature was measured at 0- to 25-cm depths in two plots mulched with black and in four plots mulched with the clear film (Chase et al., 1997). Some clear mulches (Table 1) were painted with black latex paint a week before transplanting. 'Chandler' was transplants on Oct.21 and Nov.7, respectively.

Transplants were sampled for fungal colonization of roots on 24 Oct. Fruits were harvested twice weekly, graded and weighed. Soil and plants were sampled for nematode and fungal populations at Gainesville at the end of the harvest season on May 18.

Fungi were isolated from the roots and crown tissue of every plant sampled before transplanting. VA mycorrhizal fungi were observed on 95% of the root samples. *Alternaria, Fusarium, Rhizoctonia, Trichoderma,* and an unknown pycnidial forming fungus were isolated from 5, 35, 25, 5, and 5% of the crown pieces, respectively. Although some species of *Alternaria, Fusarium,* and *Rhizoctonia* may cause root diseases, it was not possible to determine the source of pathogens isolated from roots during the growing season and at harvest.

Solar radiation and soil temperatures decreased over the measurement periods (60 days) from Aug. to Oct. Soil temperatures by late Sept. were insufficient to control nutsedge tubers or to kill emerged shoots trapped under the clear film. Maximum soil temperatures exceeding 45 C with the clear mulch at 10 cm occurred on less than 2 % of the days. By the sixth week of solarization at Gainesville, nutsedge density in the control treatment was 22 plants m^{-2} (Table 1). Metam-Na + napropamide was the only treatment that failed to reduce the number of nutsedge plants penetrating the mulches. All other treatments significantly reduced nutsedge densities to levels that were statistically similar to that

with M Br-Pic. By Dec., it was apparent that painting the solarization film before transplanting was necessary. The clear film maintained soil temperatures that were sufficiently warm to promote the sprouting of tubers but not hot enough to cause foliar scorching of the shoots trapped under the film. The cooler temperatures apparently induced nutsedge dormancy since the nutsedge pressure was relatively low with all other treatments. By the end of the crop, nutsedge densities under the solarization films were greater than with the black films (Table 1).

At Gainesville, strawberry plant growth was slow, possibly due to the high occurrence of rainfall after transplanting. Rainfall exceeded average in each month from transplanting through Mar. Rainfall in Feb. was approximately three times the average. Plant vigor ratings were made on Jan. 29, 1998 (Table 1). Plant growth rating of plants with no treatment was only 2.5 on a rating of 1 (poor growth) to 10 (excellent growth) and was significantly greater with all treatments except that with the unpainted clear mulch solarization treatment. Marketable fruit yields were significantly higher with M Br-Pic than with no fumigant, metam-Na with and without Pic, and with the soil solarization treatment-mulch painted black before transplanting (Table1). Total fruit yields in flats/ha were highest with M Br-Pic (4,131), C-17 (3,620), C-35 (3,541), Pic (3,311), and soil solarization with metam-Na-mulch painted (3,002), and significantly lower with metam-Na (2,552), soil solarization-mulch painted (2,710), metam-Na + Pic (2,199) and the check (1,705). Fruit was not obtained with soil solarization-clear mulch not painted. Weed growth under the clear mulch was extensive and picked the mulch up 7.5 to 15 cm over the soil; thus, strawberry plants did not survive the weed competition.

At Quincy, strawberry plant growth was excellent and fruit yields are shown in Table 2. Total fruit yield (in flats/ha) was highest with Pic (4,040), M Br-Pic(3511), C-35 (3,553), C-17 (3,333), Metam-Na + Pic (3,279), Dazomet (3,620), and Dazomet + solarization-mulch painted (3,543). Lower yields were obtained with solarization-mulch painted (3,210) and with metam-Na + Pic (3,116) and with no fumigant (2,417). The lowest yield was obtained with solarization- mulch not painted (815). Fruit size response to fumigant treatment was similar to that obtained for total yield. Marketable fruit were larger with treatments that resulted in higher yield and smaller with treatments that resulted in lower yields. Nutsedge counts were made on Sept. 30 and data are shown in Table 2. Nutsedge control was excellent with M Br-Pic, and with all solarization treatments, intermediate with C-17 and C-35 + napropamide, and poorest with Pic, metam-Na-Pic and dazomet each + napropamide and with the untreated check.

Roots and crowns of strawberry plants in all soil treatments at the end of the season were colonized by a wide array and diversity of fungi, and high incidences of colonization were attained by many of them. Fungi in 18 genera were isolated from roots. Incidences of infection of plants by potentially damaging pathogenic fungi included 29-59% with *Alternaria* spp., 13-42% with *Sclerotium rolfsii*, 50-83% with *Colletotrichum* spp., 13-59% with *Curvularia* spp., 75-96% with *Fusarium oxysporum*, 4-17% with *Macrophomina phaseolina*, 29-79% with *Phoma* spp., 17-42% with *Pythium aphanidermatum*, 4-17% with other *Pythium* spp., and 67-92% with *Rhizoctonia solani*.

In general, the high incidence of pathogenic fungi, especially *S. rolfsii, F. oxysporum, P. aphanidermatum*, and *R. solani*, and the severity of root disease caused by them undoubtably contributed to poor plant growth, early plant mortality, and low yields at Gainesville. Fumigation probably delay the time of infection by fungi dispersed into treated soil and allowed increased yields before pathogenic fungi reach high levels of colonization and cause severe disease.

The population densities of the highly pathogenic sting (*Belonolaimus longicaudatus*) nematode at the end of the season at Gainesville was low and not affected by treatment (data not shown).

In summary, early plant growth and yields at Gainesville were poorer than expected, probably due the high incidences of fungal root diseases and to excessive rainfall during Dec. to Mar. The yield with M Br-Pic was statistically similar to that with 1,3-dichloropropene + 17% Pic (C-17), C-35, Pic, and soil solarization + metam-Na-mulch painted black before transplanting. Yields were significantly lower with the check, metam Na, metam Na + Pic, and soil solarization-mulch painted before planting than with M Br-Pic. At Quincy, plant growth was excellent and yields were statistically similar with Pic, M Br-Pic (67:33), dazomet, solarization + dazomet-mulch painted black, C-17, C35, and metam-Na + Pic. Lowest yields were obtained with the untreated check and solarization-mulch not painted black before planting. Soil solarization with the mulch painted black suppressed nutsedge but regrowth occurred with the mulch left clear. At Gainesville, treatment had no effect on sting nematode (*Belonloaimus longicaudatus*) population densities extracted from soil samples or fungi populations on plant roots at the end of the season.

Literature Cited

Chase, C.A., T. R. Sinclair, S.J. Locascio, J.P. Gilreath, J.P. Jones, and D.W. Dickson. 1997. An evaluation of improved polyethylene films for cool-season soil solarization. Proc. Fla. State Hort. Soc. 110:326-329.

Locascio, S. J., S. M. Olson, C. A. Chase, T. R. Sinclair, D. W. Dickson, D. J. Mitchell, and D. O. Chellemi. 1999. Strawberry production with alternatives to methyl bromide fumigation. Proc. National Agric. Plastics Congress. 28:148-154.

Table 1. Effect of fumigant treatments on fruit yield, plant vigor, and nutsedge counts of 'Chandler' strawberries. Gainesville, FL. 1997-98

Treatment	Rate/ha	Yield (flats/ha)	Plant vigor[v] (Jan 29)	Nutsedge (plants/m^2) Through film May 18	Through film Oct 8	Under film May 18
Untreated		1705 d[w]	2.5 d	21.5 a	8.5	16.1 bc
Methyl bromide /Pic (67/33)	390 kg	4131 a	10.0 a	0.0 b	0.0	0.9 c
Chloropicrin (Pic)[z]	340 kg	3311 abc	6.8 bc	5.0 b	4.5	1.3 c
1,3-D + 35 % Pic[z]	327 L	3541 ab	8.0 b	3.4 b	0.9	4.0 c
1,3-D + 17 % Pic[z]	327 L	3620 ab	6.8 bc	0.8 b	20.2	16.6 bc
Metam-Na[z]	300 L	2552 bcd	5.5 c	28.4 b	12.1	13.9 bc
Metam-Na + Pic[z]	300 L + 170 kg	2199 cd	4.8 c	5.0 b	1.3	6.3 c
Solarization[x]		----	1.0 d	0.0 b	0.0	45.7 ab
Solarization[y]		2710 bcd	5.5 c	0.4 b	0.0	49.3 a
Metam-Na + Pic + solarization[y]	300 L + 170 kg	3002 abcd	6.0 bc	0.0 b	0.5	44.8 ab

[z] Napropamide applied at 4.4 kg/ha.
[y] Mulch painted black before planting.
[x] Mulch left clear and due to excessive weed growth, fruit was not harvested.
[w] Mean separation Duncan's Multiple Range Test, 5 % level.
[v] Plant vigor ratings with 10 = maximum growth and 1 = 10 % of maximum growth.

Table 2. Effect of fumigant treatments on yield, fruit weight, and nutsedge counts of 'Chandler' strawberries. NFREC, Quincy, FL. 1997-98

Treatment	Rate/ha	Yield (flats/ha)	Size (g/fruit)	Nutsedge (plants/m^2)[v]
Untreated		2417 c[w]	15.5 c	29.3 ab
Methyl bromide /Pic (67/33)	390 kg	3511 ab	17.0 ab	1.0 d
Chloropicrin (Pic)[z]	340 kg	4040 a	17.4 ab	20.2 abc
1,3-D + 35 % Pic[z]	327 L	3553 ab	17.1 ab	10.1 cd
1,3-D + 17 % Pic[z]	327 L	3333 ab	17.2 ab	13.2 bcd
Metam-Na + Pic[z]	300 L + 170 kg	3279 ab	16.9 abc	19.8 abc
Metam-Na[z]	300 L	2933 bc	16.1 bc	30.4 a
Dazomet[z]	440 kg	3620 ab	17.1 ab	33.7 a
Solarization[x]		815 d	13.4 d	0.1 d
Solarization[y]		3210 b	16.2 abc	0.2 d
Metam-Na + Pic + solarization[y]	300 L + 170 kg	3116 bc	16.1 bc	0.1 d
Dazomet + solarization[y]	440 kg	3543 ab	17.7 a	0.1 d

[z] Napropamide applied at 4.4 kg/ha.
[y] Mulch painted black before planting.
[x] Mulch left clear.
[w] Mean separation Duncan's Multiple Range Test, 5 % level.
[v] Nutsedge shoots penetrating mulch counted on Sept. 30.

Pergamon

Chemosphere, Vol. 37, No. 8, pp. 1557–1569, 1998

0045-6535/98 $19.00+0.00

PII: S0045-6535(98)00143-X

EFFECTS OF OZONE TREATMENT ON THE SOIL ORGANIC MATTER ON CONTAMINATED SITES

G. Ohlenbusch, S. Hesse, F. H. Frimmel*

Engler-Bunte Institute, Water Chemistry Division, Technical University of Karlsruhe
Richard-Willstätter-Allee 5, D-76131 Karlsruhe, Germany

(Received in Switzerland 2 December 1997; accepted 20 April 1998)

Abstract

In recent years, the ozone treatment technique has been discussed for remediation of contaminated sites. In this paper, data on the effects of ozonation of soil organic matter in ground and pore waters are presented.

An aqueous soil extract was ozonated in a stirred tank reactor and characterized by size-exclusion chromatography with online UV and dissolved organic carbon detection. Additionally, degradation experiments were carried out with these extracts in a bacteria regrowth measurement system.

After ozonation, a decrease of the humic acid fraction and a reduction of its average molecular size could be recognized. In contrast to this, there was an increase of the building block fraction and the low molecular acid fraction. These two fractions were readily degradable by microorganisms. Therefore, the bacteria regrowth increased with ozonation time. As a result of this, a fast and high regrowth of bacteria can be expected after the ozone remediation technique has been applied.

The UV absorbance of extracts at a wavelength of 254 nm showed no significant changes before and after biological degradation. It can be supposed that the microorganisms were not able to degrade chromophoric groups absorbing at a wavelength of 254 nm.

1 Introduction

High remediation costs, which are caused in general by long remediation times, often hinder an active remedial treatment. The remediation time depends on several factors. Low bioavailability of the pollutants in bioremediation or their low volatility in soil air venting techniques are common reasons. Therefore, ozone treatment techniques have been discussed as an alternative in recent years [1-4]. The ozone treatment technique can be applied in-situ or on-site. In-situ ozonation of PAH contaminated sites leads to an increase of hydrophilic substances. These substances are mostly better bioavailable and can be degraded faster by mi-

croorganisms [2, 3, 5]. Using the air venting technique, poorly volatile substances as PAH, aliphatic hydrocarbons, phenols, and pesticides can be oxidized to volatile products by ozonation. This leads to shorter remediation times. However, ozonation does not only lead to oxidative degradation of xenobiotics. The complex soil matrix is also affected [6].

The aim of this work was:

1. to elaborate an experimental procedure for the ozonation of soil organic matter (SOM) from ground and pore waters,
2. to characterize the changes in SOM by size-exclusion chromatography
   and
3. to determine the biodegradability of the ozonation products of SOM.

2. Materials and methods

Soil extract

The soil (standard soil No. 2.3; charge No. Sp 32693) was purchased from the "Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer". The organic C-content was 1.34 ± 0.14 mass percent. The pH-value was 6.6, and the maximal water retention capacity was 35.3 g per 100 g dry mass.

Extraction was made according to DIN 38414 part 4 using deionized water as extraction solvent [7]. 100 g dry mass of the homogenized soil were extracted with 1 L of the extraction solvent in a 2-L glass bottle using a benchtop orbital shaker (SM 25 Bühler; 125 rpm). Extraction time was 24 h, and extraction temperature was 20 ± 2 °C. After extraction, the sample was decanted and subsequently centrifuged (Variofuge 3.2RS, Heraeus) for 30 min at 4500 rpm. The supernatant was firstly filtrated over a prewashed (500 mL H_2O demin.) cellulose-nitrate filter (0.45 μm) and subsequently over a prewashed polycarbonate filter (0.2 μm).

Ozonation

Ozonation was carried out in a gasable stirred tank reactor where the ozone concentration in the gas flow was measured at the reactor in- and outlet with a UV detection cell. Ozone concentration in the gas flow, pH-value, and temperature of the sample were recorded continuously by a PC (Fig. 1). The amount of ozone absorbed by the sample per time period was determined through a mass balance of the gas phase.

The unknown parameters ($dN_{O_3,gas}/dt$; $\dot{N}_{decomp}$) of the balance equation (Eq. 1) were determined experimentally.

$$\dot{N}_{abs,O_3} = \dot{V}_{gas}(c_{O_3,gas-in} - c_{O_3,gas-out}) - \dot{N}_{O_3,decomp} - \frac{dN_{O_3,gas}}{dt} \qquad (1)$$

$\dot{N}_{abs,O_3}$	: absorbed ozone mass flow	$mg \cdot s^{-1}$
$\dot{V}_{gas}$	: gas flow	$L \cdot s^{-1}$
$c_{O_3,gas-in}$	: ozone mass concentration at the reactor inlet	$mg \cdot L^{-1}$
$c_{O_3,gas-out}$	: ozone mass concentration at the reactor outlet	$mg \cdot L^{-1}$
$\dot{N}_{O_3,decomp}$	: decomposed ozone mass flow in the gaseous phase	$mg \cdot s^{-1}$
$\frac{dN_{O_3,gas}}{dt}$	: accumulated ozone mass in the gaseous phase per time	$mg \cdot s^{-1}$

The absorbed amount of ozone ($\dot{N}_{abs,O_3}$) was calculated by integration over small intervals [8]. The aqueous soil extract was ozonated for different time periods in the reactor.

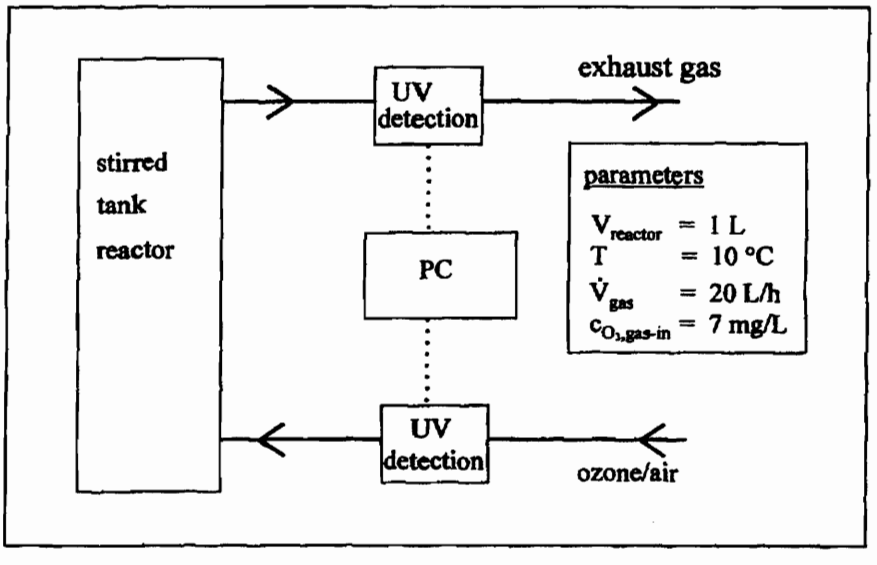


Fig. 1: Apparatus scheme for ozonation.

Size-exclusion chromatography

Chromatographic characterization of the ozonated soil extracts was performed by the LC-DOC/UV system (LC-DOC/UV = Liquid Chromatography-Dissolved Organic Carbon /UV detection) which was developed at the Engler-Bunte-Institute and the DVGW Research Center in Karlsruhe [9-11].

The combination of size-exclusion chromatography and multidimensional detection leads to a new approach for more detailed characterization of dissolved organic matter. The high sensitivity of the system allows a

direct injection of surface water and groundwater without the necessity of preconcentration or isolation, respectively. Therefore, the risk of denaturing and artifact formation can be minimized, and the analysis time can be reduced drastically as well [12].

An outline of this system is shown in Figure 2. An automatic injection system (Abimed, Bio 231) with various sample loops (0.1 to 2 mL) allowed the reproducible injection of various sample volumes. The temperature of the rack was kept to 4° C to minimize biological activity. A HPLC piston pump (Shimadzu LC 9A, Germany) ensured a constant flow (1 mL/min, isocratic) of eluent (phosphate buffer solution:1.5 g/L $Na_2HPO_4 \cdot 2H_2O$, Merck/6580; 2.5 g/L KH_2PO_4, Merck/4873) and sample through the analytical system.

A column (250 mm x 20 mm ID; theoretical plates >3000; Chrom, Germany) filled with a gel consisting of a copolymer of oligoethylene glycole, glycidylmethacrylate, and pentaerythrol-dimethacrylate (TSK HW 50 S; Merck, Germany) was used for the chromatographic analysis. Two 6-way HPLC valves (Knauer, Germany) enabled the selection of the column or optional bypass without the necessity of reconstructions.

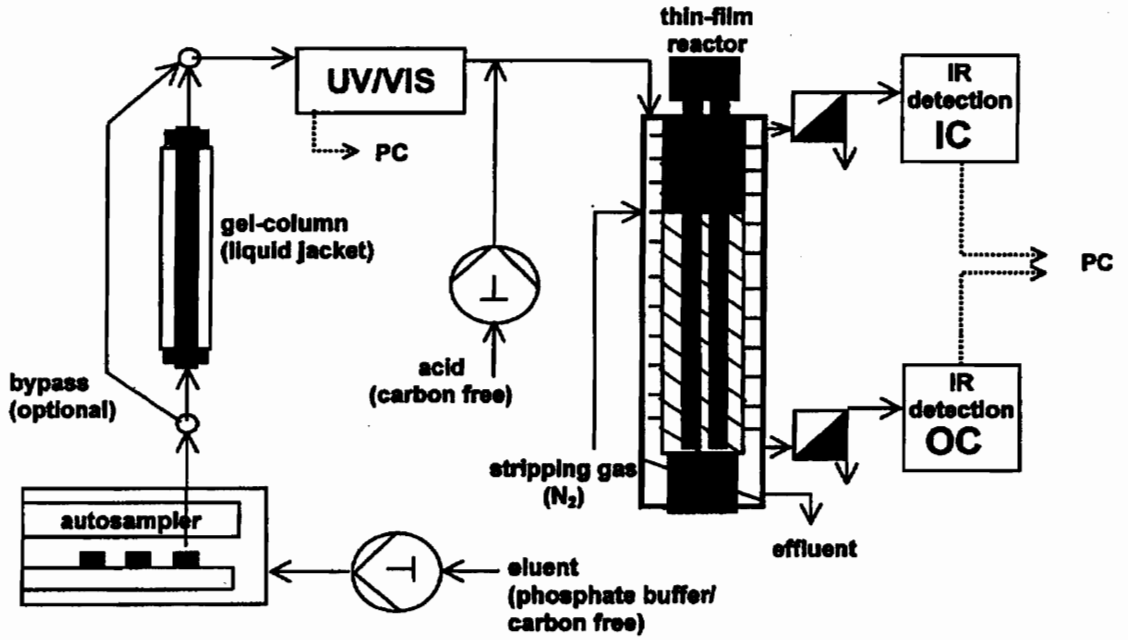


Fig. 2. LC-DOC/UV system (schematical).

The detection was carried out by a multi wavelength UV/VIS detector (Model 502, Linear Instruments, USA). For online detection of inorganic carbon (IC) and organic carbon (OC), a cylindrical thin-film reactor (Gräntzel, Germany) was used consisting of two glass cylinders and a central low-pressure mercury lamp (300 mA; 450 V; 254 nm: 82 %; 185 nm: 18 %). The revolving inner cylinder was divided in a UV shielded and in a UV irradiated part. The separation of IC was performed in the UV shielded part by continuous acidification with 0.085 vol % H_3PO_4 (1.88 mL/min; Shimadzu LC 9A, Germany) and CO_2 stripping with nitrogen gas (N_2 5.0, 20 L/h). CO_2 detection was carried out by a non-disperse infrared (IR) detector (Ul-

tramat 3, Siemens, Germany). In the irradiated part of the reactor, the organic share of the sample was mineralized to CO_2 within the gravity-flow falling film. This technique is used to get an efficient irradiation and consequently an efficient decomposition of the organic matter. The released CO_2 was stripped and quantified by a second IR detector (Ultramat 5, Siemens, Germany).

The buffer solution and the solution for the acidification were purified by irradiation with UV for 24 h in a continuous mode to eliminate organic impurities. Data logging, data processing, and data presentation were realized by Rhrodron (Germany) on ATARI (USA) compatible computers.

The size-exclusion chromatography and online detection of organic carbon (LC-DOC analysis) allowed a subdivision of DOC into operationally defined fractions such as cDOC (chromatographable, consequently hydrophilic part) and HOC (hydrophobic part). The cDOC values were obtained by evaluation of the chromatographic data. HOC resulted in the difference of DOC (column bypass) and cDOC.

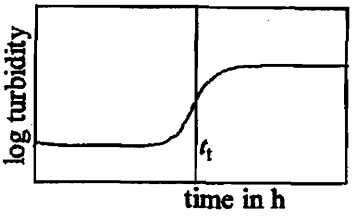
The chromatographic evaluation was carried out by determination of the peak maximum and by integration of the respective peak area using curved integration. Applying suitable calibration substances (polymethacrylic acid, polyacrylic acid, polystyrene sulfonates, dextrane, polyethylene oxides, polysaccharides) and standards of the International Humic Substances Society (IHSS), the predominant nominal average molar masses of the single fractions were calculated. At present, the LC-DOC analysis by means of TSK gels allows to differentiate between up to 5 DOC fractions as described later [9].

Degradation experiments

Degradation experiments were carried out in a bacteria regrowth measurement system according to the method of Werner [13]. In this system, bacterial growth changes the sample's turbidity measured by light scattering. The measurement scheme of this method is shown in Table 1.

To 300 mL of soil extract, 30 mL of trace element solution were added. The trace element solution contains the following substances in 1 liter: 100 mg NH_4Cl, 100 mg $Ca(NO_3)_2 \cdot 4H_2O$, 100 mg $CaCl_2 \cdot 2H_2O$, 500 mg $MgSO_4 \cdot 7H_2O$, 100 mg KH_2PO_4, 50 mg $Na_2SiO_3 \cdot 9H_2O$, 10 mg $Al_2(SO_4)_3 \cdot 18H_2O$, 0.1 mL A-Z-solution (Hoagland). Subsequently, the sample was filtered sterile using a polycarbonate filter (0.2 µm) and filled into a cuvette. After inoculation with a mixed bacteria culture which was obtained from a surface water, a sample for LC-DOC/UV analysis was taken, and the bacteria regrowth measurement system was started. At the end of the experiment, another sample was taken. Each sample for LC-DOC/UV analysis was filtered sterile (0.2 µm) and analyzed immediately after sampling.

Table 1: Bacteria regrowth measurement scheme.

sample preparation	growth curve monitoring	growth curve interpretation
soil extract **sterilize by filtration** (0.2 µm pore size) **sample cuvette** 300 mL sterilized sample 30 mL trace element solution **inoculum** mixed bacteria culture from a surface water in NaCl-solution (0.9 %)	**turbidity adjustment** by drops inoculum into the sample cuvette until equivalent to 0.03 ppm SiO_2 **turbidity measurement** (12° forward scattering) automatically every 30 min over 60 h determination of DOC (LC-DOC/UV) at beginning and end of experiment	 **growth rate** at inflection point t_t $\mu = \frac{d}{dt}\ln(turb)\ ;\quad t = t_t$ **growth factor** $f = \frac{turb_{(end)}}{turb_{(beginning)}}$

3. Results and discussion

The principle of chromatographic fractionation applying gels is based on a hybrid mechanism. Besides steric exclusion, ionic exclusion and hydrophobic interaction processes also play an important role as pointed out recently [14].

In the set up used in this work, mainly high molecular substances which have low interaction with the gel elute with the fraction 5 (R_t = 25 - 30 min). These substances show a low UV absorption at a wavelength of λ = 254 nm and can be assigned to aliphatic polysaccharides and matter of algae and bacteria. The dominating fraction 1 (R_t = 30 - 40 min) comprises the humic substances (HS). The shape of the peak, the position of the peak maximum, and the relation of UV absorption and DOC concentration allow conclusions about distribution, average molar size, and share of unsaturated aromatic structures (see Figure 3). The shoulder on the HS peak represents highly substituted aromatic substances and building blocks of HS (fraction 2, R_t = 40 - 45 min). Within the fraction 3 (R_t = 45 - 50 min), low molecular organic acids elute typically premature. This peak is designated as the 'salt fraction' which is caused by the different electrolyte concentration of the eluent and the sample.

Following within the fraction 4 (R_t = 50 - 80 min), amphiphilic substances retard like a wide front due to definite interactions with the gel. In this fraction, mainly surfactants and nitrogen containing substances (proteins, peptides, amino acids) are found [9].

Ozonation (at different mass ratios m(O_3)/m(DOC), and a reaction time of 30 min) leads to oxidation of high molecular to low molecular substances. This is shown in the LC-DOC chromatograms of Figure 3

which points out a decrease of the humic acid fraction (fr. 1; R_t = 30 - 40 min.) with increasing ozonation time.

Additionally, a marked increase of the building-block fraction (fr. 2; R_t = 40 - 45 min) and the low molecular acid fraction (fr. 3; R_t = 45 - 50 min) can be noticed. Whereas, the polysaccharide fraction (R_t = 25 - 30 min) shows no changes during ozonation. It can be seen that the humic acid fraction maximum is shifted towards longer retention times with increasing ozonation time. This is due to a reduction of the average molar mass size from 7900 g/mol to 6376 g/mol (nominal masses according to dextrane standard). In Table 2, concentrations of all fractions are shown in detail. An increase of the hydrophobic organic carbon (HOC) as a part of DOC can be recognized with increasing ozonation time. Similar results have been obtained by oxidation of a humic acid solution using UV light. It is plausible to assume that ozonation increases the number of hydroxyl groups in SOM-molecules. Thus more hydrogen bonds with the ether groups of the column gel are formed. As a result more DOC is adsorbed to the column which is calculated as HOC.

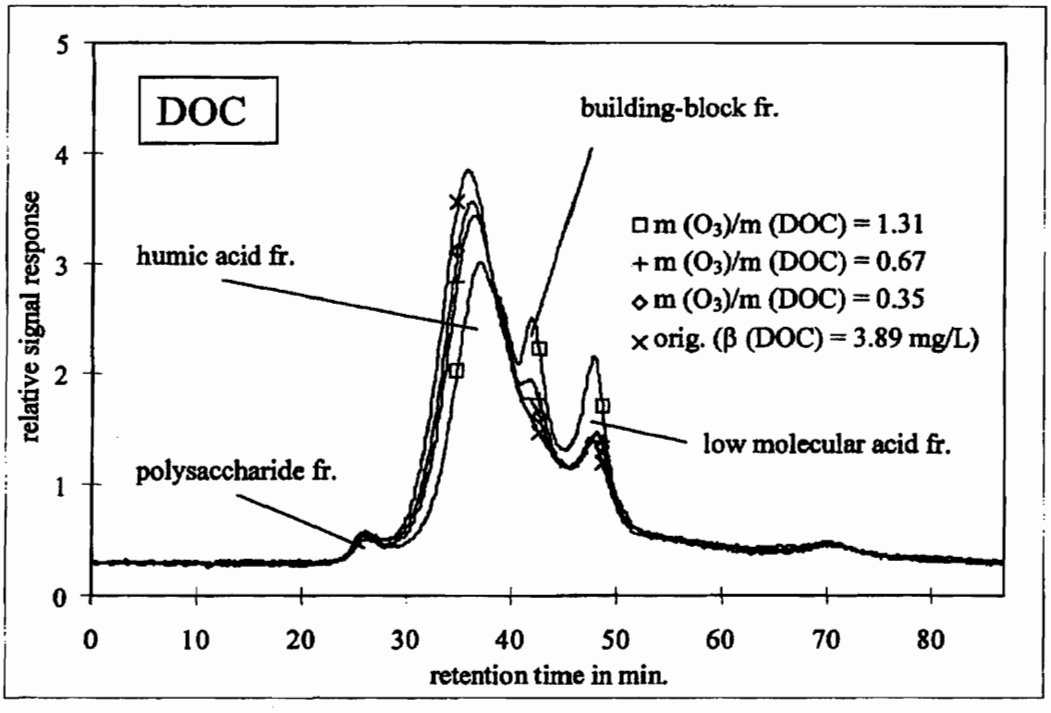


Fig. 3: LC-DOC chromatograms of an aqueous soil extract after different ozonation doses.

Table 2: Soil extract fractions after different ozonation doses measured by the LC-DOC system.

		without ozone	$\frac{m(O_3)}{m(DOC)} = 0.35$	$\frac{m(O_3)}{m(DOC)} = 0.67$	$\frac{m(O_3)}{m(DOC)} = 1.3$
fraction 5	DOC in mg/L	0.04	0.04	0.03	0.04
	(% of DOC)	(1)	(1)	(1)	(1)
fraction 1	DOC in mg/L	2.60	2.38	2.26	1.96
	(% of DOC)	(67)	(64)	(61)	(52)
fraction 2	DOC in mg/L	0.12	0.13	0.18	0.27
	(% of DOC)	(3)	(4)	(5)	(7)
fraction 3	DOC in mg/L	0.36	0.37	0.37	0.52
	(% of DOC)	(9)	(10)	(10)	(14)
fraction 4	DOC in mg/L	0.38	0.32	0.30	0.36
	(% of DOC)	(10)	(8)	(8)	(9)
β(DOC)	in mg/L	3.50	3.24	3.14	3.15
	(% of DOC)	(90)	(87)	(84)	(83)
HOC	in mg/L	0.39	0.50	0.58	0.66
	(% of DOC)	(10)	(13)	(16)	(17)
DOC (total)	in mg/L	3.89	3.73	3.72	3.81

Figure 4 shows the UV detection chromatograms of the soil extracts. A strong decrease of UV absorbance (254 nm) during ozonation of all fractions can be noticed. This can be explained mainly by an oxidation of double bonds and other groups with UV absorbance.

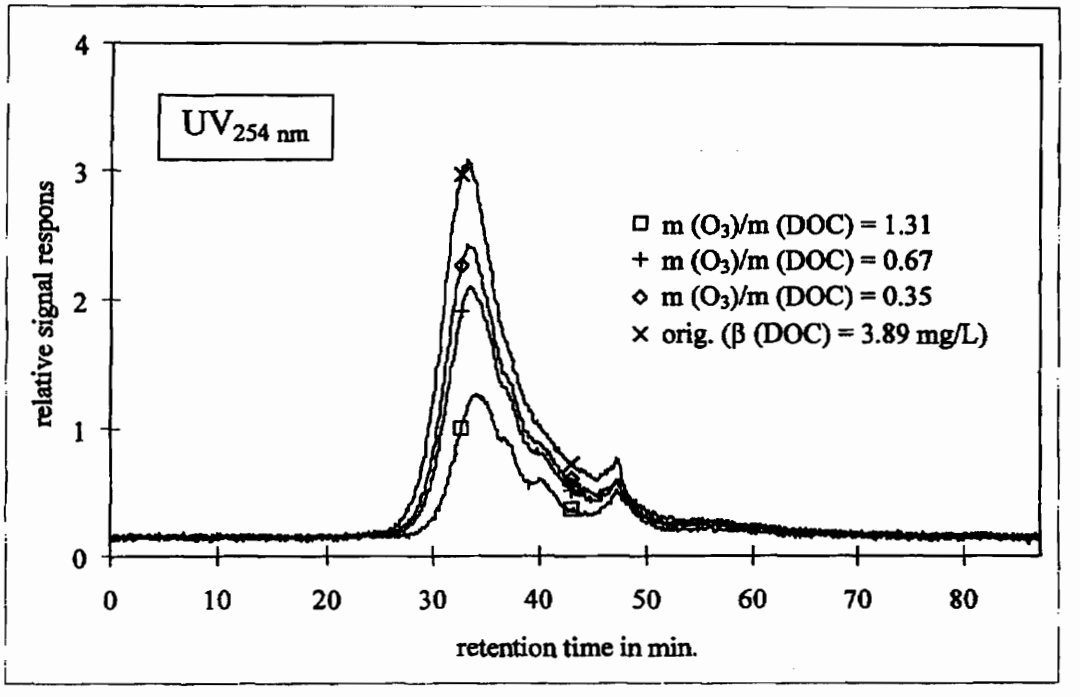


Fig. 4: LC-UV chromatograms of an aqueous soil extract after different ozonation doses.

Additionally, the building-block fraction and the low molecular acid fraction show a low UV absorbance, whereas the polysaccharide fraction shows, as expected, no UV absorbance at a wavelength of 254 nm.

Figure 5 depicts bacteria regrowth curves of soil extracts after application of 4 different ozone doses. Ozonation leads to an increasing *f*-value (defined in Table 1) which is proportional to the amount of DOC degraded and thus a measure for the microbiological degradation. One curve in Figure 5 shows a longer lag-phase then the others. This can be explained by the inhibition caused by a small amount of residual ozone, remaining in the extract after ozonation.

These results show that after remediation of contaminated sites with ozone, a fast regrowth of bacteria can be expected. It is likely that this will lead to a higher germination number in soil as before. Whether there is a selection of some bacteria during this process or not, has not been examined.

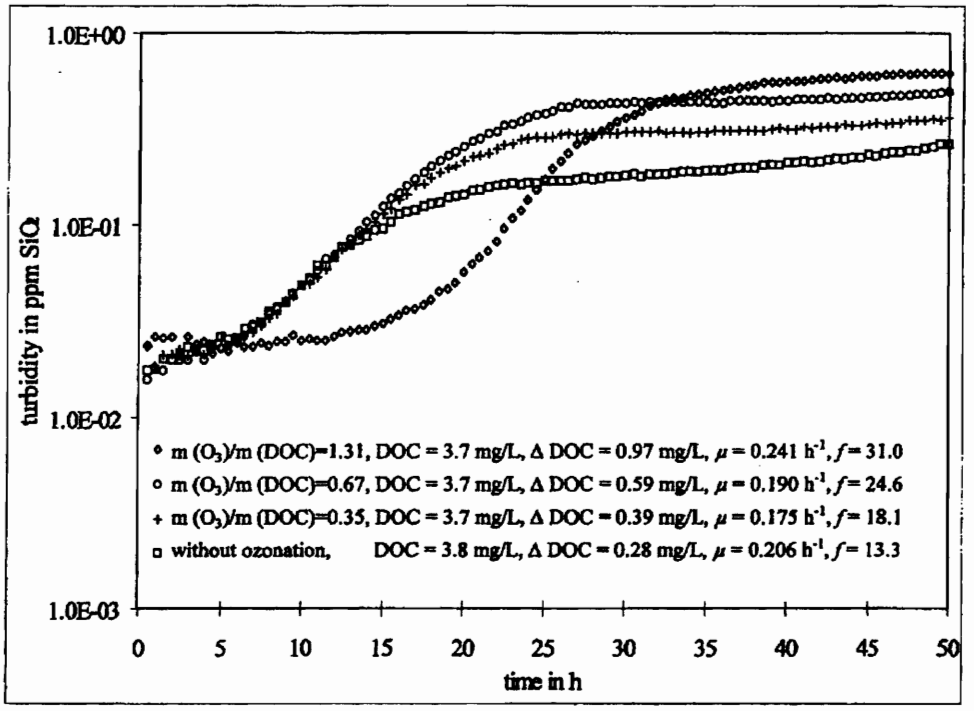


Fig. 5: Bacteria regrowth curves of an aqueous soil extract after different ozonation doses.

During microbial degradation of the ozonated and the original extracts, mainly the low molecular acid and the building-block fraction are assimilated. The humic acid fraction is degradable only to a small percentage. This can be seen in Figure 6 which shows the LC-DOC chromatograms of the extract with the highest ozone dose applied before and after biological degradation.

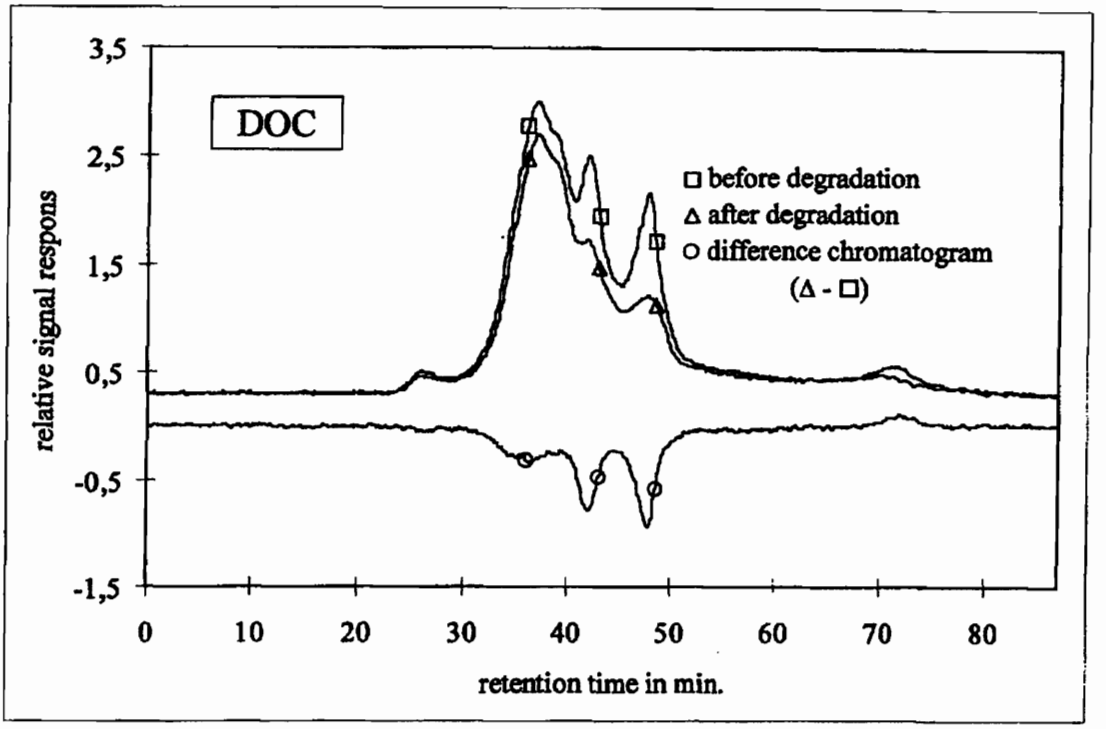


Fig. 6: LC-DOC chromatograms of a soil extract ozonated with 1.31 mg O_3 per milligram DOC before and after degradation.

The reduction of the DOC concentration of each fraction is shown in Table 3.

Table 3: Reduction of DOC concentration by microbial degradation quatified by the LC-DOC system.

		without ozon	$\frac{m(O_3)}{m(DOC)} = 0.35$	$\frac{m(O_3)}{m(DOC)} = 0.67$	$\frac{m(O_3)}{m(DOC)} = 1.3$
fraction 5	Δ DOC in mg/L	0	0	0.01	0.01
	% of fr. 5	(0)	(0)	(33)	(25)
fraction 1	Δ DOC in mg/L	0.03	0.13	0.14	0.31
	% of fr. 1	(1)	(5)	(6)	(16)
fraction 2	Δ DOC in mg/L	0	0	0.04	0.12
	% of fr. 2	(0)	(0)	(22)	(44)
fraction 3	Δ DOC in mg/L	0.08	0.08	0.08	0.20
	% of fr. 3	(22)	(22)	(22)	(38)
fraction 4	Δ DOC in mg/L	0	0	0	0
	% of fr. 4	(0)	(0)	(0)	(0)

Biological degradation has no significant effects on the UV absorbance (254 nm) of the ozonated soil organic matter (see Fig. 7). The same result is observed for the biological degradation of the original extract. It is supposed that chromophoric groups absorbing at 254 nm are quite resistent against microbial degradation.

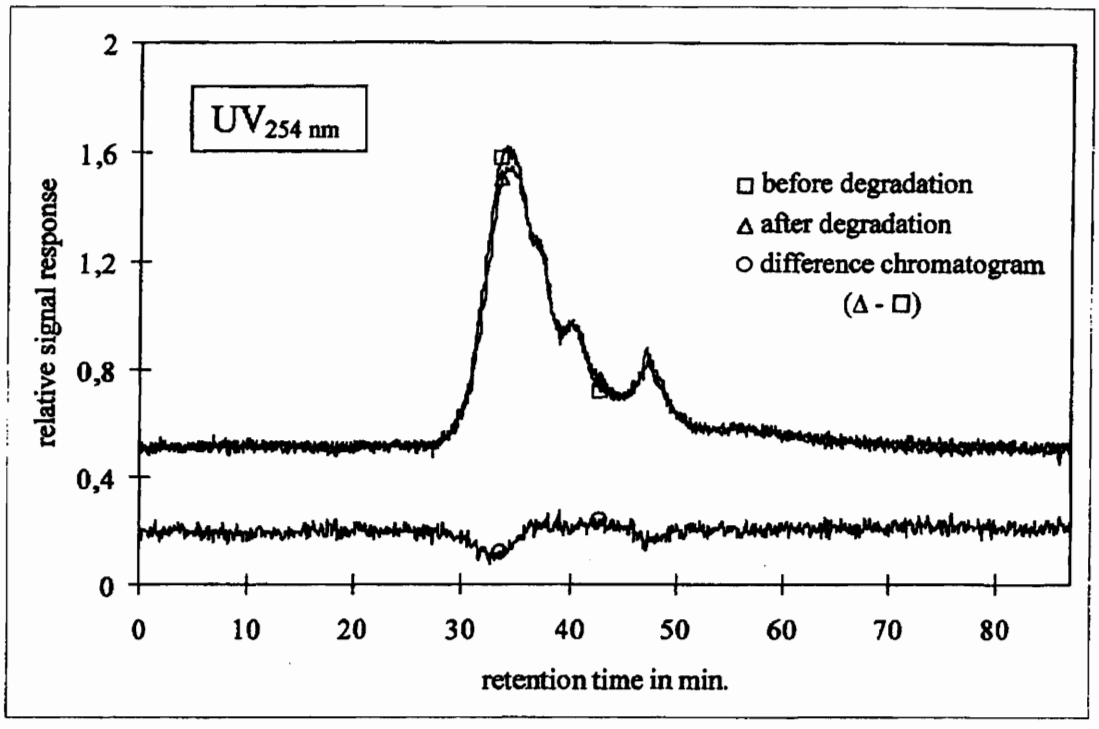


Fig. 7. LC-UV chromatograms of a soil extract ozoned with 1.31 mg O_3 per milligram DOC before and after degradation.

4. Conclusions

Ozonation leads to a decrease of the humic acid fraction and a shift of its maximum towards longer retention time. This means that a reduction of the average molecular size of this fraction took place. On the other hand, there was an increase of the building-block fraction and the low molecular acid fraction. These products show a good microbial degradability. This can lead to a fast and high bacteria regrowth after ozonation of a contaminated site. There might be a special lag-phase and a selection of bacteria during this regrowth process.

Further on, there is a high decrease of UV absorbance (254 nm) during ozonation. This is caused by a preferred oxidation of chromophores. In the biological degradation experiments, there was no great difference between UV absorption before and after microbial degradation. That means bacteria were not able to degrade these chromophoric structures, or less likely new chromophores were synthesized with a similar rate as the one of their degradation.

Acknowledgement

The authors thank the Deutsche Forschungsgemeinschaft for supporting this research. Special thanks refer to Andreas Wolf who carried out the ozonation.

Literature

[1] B. Etrich, Chemisch-technische Untersuchungen zur Ozonbehandlung von Feststoffen am Beispiel Teerkontaminierter Böden, *Wissenschaftliche Berichte FZKA 5791*, Forschungszentrum Karlsruhe, 1996.

[2] M. Stieber, Untersuchungen zum mikrobiellen Abbau von polycyclischen aromatischen Kohlenwasserstoffen Ph. D. Thesis, Technical University of Dresden, 1995.

[3] F. Seibel, M. Stieber, P. Werner, and F. H. Frimmel, Characterization of degradation products of PAH contaminated soil after ozone treatment, *SPIE* **2504**, 86-97 (1995).

[4] E. Weßling, Erfahrungen bei der Anwendung der Ozontechnik zur Zerstörung organischer Schadstoffe an der festen Phase in-situ und on-side, *Erzmetall* **44**, 196-200 (1991).

[5] H. Ruholl and C. Wortmann, Der Abbau organischer Schadstoffe mit Ozon / Untersuchung der Abbauprodukte polycyclischer aromatischer Kohlenwasserstoffe. In *Erkundung und Sanierung von Altlasten* (Edited by H. L. Jessberger), pp. 105-111, A. A. Balkema, Rotterdam, 1990.

[6] C. Kautt, Über die Auswirkungen einer Ozonbehandlung von Boden auf die natürliche organische Bodenmatrix, Ph. D. Thesis, University of Karlsruhe, 1996.

[7] Fachgruppe Wasserchemie in der Gesellschaft Deutscher Chemiker in Gemeinschaft mit dem Normenausschuß Wasserwesen (NAW) im DIN Deutsches Institut für Normung e. V. (Hrsg.): *Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, Verfahren S4*: Bestimmung der Eluierbarkeit mit Wasser (German standard methods for the examination of water, waste water and sludge; sludge in sediments (group S); determination of the leachability by water (S4)). (DIN 38414 Teil 4). 13. Lieferung, Verlag Chemie, Weinheim, und Beuth Verlag, Berlin, 1984.

[8] A. Wolf and F. H. Frimmel, The Ozone Absorption Index (OAI) - A Fast Screening Method to Determine the Efficiency of Ozonation Processes, *Proceedings of the 7th International Symposium: Chemical Oxidation-Technology for the Nineties*, Nashville, TN (in press).

[9] S. A. Huber and F. H. Frimmel, Gelchromatographie mit Kohlenstoffdetektion (LC-DOC): Ein rasches und aussagekräftiges Verfahren zur Charakterisierung hydrophiler organischer Wasserinhaltsstoffe, *Vom Wasser* **86**, 277-290 (1996).

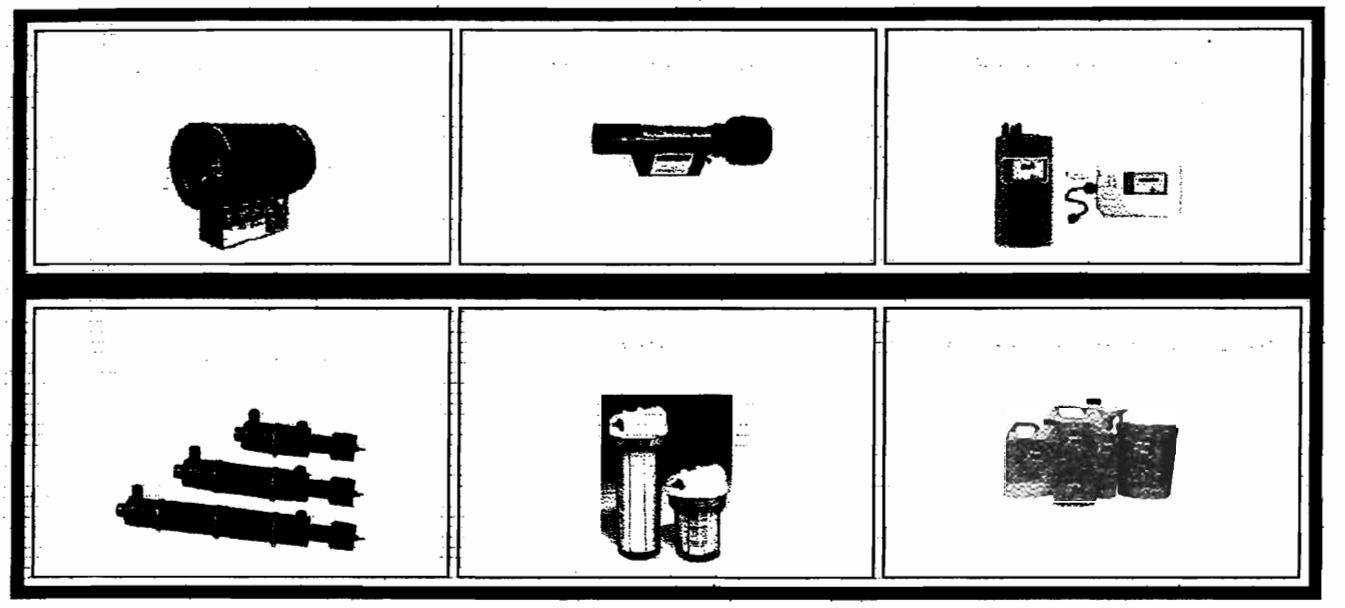
[10] S. A. Huber and F. H. Frimmel, Direct gel chromatographic characterization and quantification of marine dissolved organic carbon using high-sensitivity DOC detection, Environ. Sci. Technol. **28**, 1194-1197 (1994).

[11] S. A. Huber and F. H. Frimmel, Flow injection analysis of organic and inorganic carbon in the low-ppb range, *Anal. Chem.* **63**, 2122-2130 (1991).

[12] F. Fuchs, Gelchromatographische Trennung von organischen Wasserinhaltsstoffen, *Vom Wasser* **66**, 127-136 (1986).

[13] P. Werner, Eine Methode zur Bestimmung der Wiederverkeimung von Trinkwasser, *Vom Wasser* **65**, 258-260 (1985).

[14] I. V. Perminova, F. H. Frimmel, D. V. Kovalevskii, G. Abbt-Braun, A. V. Kudryavtsev, and S. Hesse, Development of a predictive model for calculation of molecular weight of humic substances, Wat. Research, (in press).



We have all experienced the exhilarating effects and clean fresh aroma of the air after a thunder storm. The spice of this fragrance is ozone. Ozone occurs in nature as a result of many environmental circumstances, certainly lightning being one of the most significant. Ozone plays a very important role in the maintenance of our atmosphere. Ozone is also created as ultraviolet light passes into the earth's stratosphere. Ozone is natures way of cleaning the air. It only makes sense that we should use the same principles to our own

advantage.

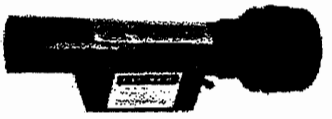
When electrical charges pass through an oxygen atmosphere, stable pairs of oxygen (O2) molecules are ripped apart and reform as a slightly modified form of oxygen, whereas it becomes ozone (O3). A negatively charged ion is formed when an electron attaches itself to an oxygen molecule. Having acquired a third oxygen atom it becomes a strong oxidant, which is highly reactive with hydrocarbons and other such unsaturated molecules. Ozone will act readily to oxidize these types of organic matter. These molecules include such pollutants as chemical fumes, smoke, trepans, and many other such gases. Ozone destroys with extreme efficiency the spores of moulds, amoebae, mildew, viruses and bacterium, as well as a variety of pathogenic and saprophytic germs. These microorganisms are protected within an outer membrane or shell. These membranes are composed of proteins which readily degrade in the presence of ozone. Without this protective coating the organism dies leaving only oxygen in it's place. Levels of 0.1 PPM have been found to be sufficient to kill most pathogens. In addition to the suppression of moulds, viruses and bacterium, ozone provides a germicidal effect, aiding in the healing of plant wounds and enhancing resistance to infection. These unsaturated molecules have an open structure and will readily combine with oxygen. As the ozone is absorbed by the host molecule it fragments the structure of the particle until it breaks up into its basic components, primarily CO2 and H2O. The ozone, having released its third atom in this process, defaults back to stable oxygen. The once offensive pollutant has effectively been dismantled and no longer exists as a contaminant. This process of neutralization is a very efficient sterilizer and purifier, literally destroying odors as nature intended it to happen. Beware however, that excessive levels of ozone can be destructive to living organisms in much the same way as described above. Ozone reacts with most substances by nature of it's oxidizing properties. Materials such as rubber, some plastics, fabrics, paints and textiles are susceptible to deterioration after time and extended periods of exposure. Metals such as iron, zinc, nickel, mercury and silver are highly reactive catalysts to ozone. Gold, platinum, stainless steel, aluminum (especially anodized) and glass are very resistant to ozone breakdown.

Naturally forming surface levels of ozone remain about 0.1 PPM depending upon atmospheric conditions and location. Levels occurring in cities, due to automobile and industrial by-products can be as high as 0.5 PPM. This is considerably higher than the E.P.A., F.D.A., C.S.A. or any of those other A's suggest is suitable for continuous breathing. Although not proven to be dangerous, some people may experience throat or nasal discomfort at prolonged levels above 0.3 PPM.

The UVO3NAIR ozone generator is ideally suited for small enclosed grow spaces. It utilizes a lamp source which bombards oxygen molecules with ultraviolet light, causing them to mutate into the tri-oxygen form, known as ozone. A small fan directs air past the lamp and out into the air mix. Immediately the ozone begins to neutralize odors and pollutants. As this oxidization occurs, the ozone reverts back into oxygen. Ozone also acts as an ionizer. The negative ions attach themselves to a positively charged dust or smoke molecule causing them to fall to the floor. At the same time, the ozonator will help restore the natural negative/positive ion balance in the air. Ozone is unstable and has a half life of only about 30 minutes. For this reason it must be continuously produced to maintain a constant level.

Locate the UVO3NAIR where ozone will be evenly distributed throughout the entire area by circulation fans. Allow the ozone generator to operate continuously, unless the concentration becomes so high that it is intolerable to breath. Timers may be used to cycle the generator at 10 or 15 minute intervals to reduce concentrations. Or you may choose to set a timer to defeat the ozonator 1/2 hour before you plan to visit the area. Another common installation method is to introduce ozone in the path of exhaust system to cycle with ventilation.

Ozone is believed to be nontoxic to plants and animals, although in high concentrations it can irritate tissue in either case. Ozone is highly reactive to all organic matter. Some people are hypersensitive to ozone or just don't like the smell, but it will not harm them. Do not remain in the room if dizziness, headaches, burning eyes, or nose or throat irritation is experienced. These symptoms will disappear after leaving the area.



This model is the best suited for small areas. A suitable enclosure would be 1500 cubic feet. Great for hobbyists or use in your home.

This model is ideally suited for enclosures up to 3000 cubic feet in area. This model can be applicable to very small spaces if controlled with a cycle timer to sequence operation periods.

This model has the output capacity to treat larger areas than the standard model. It is suitable for areas up to 5000 cubic feet. Multiple units can be used to accommodate much larger spaces very efficiently and economically.

Electrical Testing Labs (E.T.L.) certified

Models available for 120 volt and 240 volt applications. Foreign voltages available upon request.

Locate generator where adequate circulation of ozone is present throughout entire area. After two hours of operation, open the ECO patch kit (included) and place the test card in the center of the room, at least six feet from the emitting end of the ozonator. Continue operation for a one hour test period. Compare test card to color strip to determine parts per billion (ppb) value. Divide results by 1000 to convert to parts per million.

All UVO3NAIR models covered by a one year warranty on parts and labor. Replacement parts available from your authorized dealer.

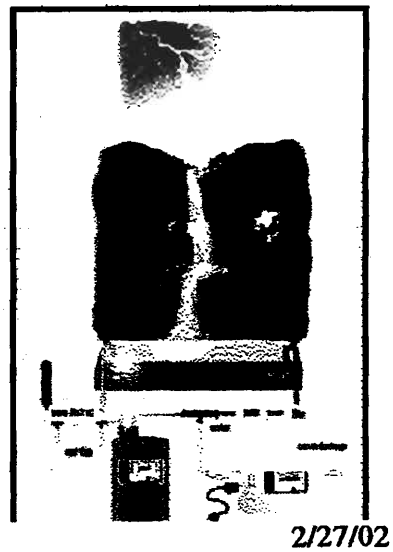
Key Benefits

- Eliminates unwanted odors quickly
- Dramatically improves air quality
- Breaks down and destroys air pollutants
- Discourages moulds and algae growth
- Inhibits pathogens,viruses and bacterium
- A powerful sterilant and germicide
- Restores natural ion balance in the air
- Completely safe and non toxic
- Low cost and easy to install
- Reliable and trouble free
- Requires no service or maintenance
- Resistant to corrosive atmospheres
- Sturdy impact resistant ABS construction
- Operates for less than a 60 watt light bulb
- Quiet and efficient operation
- Ideal for greenhouse or grow room use

Nutrient Solution Ozonator

hydropure.com

Ozone Treatment for Hydroponic Grow Systems



- 1. Minimizes the risk of disease (disinfects pathogens)
- 2. Reduces system organic loading, a source of energy for microorganisms and source of potential toxins.
- 3. Reduces biofouling (deposits, biofilms, sludge) of irrigation lines and hardware surfaces, reducing maintenance requirements and improving sanitation.
- 4. Continuously cleans and disinfects the nutrient solution, increasing the time that the solution can be re used (providing fertilizers are monitored and adjusted)
- 5. Less solution dumping means fertilizer cost savings and less discharges into the environment.
- 6. No harmful residual byproducts – ozone is triatomic oxygen

- 7. Systems are available for commercial greenhouses [redacted]

Several million cubic meters of water are ozonated every day throughout the world. In France, drinking water has been disinfected with ozone since 1906. Today, at least 1300 water treatment plants worldwide are using ozone, including Montreal that ozonates 1.2 million m3 of water daily.
Ozone (03) in water functions both as a microbial disinfectant and a strong oxidizing agent of organic compounds. As a disinfectant, ozone kills microorganisms (including plant pathogens) by oxidizing cell walls and certain essential components inside, such as enzymes, proteins, DNA and RNA. As an oxidizing agent, ozone is one of the most powerful compounds known, having an oxidation potential about 1.5 times higher than that of chlorine. In hydroponic systems, organic compounds from root exudates and organic growing mediums (ea. peat) build up in nutrient solutions. These compounds are food for microorganisms, including plant pathogens, and certain compounds can be toxic to plants over time. Ozone partially oxidizes or breaks up. these organic compounds into smaller compounds that are more biodegradable (seldom are organics totally oxidized to C02 and water). These more readily biodegradable compounds are further oxidized by ozone every time the solution cycles, they are consumed by microorganisms in the solution or in a biological filter, or they can be removed by an activated carbon filter.

Because ozone gas is unstable and cannot be stored, it is produced on-site by corona discharge, using an ozone generator that passes air between electrodes separated by a dielectric and a narrow discharge gap. The result is the conversion of oxygen O2 into ozone O3. The ozone is dissolved by mass transfer into the nutrient solution using a venturi injector. Mass transfer efficiency of 90-955'o is typical, with the remaining 5-10% being vented as off-gas. The undissolved ozone is separated from the water in the degasser unit and the ozone in the off-gas is destroyed by the ozone filter.
Organic loading and microorganisms together make up the oxidizable component referred to as the ozone demand. Dissolved ozone residual appears only after the applied dose surpasses a critical amount corresponding to the ozone demand. Prior to the appearance of a residual, organic compounds are oxidized, and at the same time, microorganisms are inactivated. The presence of residual ozone and sufficient contact time before the ozone is degassed are key requirements for nutrient solution disinfection. The degassing unit doubles as reaction vessel, enabling a period of contact or reaction time before the solution is degassed.

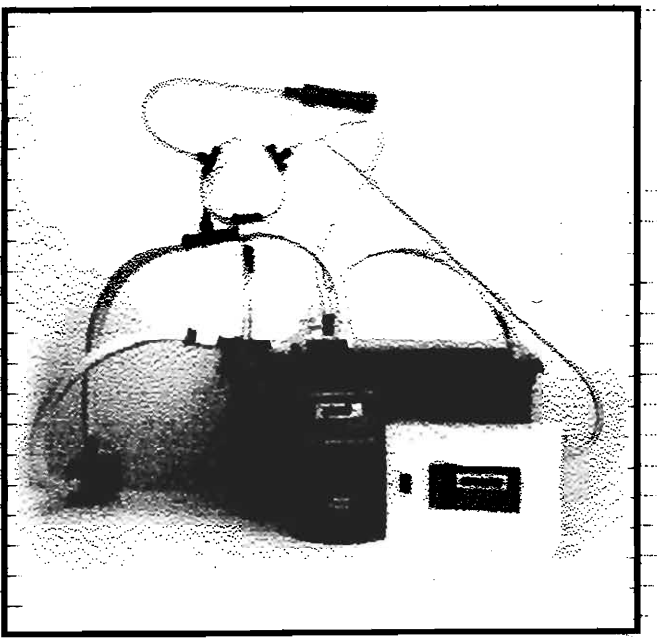
Preliminary evaluations of ozone for soilless culture disinfection have been made. Studies show that ozone is effective in inactivating the plant pathogens tested, including Corynebacterium michiganense, Erwinia carotovora, Pseudomonas solanacearum. Fusarium oxysporum, Verticilllium alboatrum, TMV, and CGMMV. In general, bacteria species are more easily killed by ozone than viruses. Ozone can also oxidize some forms of iron in the nutrient solution. When using ozone. the best iron stability is with Fe-EDTA, followed by Fe-DTPA. Fe-EDDHA has poor stability and should not he used.

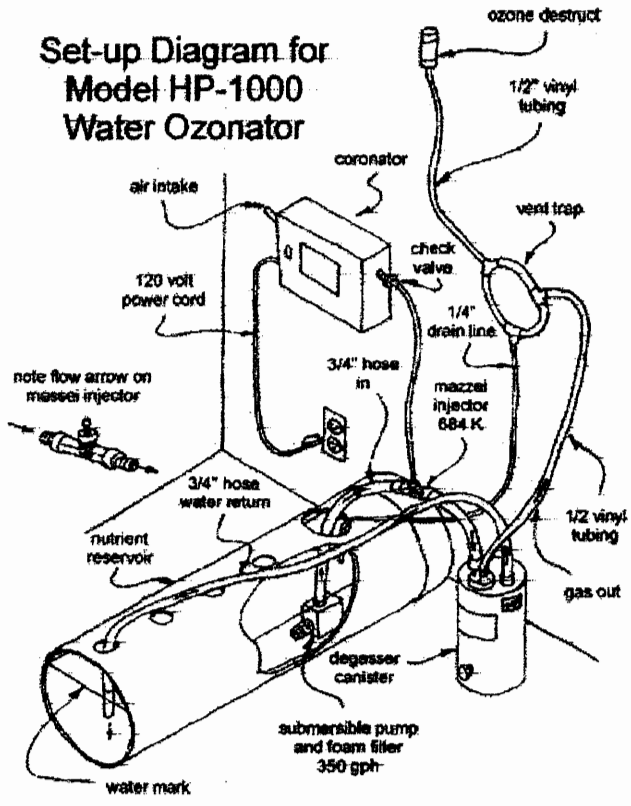
The effective ozone dose for disinfection, and oxidization is time related and therefore cumulative (dosage = concentration of ozone x time). Some growing systems operate continuously (e.g. bare root NFT). In batch style growing systems where irrigation is applied at intervals rather than continuously, a bypass shunt enables continuous circulation of solution in the nutrient reservoir between irrigation cycles. Irrigation is then applied by opening a valve to the irrigation line as required. A second pump dedicated for irrigation can also be used.

Ozone should be applied continuously from system start-up. If brought "on-line" later, microorganisms and organic loading (the ozone demand) may build up to levels where the system ozone capacity is insufficient to provide the desired effect. The ozone system and associated filtration components keeps the solution clean and disinfected. resulting in less deposits and biofilm formation inside irrigation lines and on growing system surfaces, improving sanitation and reducing maintenance needs. Continuous cleaning of the solution means that the same solution can be re-used for longer periods before dumping (many months), providing fertilizer levels are regularly monitored and adjusted as required.

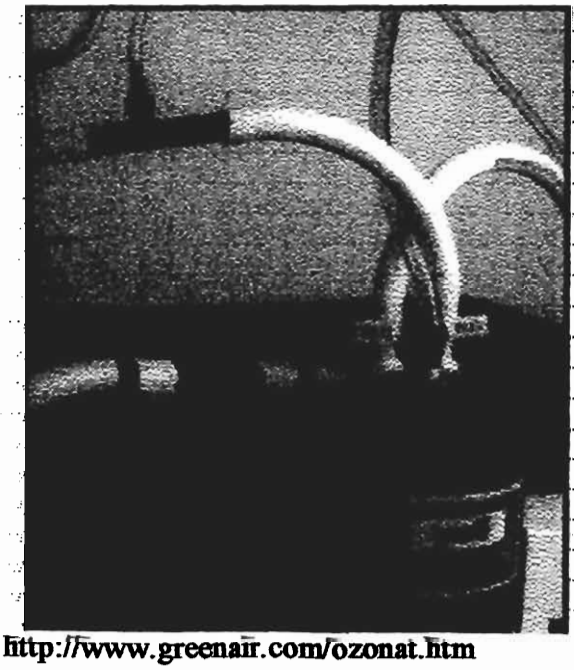
The venturi mass transfer system is also highly efficient at dissolving oxygen into the nutrient solution, ensuring near saturation levels of dissolved oxygen for plant root respiration. An additional venturi can be added after the ozone degasser to provide air bubbles in the nutrient solution supplied to the plants. This is particularly advantageous in deep flow hydroponic systems when the solution is introduced continuously in the bottom of the system and moves through a loose root zone to an overflow.

This is a photograph of the HP-1000 assembly with the hoses connected and the nutrient tank in back. The system consists of the ozonator itself, the mixing and degassing chamber, the massei injector and the gas trap with ozone destruct filter. In this example the pump is shown plumbed inline (open air) with a filter canister in front of it in the intake line.





This is how it works. Water or nutrient solution is directed from the source by a pump or diversion valve. As the solution passes through the mazzei injector it draws ozone from the ozonator which then becomes mixed into the stream. The solution continues on into the mixer/degasser canister where the ozone is further dissolved into the solution. Any remaining ozone is then removed from the flow and vented through the trap and carbon destruct filter. The treated solution is then returned to the reservoir or irrigation system. It's just that simple.



Left : Shows a close up of the plumbing from the degasser and the Massei injector.

Right : Demonstrates the condensation trap and carbon destruct. Notice the trap drain hose is looped in a

[redacted] bow to trap water and block gas escape. [redacted]

Back To Top

Thank You.....This is The END

Send mail to greenair@greenair.com with questions or comments about this web site.
Last modified: October 12, 2001

PEST MANAGEMENT GRANTS FINAL REPORT

CONTRACT NO. 99-0220

FIELD TRIALS FOR THE COMBINED USE OF OZONE GAS AND BENEFICIAL MICROORGANISMS AS A PREPLANT SOIL TREATMENT FOR TOMATOES AND STRAWBERRIES

ALAN PRYOR
SOILZONE, INC.

2001 b

AUGUST 31, 2001

PREPARED FOR CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION

DISCLAIMER - THE STATEMENTS AND CONCLUSIONS IN THIS REPORT ARE THOSE OF THE CONTRACTOR AND NOT NECESSARILY THOSE OF THE CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION. THE MENTION OF COMMERCIAL PRODUCTS, THEIR SOURCE, OR THEIR USE IN CONNECTION WITH MATERIALS REPORTED HEREIN IS NOT TO BE CONSTRUED AS ACTUAL OR IMPLIED ENDORSEMENT OF SUCH PRODUCTS.

ACKNOWLEDGEMENTS - The contributions of Drs. Becky Westerdahl of the Dept. of Nematology and John Duniway of the Dept. of Plant Pathology are gratefully acknowledged. THIS REPORT WAS SUBMITTED IN FULFILLMENT OF CONTRACT NO. 99-0220 - FIELD TRIALS FOR THE COMBINED USE OF OZONE GAS AND BENEFICIAL MICROORGANISMS AS A PREPLANT SOIL TREATMENT FOR TOMATOES AND STRAWBERRIES UNDER THE SPONSORSHIP OF THE CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION. WORK WAS COMPLETED AS OF JULY 31,2001.

TABLE OF CONTENTS

ABSTRACT

This research investigated the effect in tomatoes and strawberries of co-treatment of soils with pre-plant application of ozone gas and post-plant treatment with either fungicidal or nematicidal beneficial microorganisms. Ozone gas was injected 2-3 days before planting into bedded soil through drip tubing buried 3-4" deep along the bed length. In the tomato field trial, two nematicidal bacteria (*Bacillus chitinosporus* and *Burkholderia cepacia*) were used in soils heavily infested with root knot nematodes. In the strawberry field trial, a fungicidal bacteria (*Pseudomonas fluorescens*) and a mixture of fungicidal fungi (a mixture of five *Trichoderma harzianum* strains) were used in a field in transition to organic certification. Each type of beneficial microorganism was applied separately by drench application to transplants prior to planting. Each crop also received one additional application of microorganism that was applied to the surface of the soil and plants subsequent to planting.

Tomato harvest yields indicated that use of either microorganism on a standalone basis resulted in generally higher yields compared to untreated or chemically treated controls. Only the *B. cepacia* treatments on a standalone basis produced a statistically significantly higher yield than untreated or chemically treated controls. Strawberry harvest yields indicated that most treatments produced a numerically higher yield (ranging from 5-15%) compared to untreated controls but none of the increased yields was statistically significant.

EXECUTIVE SUMMARY

Introduction

Tomato and strawberry growers in California are highly reliant on methyl bromide as a soil fumigant. In 1997, use of methyl bromide for strawberries in California amounted to over 4,100,000 lbs. on 20,400 acres. In the same year methyl bromide use in California for tomatoes amounted to over 1,200,000 lbs. on 5,560 acres. Use of methyl bromide is being phased out by 2005 due to concerns of its deleterious long-term effects on the ozone layer. Further, the most effective chemical alternatives, Telone (containing 1,3-dichloropropene) and Vapam (containing metam sodium), include materials that have been placed on California's Proposition 65 list as known carcinogens in recent years and increasingly severe restrictions are being placed on their use. Thus, there is an urgent need for environmentally benign substitutes for preplant soil fumigation.

Paradoxically, ozone gas itself has been recently shown by the project investigators to be effective in increasing yields of crops grown in soils treated with commercially viable quantities of ozone (50 – 400 lbs./acre). This research investigated the effect of preplant co-treatment of soils with both ozone gas and

fungicidal and nematicidal beneficial microorganisms that were used subsequently for tomato or strawberry crop production.

Objectives

The following project objectives were successfully completed as part of this research:

Task 1 - Screen the effects of ozone on the selected beneficial microorganisms in the laboratory.

Task 2 - Presample field soils to determine baseline pathogen pressures.

Task 3 - Prepare fields for ozonation and subsequent planting of test crops.

Task 4 - Perform ozonation and addition of microbes and application of additional control chemicals.

Task 5 - Perform soil analysis after treatments.

Task 6 - Determine Crop Yield.

Task 7 - Preparation of Interim and Final Reports.

Methodology

The following describes the different ozone and microbe treatments employed at each trial.

Tomato Treatments

Untreated Control
Bacillus chitinosporus
Burkholderia cepacia
250 lb. O_3/acre
50 lb. O_3/acre
50 lb. O_3/acre w/ *B. cepacia*
50 lb. O_3/acre w/ *B. chitinosporus*
Telone II
Metam Sodium
Telone EC

Strawberries Treatments

Untreated Control
Trichoderma harzianum

Pseudomonas fluorescens
400 lb. O_3/acre
100 lb. O_3/acre
400 lb. O_3/acre w/ *T. harzianum*
400 lb. O_3/acre w/ *P. fluorescens*
100 lb. O_3/acre w/ *T. harzianum*
100 lb. O_3/acre w/ *P. fluorescens*

Plot sizes were 20 ft. x 32 inches for tomatoes (6 replicates per treatment) and 15 ft x 52 inches for strawberries (3 replicates per treatment).

Ozone gas was injected through drip tubing buried 3-4" deep into preirrigated or moistened soil that averaged 11-13% for the tomatoes and 12-14% for the strawberries.

In the tomato field trial, two nematicidal bacteria (*Bacillus chitinosporus* and *Burkholderia cepacia*) were used in soils heavily infested with root knot nematodes. In the strawberry field trial, a fungicidal bacteria (*Pseudomonas fluorescens*) and a mixture of fungicidal fungi (a mixture of five *Trichoderma harzianum* strains) were used in an organically certified field. Each type of beneficial microorganism was applied separately by drench application to transplant roots prior to planting. Each crop also received one additional application of microorganism (except for *Pseudomonas fluorescens* in strawberries) that was applied to the surface of the soil subsequent to planting. The microorganisms were applied in an amount and concentrations recommended by the manufacturers.

In the tomato field trial, end-of-season harvest yields, root gall ratings, and soil pathogen counts were obtained as well and soil beneficial microorganism obtained during the growing season. In the strawberry field trial, weekly harvest yield were obtained as well and soil beneficial microorganism obtained during the growing season.

Results and Conclusions

Crop yields and soil pathogen and beneficial microorganism concentrations were monitored to gauge the effectiveness of the treatments.

Tomato harvest yields indicated that use of either microorganism on a standalone basis resulted in generally higher yields compared to untreated or chemically treated controls. Only the *B. cepacia* treatments on a standalone basis produced a significantly higher yield than untreated or chemically treated controls. Strawberry harvest yields indicated that most treatments produced a small yield increase (ranging from 5-15%) compared to untreated controls but none of the increased yields was statistically significant.

Outreach and Budget

A field day was held on March 21st at Mallard Bend Farms in which the ozonation technology was displayed and discussed in conjunction with organic farming practices. The Field Day was held as part of a seminar on sustainable farming practices sponsored by the Ecological Farming Association and the USDA Natural Resources Conservation Service.

The project was completed within budget. A no-cost extension of time was made to complete the strawberry field trial.

REPORT

Methodology

Two field trials were performed under these research contracts. The crops, California location, and collaborators are listed below.

Crop	Location	Collaborator
Tomatoes	Irvine	Dr. B. Westerdahl, UCD
Strawberries	Camarillo	Conway Farms Dr. John Duniway, UCD

All ozone was injected through standard 1/2" PVC drip tubing (Drip In, Madera, CA) with 0.5 gph emitters on 12" spacing. Tubing was buried 3.0 – 4.0" deep in 40" (tomatoes) or 52" (strawberries) beds. Injection tubing so used was left in place throughout the duration of the trial and was used for subsequent irrigation in the tomato trial. A single drip tube centered in the bed was used for the tomato trials. Two tubes were used for each strawberry bed with each tube placed 6-8" from the edge of each bed. Both field trial applications utilized ozone produced in air. Ozone treatments were under moistened conditions generally at about half of field capacity and proceeded planting by 1-5 days. Plots were laid out in randomized blocks. Upon harvest of the crops, yields were segregated and weighed.

Ozone gas was injected into preirrigated or moistened soil that averaged 11-13% for the tomatoes and 12-14% for the strawberries. Plot sizes were 20 ft. x 32 inches for tomatoes (6 replicates per treatment) and 15 ft x 52 inches for strawberries (3 replicates per treatment). The following describes the different ozone and microbe treatments employed at each trial.

Tomato Treatments

Untreated Control
Bacillus chitinosporus
Burkholderia cepacia
250 lb. O_3/acre
50 lb. O_3/acre
50 lb. O_3/acre w/ *B. cepacia*
50 lb. O_3/acre w/ *B. chitinosporus*
Telone II
Metam Sodium
Telone EC

Strawberries Treatments

Untreated Control
Trichoderma harzianum
Pseudomonas fluorescens
400 lb. O_3/acre
100 lb. O_3/acre
400 lb. O_3/acre w/ *T. harzianum*
400 lb. O_3/acre w/ *P. fluorescens*
100 lb. O_3/acre w/ *T. harzianum*
100 lb. O_3/acre w/ *P. fluorescens*

Laboratory exposures of all microbes were performed prior to the field trials and all were shown to be susceptible to ozone exposure compared to untreated controls (See Appendix A). As a result of this information, the researchers determined that the most advantageous mode of delivery of the microorganisms would be through a root drench application and additional spray application to the surface of the soil subsequent to ozonation.

Bacterial and fungal isolates were applied as a stand-alone treatment and in conjunction with ozone applications. In the tomato field trial, two nematicidal bacteria (*Bacillus chitinosporus* and *Burkholderia cepacia*) were used. In the strawberry field trial, a fungicidal bacteria (*Pseudomonas fluorescens*) and a mixture of fungicidal fungi (a mixture of five *Trichoderma harzianum* strains) were used. Each type of beneficial microorganism was applied separately by drench application to tomato transplant roots 2 weeks prior to planting and to strawberry transplant roots immediately prior to planting. Each crop also received one additional application of microorganism (except for *Pseudomonas fluorescens* in strawberries) that was applied to the surface of the soil subsequent to planting. The microorganisms were applied in an amount and concentrations recommended by the manufacturers as follows:

Bacillus chitinosporus - The *Bacillus chitinosporus* culture was provided by Natural Resources Group of Woodlake, CA. Two weeks prior to planting, transplant drench solution was prepared to a concentration of 3.8 x 10exp5 CFU/ml. Approximately 1 gallon of microbe solution was added as a drench to a flat of 200 tomato transplants, allowed to soak for 20 minutes, and the excess drained. Post planting solution of the same concentration was prepared two weeks after planting. 0.75 ml of this solution was immediately further diluted to 250 ml of solution and sprayed uniformly onto the surface of each plot followed by approximately 0.5 gallon of water applied in the same manner.

Burkholderia cepacia - The *Burkholderia cepacia* culture was provided by Stine Microbial Products of Adel, IA. Two weeks prior to planting, transplant drench solution was prepared to a concentration of 3.4 x 10exp5 CFU/ml. Approximately 1 gallon of microbe solution was added as a drench to a flat of 200 tomato transplants, allowed to soak for 20 minutes, and the excess drained. Post

planting solution of the same concentration was prepared two weeks after planting. 0.75 ml of this solution was immediately further diluted to 250 ml of solution and sprayed uniformly onto the surface of each plot followed by approximately 0.5 gallon of water applied in the same manner.

Pseudomonas fluorescens - The *Pseudomonas fluorescens* culture was provided by Dr. John Duniway of the Department of Plant Pathology at UC Davis. Transplant dip solution was prepared to a concentration of 5.0 x 10exp9 bacteria/gallon. Bare root strawberry transplant roots were fully submerged in the solution for 10 minutes on October 20 and immediately planted. No post planting application of this microbe was made.

Trichoderma harzianum strains (5) - A mixture of *Trichoderma harzianum* strains were provided by NBT of Seville, Spain. Transplant dip solution was prepared to a concentration of 3.4 x 10exp10 conidia/gallon. Bare root strawberry transplant roots were fully submerged in the solution for 2 minutes on October 20 and immediately planted. Post planting solution was prepared to a concentration of 8.9 x 10exp9 conidia/gallon. 15 ml of solution was applied directly to the exposed root crown of each transplant on January 2.

Results

Tomatoes

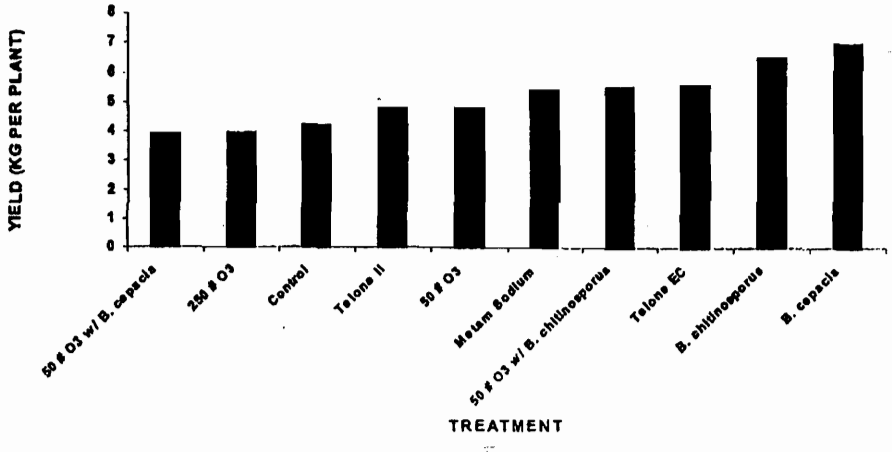
The increase or decrease in yield and Root Gall Rating resulting from each treatment compared to untreated and chemically treated controls is shown below.

Effects of ozone and *Bacillus chitinosporus* and *Burkholderia cepacia* on yield and root gall rating in the tomato field trial

Treatment	kg/Plant	% +/- Control	Gall Rating
50 lb. O_3/acre w/ *B. cepacia*	3.956 - a	-7.4%	8.933 - cd
250 lb. O_3/acre	4.012 - ab	-6.1%	9.333 - d
Control	4.272 - ab	0.0%	9.333 - cd
Telone II	4.840 - ab	13.3%	2.733 - a
50 lb. O_3/acre	4.892 - ab	14.5%	8.267 - cd
Metam Sodium	5.484 - ab	28.4%	9.133 - d
50 lb. O_3/acre w/ *B. chitinosporus*	5.608 - ab	31.3%	8.667 - cd
Telone EC	5.688 - ab	33.1%	5.533 - b
B. chitinosporus	6.576 - bc	53.9%	6.267 - cd
B. cepacia	7.096 - c	66.1%	9.467 - d

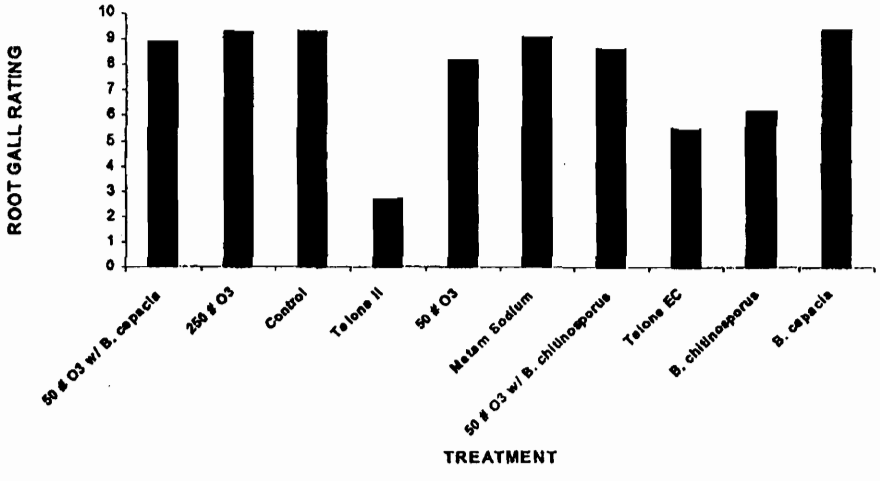
(Separation of means performed with Fisher's Protected LSD at .05 Significance Level for previous and all subsequent analyses)

2000 OZONE FIELD TRIALS
TOMATOES



O_3 no sig effect on yield or galling

2000 OZONE FIELD TRIALS
TOMATOES



Soil levels of *Bacillus chitinosporus* and *Burkholderia cepacia* 4 weeks after planting

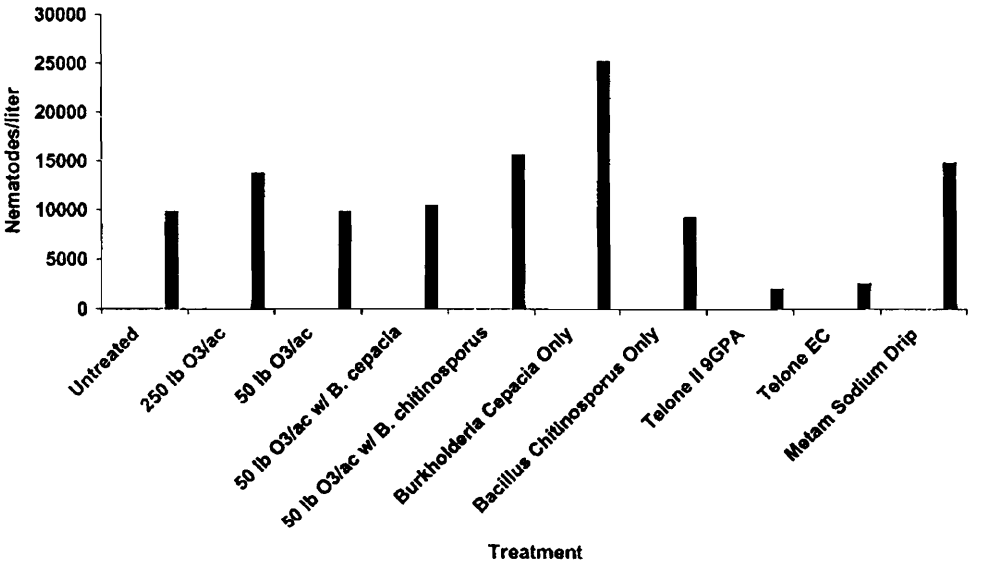
TREATMENT	*B. cepacia* (cfu/gdw soil)	*B. chitinosporus* (cfu/gdw soil)
Untreated control	348,000	32,000,000
Microbe Only	305,000	47,000,000
Microbe + Ozone @ 50 lb./acre	331,000	36,000,000

Effect of Treatments on Soil Root Knot Nematodes at Harvest

Treatment	RK Nemas / Liter
Untreated Control	9,940
250 lb. O_3/acre	13,790
50 lb. O_3/acre	9,870
50 lb. O_3/acre w/ *B. cepacia*	10,490
50 lb. O_3/acre w/ *B. chitinosporus*	15,650
Burkholderia cepacia	25,300
Bacillus chitinosporus	9,350
Telone II	2,140
Telone EC	2,660
Metam Sodium	14,930

tomatoes increase of nematodes with O_3 and microbials

2000 OZONE FIELD TRIALS
TOMATOES



Strawberries

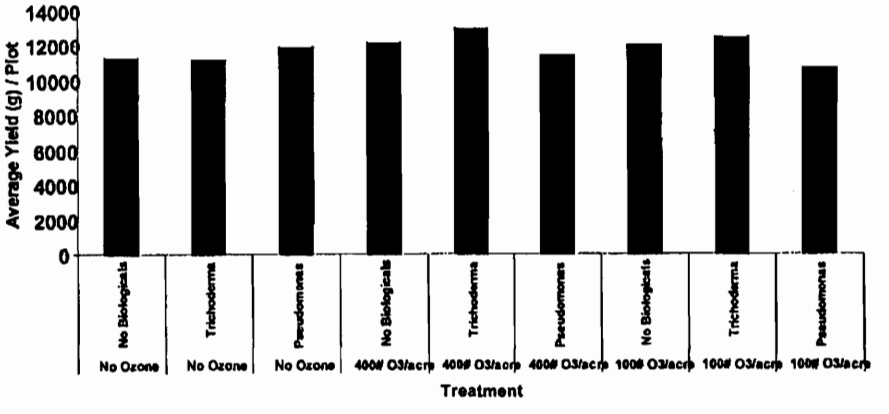
The increase or decrease in yield resulting from each treatment compared to untreated controls is shown below.

Effects of ozone and *Trichoderma harzianum* and *Pseudomonas fluorescens* on yield in the strawberry field trial

Ozone	Yield g / Plot	% of Control
Untreated Control	11378 - a	100%
Trichoderma harzianum	11267 - a	99%
Pseudomonas fluorescens	11968 - a	105%
400 lb. O_3/acre	12246 - a	108%
100 lb. O_3/acre	13090 - a	115%
400 lb. O_3/acre w/ *T. harzianum*	11573 - a	102%
400 lb. O_3/acre w/ *P. fluorescens*	12149 - a	107%
100 lb. O_3/acre w/ *T. harzianum*	12598 - a	111%
100 lb. O_3/acre w/ *P. fluorescens*	10854 - a	95%

no sig increase in strawberry yield

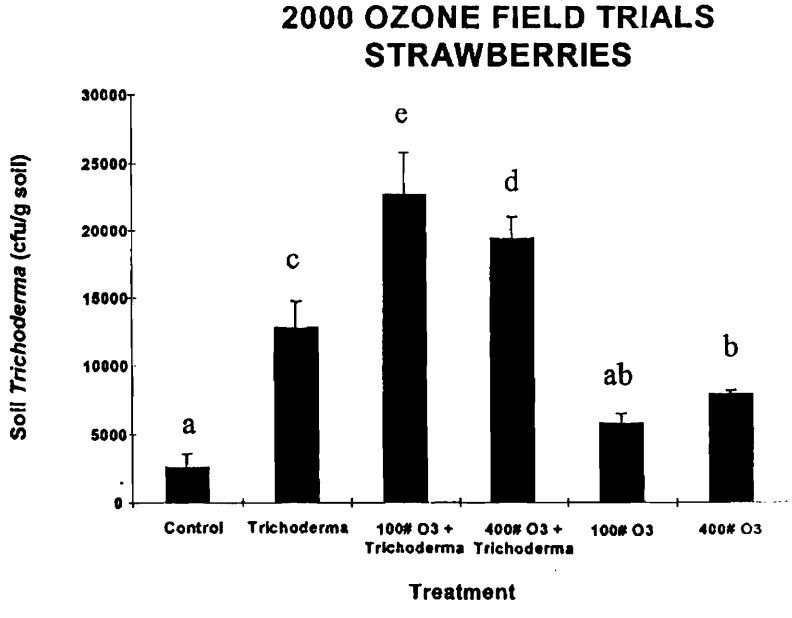
2001 OZONE FIELD TRIALS
STRAWBERRIES



Post Treatment Soil Colonization by Trichoderma (CFU/Gram Wet Soil)

Treatment	Average (cfu/g wet soil)
Control	2,600 -a
Trichoderma sp.	12,778 - c
Trichoderma sp.+ 100lbs ozone/acre	22,667 - e
Trichoderma sp.+ 400lbs ozone/acre	19,333 - d
100lbs ozone/acre	5,800 - ab
400lbs ozone/acre	7,933 - b

Improved colonization of Trichoderm



Discussion and Conclusions

Tomato harvest yields indicated that use of either microorganism on a standalone basis produced the highest yields compared to untreated and chemically treated controls. Ozone at 50 lb. O_3/acre on a standalone basis or in conjunction with *B. chitinosporus* resulted in higher yields (approximately 15% and 30%, respectively) compared to untreated controls and was generally equivalent to chemically treated controls. Only the *B. cepacia* treatments on a standalone basis produced a significantly higher yield than untreated and all chemically treated controls.

The overall differences in yields between ozone treatments and untreated controls were substantially less this year than in previous year, however.

Following is the results of applications of 250 lb. and 50 lb. ozone per acre in tomatoes at the same site in previous years compared to the results achieved in 2000.

Comparative Tomato Yields

Treatment / Year	1997	1998	1999	2000
250 lb. 03/acre	+ 79 %	+ 44 %	+ 20 %	-6.5%
50 lb. 03/acre	NA	+ 19 %	+ 17 %	+15.5%

The differences are partly due to the overall increase in yield in the untreated plots that had the effect of reducing apparent differences in yield compared to years in which soil borne pathogens pressures were greater (i.e. yields in untreated controls were particularly low in 1997 and 1998). The decreased differences in yields also seen between chemically treated and untreated controls over the recent years are also indicative of reduced pathogen pressures. However, it was recently determined that soils from different parts of the field in which these field trials were performed have substantially different characteristics with respect to moisture retention and their ability to allow ozone to pass through the soil without breaking down into oxygen. This may also have been a contributing factor and further research into the underlying causes of these discrepancies needs to be performed.

Colonization of soil by the bacteria, root gall rating, and final soil nematode concentrations were not accurate predictors of final yields. The yields in the standalone biological treatments were the highest in the trial yet also showed little colonization of the soil and generally high root gall ratings and root knot nematode soil counts at the end of the trial.

Strawberry harvest yields indicated that most treatments produced a small increase in yield (ranging from 2-15%) compared to untreated controls but none of the increased yields was statistically significant.

Good colonization of the soil by the *Trichoderma* sp. fungi was achieved with all Trichoderma treatments showing statistically increased soil levels compared to untreated plots. Trichoderma added to ozone treated plots also showed statistically significant increases compared to Trichoderma used on a standalone basis. The uniformity of yield over the ozone treated plots indicates that soil variation was probably not a factor in these field trials.

Problems Encountered and Solutions

i) Tomatoes - Tomato transplants raised for the project at the UC South Coast Extension Station suffered an infestation immediately prior to the planned planting. Almost all alternative sources of transplants in the

marketplace were depleted due to the late date in the planting season. Acceptable alternatives were finally obtained but they were larger than planned due to the late date. Extra efforts were made by the various researchers and staff to ensure that a successful planting occurred and very few transplant losses were noted.

ii) Strawberries - Space for the planned strawberry field trials in Watsonville was not available as anticipated. An acceptable alternative site in Camarillo was secured although soil pathogens were not present as originally represented by the grower. The late date securing the site prevented confirmation of the pathogens in the soil prior to planting because of the length of time required for Verticillium or Phytopthora analyses.

APPENDICES

A. Effects of Ozone on Microbes in a Laboratory Experiment

I. Effects of ozone on *Bacillus chitinosporus* and *Burkholderia cepacia* in a laboratory experiment

TREATMENT	*B. cepacia* (cfu/gdw soil)	B. chitinosporus (cfu/gdw soil)
Untreated control	6,740,000	3,190,000
Ozone @ 50 lb./acre	5,570,000	3,450,000
Ozone @ 250 lb./acre	3,260,000	1,930,000

II. Effects of ozone on Pseudomonas *fluorescens* and *Trichoderma harzianum* in a laboratory experiment

TREATMENT	*P. fluorescens* (cfu/gdw soil)	*T. harzianum* (cfu/gdw soil)
Untreated control	9,600,000	12,100,000
Ozone @ 100 lb./acre	11,500,000	13,300,000
Ozone @ 400 lb./acre	6,900,000	14,700,000

not much effect on T. harzianum

continuously cultured in the absence of *Rk* on susceptible plants. The influence of host selection on the occurrence of the virulent phenotype in *M. incognita* is discussed.

Termination of obligate developmental dormancy in an ascarid. E. G. PLATZER (1), L. T. Luong (2), and N. Hinkle (3). (1) Dept. of Nematology, University of California, Riverside, CA 92521; (2) Dept. of Nematology, University of California, Davis, CA 95616; (3) Dept. of Entomology, University of California, Riverside, CA 92521. Phytopathology 91:S141. Publication no. P-2001-0089-SON.

Allodapa suctoria is a subulurid nematode in the Order Ascaridida. As an adult it is common in the cecum of poultry. Infective juveniles (J2) are encapsulated in the hemocoel of coleopterans, dermapterans, and orthopterans. Encapsulated J2's were obtained by dissection from *Alphitobius diaperinus* (darkling beetle) and placed in physiological saline at 38 C in a 95 percent nitrogen: 5 percent carbon dioxide atmosphere. No excystment took place. In a second experimental series, the J2's were released from the capsule when trypsin was included in the saline. However, the J2's remained inactive. Inclusion of bile salts (sodium tauroglycolate) in the incubation conditions used in the second experiment resulted in the release of active J2's. In this case, bile salts were the physiological trigger required to terminate the obligate developmental dormancy in the J2's of *Allodapa suctoria*.

Biofumigation and soil heating to control *Meloidogyne incognita* and *M. javanica*. A. T. PLOEG. Department of Nematology, University of California, Riverside, CA 92521. Phytopathology 91:S141. Publication no. P-2001-0090-SON.

Plastic vials (250 ml) were filled with *M. incognita* or *M. javanica* infested sand. Treatments consisted of adding or not adding finely chopped fresh Broccoli leaf and stem material (2 g/100 g sand) to the sand, and placing the vials in waterbaths at 40, 35, 30, 25 and 20 C. Vials were removed from the waterbaths after 1, 3, 10, 15 and 20 days, melon (var. Durango) was then seeded in each vial and the melon seedling was grown for 4 weeks. After 4 weeks, root and top fresh weight, root galling, and number of egg masses were recorded. Results were similar for *M. incognita* and *M. javanica*. At temperatures above 25 C, addition of broccoli plant refuse to the soil significantly reduced galling of melons. In a related study on a *M. incognita* infested field, the combination of solarization and incorporation of broccoli refuse, reduced melon root galling and population levels at harvest, and increased fruit yield compared to the non-treated fallow control.

Host status and susceptibility of Lisianthus to three *Meloidogyne* species. A. T. PLOEG. Department of Nematology, University of California, Riverside, CA 92521. Phytopathology 91:S141. Publication no. P-2001-0091-SON.

Lisianthus (*Eustoma grandiflorum*), a plant native to the USA, is gaining in popularity in the floriculture industry. Root-knot nematodes were suspected as a cause of poor stand, yellowing and wilting of Lisianthus in greenhouse operations in Southern California. As information on the host status and susceptibility of Lisianthus to root-knot nematodes is not available, greenhouse experiments were initiated to provide such information. Transplants of Lisianthus var. Mariachi - Lime Green or four-week-old tomato var. Tropic seedlings were planted into pots containing 3 kg steam-sterilized soil. One week later, second-stage juveniles (J2) of the root-knot nematodes *Meloidogyne incognita*, *M. javanica* or, *M. hapla* were added to the pots (10^2, 10^3, 10^4 juveniles per pot). Pots without nematodes were included as controls,. Data on the host status and susceptibility of Lisianthus to the three *Meloidogyne* species will be presented and compared to tomato.

Functional analysis of a nematode induced cell cycle gene, cdc2a, through antisense and cosuppression. E. PLOVIE (1), E. Huyck (1), and G. Gheysen (1,2). (1) VIB, Dept of Plant Genetics, Ghent university, K.L Ledeganckstraat 35, 9000 Ghent, Belgium; (2) Present address: Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure links 653, 9000 Ghent, Belgium. Phytopathology 91:S141. Publication no. P-2001-0092-SON.

Sedentary plant parasitic nematodes are economically important because they cause yield and quality losses in many crops. Juveniles emerging from the eggs infect plant roots by penetration, migration to specific tissues and they induce a redifferentiation of plant root cells into specialised feeding cells. These feeding cells are indispensable for the growth and reproduction of the nematodes. Cyst nematodes generate syncytia by cell wall degradation and root knot nematodes induce giant cells by mitosis without cytokinesis. Feeding site formation by cyst and root knot nematodes is accompanied by cell cycle activation and the multinucleate nature of these feeding cells is generated by incomplete cell cycles. During the last years several cell cycle genes have been identified that show much higher expression level in the nematode feeding cells compared to normal root cells. In this study we want to inhibit the expression of a cell cycle gene, cdc2aAt, that is strongly activated in the feeding sites, by antisense and cosuppression. In this way we hope to block the formation or the functioning of the feeding cells, so we can learn something about the role of the cell cycle and in the mean time try to make the plant resistant against infection by sedentary nematodes. Several constructs have been made and transformed in A. thaliana. Nematode resistance tests with the cyst nematode Heterodera schachtii and the root-knot nematode Meloidogyne incognita were performed and the most interesting lines will be analyzed at the molecular level.

Evaluating the potential for antagonism between an aphid predator and entomopathogenic nematodes. J. R. POWELL and J. M. Webster. Dept. Biological Sciences, Simon Fraser Univ. Phytopathology 91:S141. Publication no. P-2001-0093-SON.

Antagonism (an interaction between members of different species that reduces the ability of one or both agents to manage a host population) can occur in integrated pest management systems if the control agents are not compatible. An inundative application of entomopathogenic nematodes (EPNs) generally is believed to have no impact on beneficial arthropod populations, but this assumption has not been rigorously tested. The aphid midge, *Aphidoletes aphidimyza*, is used to manage aphids in greenhouse, orchard, and field crops, and several stages in its life cycle are vulnerable to EPN infection. Filter paper bioassays indicate that the level of aphid midge mortality differs with EPN species applied (>80% for *Steinernema carpocapsae*, <25% for *Heterorhabditis bacteriophora*; ~500 IJs for 5 days), and is lower if the midge is cocooned during exposure (>20% (uncocooned), <2% (cocooned) for *Steinernema feltiae*; ~500 IJs). Non-target insects, such as the aphid midge, may act as alternate hosts and/or disrupt host cue gradients, thus interfering with target-host finding by EPNs. The potential for antagonism in the EPN-aphid midge system will be discussed.

Interaction between the reniform nematode and thrips on Mississippi cotton. W. O. PRICE, G. W. Lawrence, and J. T. Reed. Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762. Phytopathology 91:S141. Publication no. P-2001-0094-SON.

Tests were established in field micro plots to examine the interaction between the reniform nematode (*Rotylenchulus reniformis*) and thrips on seedling cotton. Cotton cv. Bollgard 33B was inoculated with initial population numbers (Pi) of 0, 500, 1000 and 5000 reniform nematodes / 500 cm^3 of soil. When plants emerged, thrips were infested at Pi = 0, 2, 5 and 10 thrips / plant and immediately caged. At harvest, plant height, total nodes, location of bolls by node and position, number of aborted terminals, number of vegetative bolls and cotton yields were recorded. Initial population levels of the reniform nematode and thrips had a significant effect on cotton plant growth parameters. Both the reniform nematode and thrips reduced the number of nodes per plant. A greater number of cotton plants with aborted terminals were recorded in the reniform infested plots. Seed cotton yields decreased as the reniform Pi levels increased. No significant interaction between the reniform nematodes and thrips was observed for seed cotton yields.

Reduction of root-knot nematode, *M. javanica*, in soil treated with ozone. J. J. QIU (1), B. B. Westerdahl (1), A. Pryor (2), and C. E. Anderson (1). (1) Dept. Nematology, University of California, Davis, CA 95616; (2) SoilZone, Inc., Davis, CA 95616. Phytopathology 91:S141. Publication no. P-2001-0095-SON.

Ozone gas (O3), has been extensively used to disinfect water. O3 rapidly breaks down into oxygen following application. The use of O3 for soil-fumigation was investigated in laboratory trials. O3 was produced at a concentration of 1% in air by a conventional electrical discharge O3 generator. In preliminary trials, O3 mass transfer rate was influenced by soil texture and moisture levels. Two ozone dosage levels and 3 gas flow rates were tested against resident populations of *M. javanica* (MJ) and free-living nematodes (FL) in a field collected sandy loam soil with a moisture level of 11% (w/w). FL consisted of 58% Cephalobidae, 21% Diplogasteridae and 5 additional families. Survival was assessed with Baermann funnel. Results from two replicated trials were consistent. The reduction for both MJ and FL was dosage dependent and flow rate independent. Compared with untreated, at a dosage equivalent to 250 kg/ha, MJ and FL, were reduced by 68% and 52% ($P = 0.05$), respectively. At a dosage equivalent to 50 kg/ha, reductions were 24% and 19% ($P = 0.05$), respectively.

① 10 mg/liter improves flow ② no effect at higher salt [Na⁺] below 15% ESP

③ increased salt leaching [cations] lowers pH

OZONE SCIENCE & ENGINEERING
Vol. 23, pp. 65-76
Printed in the U.S.A.

0191-9512/01 $3.00 + .00
International Ozone Association

The Effects of Ozonated Irrigation Water on Soil Physical and Chemical Properties

2001

Logan Raub[1], Christopher Amrhein[1*], and Mark Matsumoto[2]

[1]Department of Environmental Sciences
Graduate Program in Soil and Water Sciences
University of California, Riverside

[2]Department of Chemical/Environmental Engineering
University of California, Riverside

*Corresponding author: amrhein@ucr.edu

Received for Review: 6 July 1999
Accepted for Publication: 10 March 2000

Abstract

There are reports that ozone in irrigation water can improve crop vigor, reduce insect and disease, enhance water penetration, and reduce fertilizer needs. It has been noted that ozone treated field soils seem spongier and have less standing water. Here we report on a laboratory column study on the effects of ozonated irrigation water on hydraulic conductivity, soil hardness (aggregate strength), clay dispersion, soil swelling, and changes to the chemical composition of the leachate water. Additional batch studies were conducted to characterize the factors affecting the rate of ozone loss in soil/water suspensions and the results used in a mathematical model to predict ozone movement into a soil. We found that ozone increased the saturated hydraulic conductivity and decreased clay dispersion in a loamy soil, but not if the soil had an exchangeable sodium percentage >15%. In two other soils tested, the ozone effects were mixed or insignificant. In every soil tested, the drainage water from the ozone-treated columns had lower pH's and higher electrolyte concentrations. This is attributed to organic matter oxidation and the weak acid properties of ozone. The rate of ozone degradation in soil water could be modeled using the total organic carbon content of the soil, the pH, and the soil/water ratio. Based on the rate of ozone loss in soil/water suspensions, the calculated depth of ozone penetration during the initial wetting of the soil was <2 mm, indicating that the reaction may be limited to the surface.

Keywords

Ozone; Irrigation Water; Agricultural Applications; Soil Properties; Ozone Degradation in Soil; Tulare Clay; Milham Soil; Ramona Soil; Infiltration Rate;

Introduction

Ozone is a strong oxidant that has been used for over a hundred years to disinfect drinking water and to control undesirable color, taste, and odor in water. Ozone has also been used to oxidize and precipitate iron and manganese from groundwaters. More recently, there has been an interest in the use of ozone in irrigation water. Farmers report that low doses (<1 mg L^{-1}) of ozone added to irrigation water improve crop vigor, reduce insect and disease, enhance water penetration, and reduce the need for fertilizers (Pedersen and Redsun, 1996). Most of these reports are anecdotal and few controlled studies have been conducted.

There is good evidence that ozone can have an effect on the surface chemistry of colloidal materials in water. Several drinking water treatment plants now preozonate the raw water to improve filtration. It has been found that the ozone helps "remove clay turbidity through coagulation", although the mechanisms are not well understood (Langlais, et al., 1991, chapter III.F). It has been hypothesized that ozone reacts with the organic coating on the clay and either removes or modifies the organic matter, thereby changing the surface charge of the clay. An interesting finding is that more is not always better. Maier (1984) reported that flocculation reached a maximum when the ozone doze was between 0.5 and 1.5 mg L^{-1}.

The problems in soils of poor permeability, low hydraulic conductivity, crusting, sealing, and hard-setting are largely attributed to changes in surface charge on clays and the interaction of adsorbed cations, anions, organic matter, pH, and ionic strength. The fact that ozone has an important effect on coagulation, flocculation, and filter performance suggests there may be a beneficial effect to water management in irrigation and soil tilth.

Pedersen and Redsun (1996) interviewed farmers that had used ozone in their irrigation water and there was general agreement that the topsoil in ozone treated fields was more porous and spongy. These farmers also reported less standing water, decreased clodding, and deeper water penetration into the soils. All of these observations are in agreement with the effects one would predict from an amendment that increases clay flocculation.

In agriculture, the most common soil amendment to improve infiltration, soften hard soils, and reduce clodding and crusting, is gypsum ($CaSO_4 2H_2O$). Gypsum improves clay flocculation by increasing the Ca^{2+} concentration of the soil water, increasing the ionic strength, and replacing adsorbed sodium. Sodium on the surface of clays has a deleterious effect on soil structure and tends to increase the repulsive forces between negatively charged clay particles. Replacing exchangeable Na^+ with divalent or trivalent cations reduces clay dispersion and swelling and improves the physical properties of soils. We originally hypothesized that the flocculating effects of ozone would not be sufficient to overcome the dispersive forces induced by high exchangeable Na, similar to the results which had been found in earlier studies with synthetic polymers (Zahow and Amrhein, 1992).

In this project, we investigated the effects of ozonated irrigation water on the physical and chemical properties of soils in repacked columns. The properties measured were saturated hydraulic conductivity, aggregate strength, clay dispersion. soil swelling, and changes to the chemical composition of the leachate water. Batch studies with soil + water suspensions were performed to identify the factors that affect the rate of ozone loss in soil water. A mathematical model was constructed from the data to predict the rate of ozone loss in soils based on the soil/water ratio. the initial ozone concentration, the total organic carbon in the soil, and pH. Other variables that were evaluated included the alkalinity, clay content. and specific surface area.

Materials and Methods

Experiment 1: Column Study

Three soil types were used to investigate the effects of ozone on the physical properties of soil in the column study. The first set of soil samples, identified as "Tulare", came from an experimental field site in the Tulare Lake Basin of the San Joaquin Valley near Corcoran, CA. This soil is classified as a Tulare clay, a fine, montmorillonitic (calcareous), thermic Vertic Haplaquoll. The soil is typically cropped to cotton and safflower. The experimental field plots, from which the soil samples were collected, had been irrigated for six years with mixtures of low-salinity canal water and saline drainage water. The blended irrigation water

had total dissolved solids (TDS) of 400, 1500, 3000 and 4500 mg L^{-1}.

The second set of soil samples was collected from an experimental field site on the west side of the San Joaquin Valley, CA. The soil is classified as a "Milham" soil and characterized as a fine-loamy, mixed, thermic Typic Haplargid. This set of samples was collected from a pistachio orchard that was being used in a salt-tolerance study. The field site had been irrigated for two years with blended waters of varying electrical conductivities of 0.75, 2.0, 4.0, 6.0, and 8.0 dS m^{-1} (approx. 500, 1300, 2600, 3800 and 5000 mg L^{-1} TDS, respectively). For this experiment, the soils were labeled T-1, T-2, T-3, T-4 and T-5 for the varying treatment salinities.

The last soil studied using the columns, came from Riverside, CA area and is classified as "Ramona" and characterized as a fine-loamy, mixed, thermic Typic Haploxeralf. This soil is typically cropped to citrus and is distinct from the soils collected from the San Joaquin Valley because of the fresh organic matter content.

All soils were air dried, crushed and passed through a 2-mm sieve prior to testing. Saturation extracts of the soils were analyzed for the major cations (calcium, sodium, potassium and magnesium), pH, and electrical conductivity (EC). The sodium adsorption ratios (SAR) were calculated from extracted solute concentrations and the exchangeable sodium percentages (ESP) were measured on the Tulare and Milham soils using 1.0 M ammonium acetate extract. The ESP of the other soils were estimated from the SAR's, using a previously determined, average selectivity coefficient. Particle size analysis was determined using the hydrometer method (Klute, 1986). The alkalinity was determined by titrating a sample of the extract to pH 4.4 using 9.4 mM sulfuric acid. The total organic carbon in the soil was determined by a loss-on-ignition method (Sparks 1996), where the mass difference after heating at 400°C for 16 hours was taken as the amount of organic matter ignited. The surface area was determined using the ethylene glycol mono-ethyl ether (EGME) method (Klute 1986). The chemical and physical properties of these soils are listed in Table I.

The ozonated water was produced on an as-needed basis utilizing an Osmonics generator that produced ozone by feeding dry compressed air through a column where corona discharge produces atomic oxygen radicals that further react with oxygen molecules to produce ozone. The ozone-enriched gas was then diffused into a beaker of water for 10-15 minutes at a rate of 1 L ozonated air per minute to give concentrations of ozone of approximately 10 mg L^{-1}. The concentration of ozone in the water could be varied by changing the flow of ozonated air into the water column or by varying the amount of voltage applied in the corona discharge. For an experiment with differing concentrations of ozone, it was found that varying the voltage gave the most consistent ozone concentrations.

Ozone concentration in the applied water was determined using the iodometric test for residual chlorine, which was modified to measure residual ozone. The iodometric test was used because it is quick, easy, and accurate (Greenberg et al., 1992). Because other oxidants like chlorine, oxides of nitrogen, and manganese species interfere with the iodometric test, the water was aged prior to ozonation by leaving it open to room air for a day to allow for dissipation of residual chlorine. Normally the iodometric test is done on an acidified sample, however there was interference from Mn in the leachate samples at low pH. Eliminating the acidification step reduced the interference from manganese and other oxidizing compounds without substantially affecting the ozone determination. In general, a 50-mL aliquot of ozonated water or leachate was reacted with 0.5 g of KI for five minutes. The sample was then titrated with a standardized thiosulfate solution to a colorless end-point using a starch indicator. The reaction of ozone with KI is as follows:

$$O_3 + 2KI + H_2O = I_2 + O_2 + 2KOH \quad [1]$$

The iodine formed is then titrated with sodium thiosulfate:

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-} \quad [2]$$

The soil columns were constructed from glass cylinders with the dimensions 4.7 cm internal diameter and 20 cm length. Steel mesh was attached to one end of each column, and two layers of cheesecloth placed above the mesh to contain the soil during leaching. One hundred grams of soil was weighed, placed into each column and tamped

TABLE I. CHEMICAL ANALYSIS OF SOILS.

	Saturation Extracts										
	Electrical conductivity	pH	Na	K	Ca	Mg	SAR†	ESP‡	TOC§	PSA¶	SURFACE AREA
	dS m^{-1}		mM	mM	mM	mM	mM$^{1/2}$		(%)	Sa/Si/C	(m^2 g^{-1})
Tulare											
400	2.8	8.13	11.26	1.29	6.33	3.96	4.96	8.00	3.67	10/36/53	128.9
1500	2.7	8.33	16.25	1.00	4.02	2.31	9.14	12.40	"	"	"
3000	3.2	8.41	22.95	0.67	1.38	1.63	18.70	20.00	"	"	"
4500	3.7	8.70	37.07	1.23	3.58	1.59	16.30	19.40	"	"	"
Milham											
T-1	0.6	7.18	2.78	0.60	1.63	0.67	1.83	2.50	1.92	39/25/36	146.3
T-2	1.1	7.22	6.14	0.65	2.51	0.74	3.41	6.70	"	"	"
T-3	1.5	7.30	12.13	0.62	1.65	0.44	8.38	11.50	"	"	"
T-4	5.0	7.39	47.64	1.13	10.25	1.34	14.00	22.10	"	"	"
T-5	2.7	7.54	23.53	0.77	2.55	0.48	13.51	24.10	"	"	"
Ramona	1.12	7.90	4.20	0.62	3.87	1.11	1.92	2.80	4.16	76/13/11	51.8
Buffalo >7"	2.11	7.50	11.03	0.40	5.07	1.28	4.38	6.10	5.43	21/40/39	77.2
Buffalo 0-2"	2.89	7.46	8.12	0.79	10.91	3.85	2.11	3.00	11.99	31/45/23	75.5
Holtville	1.03	7.93	4.26	0.60	2.50	1.02	2.27	3.20	1.88	19/30/51	173.7
Hanford	1.51	8.00	5.84	0.73	3.97	1.38	2.53	3.60	0.89	84/12/4	30.1
Imperial	3.85	7.50	24.49	0.62	8.62	4.90	6.66	8.90	1.92	12/52/35	141
Etiwanda	0.86	7.50	1.77	0.74	3.08	0.95	0.88	1.30	1.11	89/10/1	29.9
Grangeville	0.47	7.15	0.52	0.34	1.23	1.04	0.34	0.50	1.26	54/35/11	47.7
Merriam	1.06	6.78	1.87	0.56	2.67	1.30	0.94	1.40	1.71	76/15/9	33.4
Coachella	0.96	7.50	4.12	0.60	2.63	0.91	2.19	3.10	0.99	86/12/1	22.2
Pachappa	2.04	7.50	5.71	0.48	7.61	1.39	1.90	2.70	1.17	75/16/9	52

† Sodium Adsorption Ratio
‡ Exchangeable Sodium Percentage
§ Total Organic Carbon. Mass percentage of soil which is organic carbon.
¶ Particle Size Analysis. A separation of the mineral fractions into sand, silt and clay sized fractions, expressed as a percentage of mass.

to a bulk density of 1.3 g cm^{-3}. A small piece of filter paper (2-cm diameter) was placed on the soil surface to decrease the energy of the applied water, which would disturb the surface layer of the soil. The constructed columns were then placed in a wooden brace that was able to hold twelve soil columns side by side. Ozone treated and control columns were run alongside each other. A funnel was placed above to deliver irrigation water to the columns and another funnel was placed below the column to catch the leach-water, which was collected in 250-ml beakers for sampling and further analysis.

The soil columns were leached (irrigated) with aliquots of aged Riverside tap water or aged, freshly ozonated tap water in increments of 10 to 20 mL. Different soils received different aliquots of water, depending on the hydraulic properties of the soil. Water was applied at a rate that approximately matched the hydraulic conductivity. For some soils with high hydraulic conductivities, water was applied as often as every 20 minutes. For the soils with lower hydraulic conductivities, water was applied two or three times a day. Each soil received equal amounts of treatment water so that comparisons could be made between control and treatment. The treatments lasted approximately two to three days and a maximum of 200 mL/column applied. Some of the soils with high ESP's received <100 mL because of very slow infiltration rates. All treatments were replicated five fold. An average of 2.7 pore volumes of leachate was collected. The leachate from the columns was analyzed for electrical conductivity (EC_{25}), pH, and turbidity by light absorption at 500-nm wavelength. The leachate waters from the columns were also analyzed for calcium, magnesium, potassium, sodium, iron, and magnesium using the ICP-optical emission spectrophotometer.

To calculate a saturated hydraulic conductivity of the soil, the columns were ponded with 200 mL (giving a hydraulic head of approximately 12 cm) of water and allowed to percolate for a measured amount of time. Measurements were taken as to the height of the soil column, the height of the water at time zero and the height of the water after an arbitrary amount of time had passed. Saturated hydraulic conductivity, K_{sat}, was then determined using the modified Darcy's Law for a falling-head permeameter [$K_{sat} = (L/t_1) * (\ln(b_0+L) - \ln(b_1+L))$], where L is the length of the soil column, b_0 is the depth of water on top of the soil at time = 0, b_1 is the depth of water after time t_1 had passed.

Following leaching, the soils were allowed to drain overnight by gravity and then were weighed. The columns were then placed in an oven at 105°C for 24 hours, cooled and re-weighed. The differences between the weights were used to measure changes in water holding capacity, and assumed to give some measure of soil swelling.

The dried soil cores were subjected to tensile strength tests to determine if the soils had changed in hardness. The tensile strength test was performed using a Chicago Soil Test unit, which compressed the cores to the breaking point using the method of Dexter and Kroesbergen (1985). In this test, the cores were laid on their sides and the highest mass required to crush the cylindrical cores was recorded. The tensile strength is linearly proportional to the loading force at failure (Dexter and Kroesbergen, 1985).

All data for the column experiments were analyzed using the Student t-test for significant difference with a one-tailed significance distribution. Data entered into the tables are the means of the five columns run for each treatment.

Experiment 2: Batch Suspension Studies

This study was done because of the difficulties we encountered when trying to measure the loss of ozone with depth in column experiments with varying masses of soil. We concluded that to predict the penetration depth of ozonated water, we needed to measure the rate of ozone loss in a well-mixed system containing samples of the soil. Once we knew the rate constant for ozone loss in a well-mixed soil water system, a mathematical model could be used to predict the depth of ozone penetration into a soil.

An additional ten soil types (plus the three from the column experiment) were selected to study the rate at which ozone is lost from a well-mixed system of soil, ozone, and water (Table I). For the experiment, a 300-mL sample of aged Riverside tap water was ozonated for 10-15 minutes using the Osmonics ozone generator and then analyzed for initial O_3 concentration. A weighed amount of soil (0.1 to 1.0 g) was introduced to the ozonated water and the soil /water suspension gently stirred with a magnetic stir

15% ESP

bar while the ozone concentration was monitored with time. Ozone concentration was monitored by withdrawing and filtering 10 mL of the solution into a beaker containing 0.5 g KI. The membrane filter excluded suspended colloids that might have interfered with the iodometric test. The acidification step was left out to reduce the interference from soluble manganese and oxides of nitrogen species. The sample was allowed to react for five minutes with the KI and then titrated with a standardized thiosulfate solution. The concentration of ozone in the sample was calculated and the loss of ozone with time was plotted. Soil solution pH was monitored throughout the experiment runs, noting a strong correlation of rate of ozone consumption to pH from preliminary experiments. The experiment was then repeated with a different soil mass.

Modeling

The model for ozone decomposition we used is based on a model constructed by Yurteri and Gurol (1988). The Yurteri and Gurol model was formulated to predict the rate constant of ozone consumption in municipal wastewater. Their model was based on the chemical properties of the wastewater, including pH, alkalinity, and total organic carbon (TOC). Our model initially used these parameters but was expanded to include suspension density (mass of soil to solution volume ratio), specific surface area of the soil, and clay content. Initially we hypothesized that to better predict the loss of ozone in our system, we needed to include the surface area of the soil in the equation, because of the potential interaction of ozone with surface coatings on soil minerals.

Results and Discussion

Experiment 1: Column Study

The effect of ozone-treated irrigation water on the saturated hydraulic conductivity of the Milham soil samples is shown in Figure 1. The K_{sat} values decreased with increasing ESP, an observation that is typically seen. Each point on the graph is an average K_{sat} of five replicated columns in each of the samples (T-1, T-2, ... etc.). The error bars drawn are the standard deviation for those five soil columns. The ESP values plotted in Figure 1 were determined from the SAR of the leachate solutions, and therefore are somewhat different than the SAR values of the saturation extracts. The relationship between SAR and ESP had previously been determined for this soil and was used to calculate the ESP.

Improves flow below 15% ESP

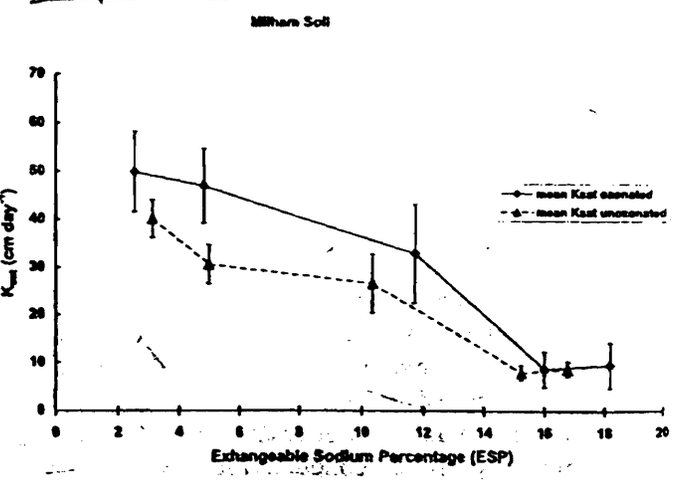


Figure 1. Saturated hydraulic conductivity of the Milham soil as a function of ESP and ozonated irrigation water treatment. The error bars are ± one standard deviation of the five replicates.

At ESP values below 15%, there was a significant increase in the K_{sat} values in the ozonated-treated Milham soil (Fig. 1). At ESP values above 15%, the dispersive effects of high exchangeable sodium completely over-rode any beneficial effects of the added ozone to hydraulic conductivity.

The Tulare soil, which is finer textured than the Milham, did not show a significant improvement in saturated hydraulic conductivity as a result of ozonation at low salinities (Table II). The K_{sat} values generally decreased with increasing ESP, which appears to be the most important factor controlling K_{sat} in this soil. The statistical analysis of the average K_{sat} values showed the ozonated treatment of the 1500 sample was significantly lower than the unozonated treatment. The more saline samples (3000 and 4500) had significantly higher K_{sat} values in the ozonated treated samples, although the increase was not enough to recommend ozone as a treatment for salt-affected soils.

TABLE II. THE EFFECT OF OZONATION ON THE SATURATED HYDRAULIC CONDUCTIVITY OF SOILS.

Soil type	K_{sat} ozonated†	K_{sat} unozonated	Significance
	(cm day^{-1})		
Tulare			
400	38.9	38.5	NS
1500	7	24.2	***
3000	0.5	0.3	*
4500	6.3	3.9	**
Milham			
T-1	49.8	44.3	*
T-2	46.7	30.6	***
T-3	32.8	26.6	**
T-4	7.7	8	NS
T-5	7.8	8.7	NS
Ramona	223.5	187.6	NS

*,**,***Significant difference for main effect at P< 0.05, 0.01, and 0.001, respectively.
NS Not significant.
† Ozone level approximately 10 mg L^{-1}.

The third soil tested was the Ramona soil, which is fairly coarse textured and had a high rate of water infiltration. The K_{sat} values measured on this soil show that the ozone-treated irrigation water soaked into the soil somewhat faster than the unozonated water, although the change was not statistically significant (Table II).

In addition to the reduced hydraulic conductivity, soils high in salt and exchangeable sodium often exhibit a phenomenon called "hard-setting". That is, upon drying the soil becomes rock-hard and difficult to cultivate. The hardness of the soil can be measured by determining the force required to break a reformed soil prism (like a core). There have been reports that ozone increases "soil softness" (Pedersen and Redsun, 1996). Table III shows the tensile strength (kPa) required to break the dried soil cores from the column studies.

Contrary to previous reports, there was no significant difference in the tensile strength test between any of the ozone-treated and untreated columns. In the Tulare soil samples, the loading required to break the cores increased with increasing salinity, a trend commonly associated with salt-affected soils. This effect was not as clear in the Milham soil, which does not tend to "hard-set". This soil is particularly favored by farmers because of its water-intake properties and ease of tillage. The soil cores of the Ramona soil failed at a loading that was below the tester's threshold. That is, as soon as the core was slightly compressed, the core crumbled. The coarse texture of the soil and low clay content, compared to the Milham and Tulare soils, did not favor the formation of hard-setting cores.

The tensile strength test is not very sensitive to small changes in soil structure. Additionally, it may take several months of leaching, drying, and cultivating before significant changes to the strength of the soil would be measured by this test.

TABLE III. THE EFFECT OF OZONATION ON SOIL STRENGTH.

Soil type	Tensile Strength ozonated†	Tensile Strength unozonated	Significance
	(kPa)		
Tulare			
400	20	21	NS
1500	34	30	NS
3000	ND	ND	ND
4500	77	92	NS
Milham			
T-1	10	9	NS
T-2	7	7	NS
T-3	11	14	NS
T-4	25	27	NS
T-5	19	15	NS
Ramona	ND	ND	NS

NS Not significant.
ND Not determined.
†Ozone level approximately 10 mg L^{-1}.

Reductions in hydraulic conductivity have been attributed to soil swelling and the plugging of conducting pores with dispersed clay. Soil swelling increases with increasing exchangeable sodium and with decreasing solution ionic strength. Under conditions of high exchangeable sodium and low ionic strength, clay particles repel each other which leads to swelling and closure of large soil pores. When a soil swells, its water holding capacity increases because there is an increase in the fine pore space between clay particles and a decrease in the larger pores, which normally would drain under the force of gravity. Thus, changes in water holding

capacity can be used as a quantitative indicator of internal soil swelling.

TABLE IV. WATER HOLDING CAPACITY OF THE SOIL

Soil type	Water Holding Cap.		Significance
	ozonated†	unozonated	
Tulare	(mL (.1 kg)$^{-1}$)		
400	60.3	62.8	***
1500	57.2	58.6	NS
3000	60.2	60.5	NS
4500	66.7	67.5	***
Milham			
T-	44.8	44.5	NS
T-	45	43.9	**
T-	41.4	40.8	NS
T-	46.4	47.4	*
T-	45.3	46.6	NS
Ramona	45.9	44.6	NS

*,**,***Significant difference for main effect at P< 0.05, 0.01, and 0.001, respectively.
NS Not significant.
† Ozone level approximately 10 mg L^{-1}.

Table V. TURBIDITY OF LEACH WATER AS INDICATED BY LIGHT ABSORBANCE.

Soil type	Relative Absorbance		Significance
	ozonated†	unozonated	
Tulare			
400	0.46	0.48	NS
1500	0.07	0.056	NS
3000	0.156	0.174	NS
4500	0.105	0.08	NS
Milham			
T-1	0.01	0.01	0
T-2	0.01	0.018	*
T-3	0.037	0.148	***
T-4	0.134	0.228	*
T-5	0.178	0.42	**
Ramona	0.95	0.91	NS

*,**,***Significant difference for main effect at P< 0.05, 0.01, and 0.001, respectively.
NS Not significant.
† Ozone level approximately 10 mg L^{-1}.

It is important to note that not all soils swell, but soils high in smectite clays are generally considered swelling soils. The Tulare soil would be classified as a swelling soil due to the amount and type of clay and the extensive cracking that occurs in the [illegible]. Milham and Ramona soils are not considered swelling soils. In the unozonated soil, all of the samples treated with ozonated water held less water after drainage, although not all of the samples were statistically different from the unozonated samples (Table IV). In the Milham and Ramona soils, there were no clear trends and largely no significant differences in water holding capacity due to ozonation.

Clay dispersion and movement through soils has been correlated with reductions in hydraulic conductivity (Shainberg and Letey, 1984). In this study, clay in the leachate (turbidity) was determined by light absorption at 500 nm wavelength. Table V shows that the absorbance increased with increasing exchangeable sodium in the Milham soil, which was expected. The ozone-treated columns had significantly less clay in the leachate, suggesting that clay flocculation was improved as a result of ozonation. Surprisingly, the Tulare soil had large amounts of dispersed clay in the lowest salinity sample (400). This soil also had high variability in the saturated hydraulic conductivity measurements. There were no significant differences in dispersed clay from the Tulare soil samples in the ozone-treated and untreated columns. The Ramona soil showed no difference in dispersed clay, but this soil also showed no significant increase in hydraulic conductivity from ozonation.

Analysis of the leach waters from the columns yielded significant differences between ozone-treated and untreated waters. There was a significant increase in the leaching of salts from all of the ozone-treated soils, as measured by solution EC (Table VI). Cation concentrations in the leachate solutions were analyzed but are not presented. In all cases, the ozonated water leached more cations (Na, Ca, Mg, and K) compared to the untreated water. The changes in the SAR values were mixed, with no apparent decrease in SAR due to ozonation (Table VI). There was an increase in the total amounts of cations leached as a result of ozonation.

There are two possible reasons for the higher electrical conductivities and higher cation concentrations in leach-water from the ozone-treated soils. First, the ozone reacted with the organic matter of the soils, causing degradation and release of the cations and anions from the organic matter. Secondly, it is known that ozone behaves

TABLE VI. THE ELECTRICAL CONDUCTIVITY, pH AND SAR OF THE LEACHED WATERS.

Soil type	pH		Significance	EC_{25}[‡]		Significance	SAR		Significance
	ozonated[†]	unozonated		ozonated	unozonated		ozonated	unozonated	
				(dS m^{-1})			($mM^{1/2}$)		
Tulare									
400	7.6	7.7	••	1.73	1.58	••	3.7	4	NS
1500	7.9	8	•••	2.46	2.01	•••	7.2	6.9	NS
3000	7.5	7.6	NS	5.13	4.77	NS	17.3	17.2	NS
4500	8.4	8.5	NS	4.29	4.25	NS	ND	ND	ND
Milham									
T-1	8.1	8.3	•••	0.88	0.61	•••	1.68	1.92	•
T-2	7.4	7.5	•••	0.93	0.85	•••	3.17	3.29	NS
T-3	8.1	8.3	•••	1.22	0.92	•••	7.71	6.82	•••
T-4	7.9	8	••	1.72	1.64	••	10.55	10.04	•
T-5	8.2	8.3	•••	2.6	2.2	•••	11.98	11.04	••
Ramona	7.2	7.3		0.7	0.56	NS	0.96	1.04	•••
Leach-water	7.7	7.8		0.58	0.49				

•,••,••• Significant difference for main effect at $P<0.05$, 0.01 and 0.001 respectively.
NS Not significant.
[†] Ozone level approximately 10 mg L^{-1}
[‡] Electrical Conductivity of the waters corrected to 25 °C.

a weak acid (Langlais et al., 1991), decreasing the pH of the irrigation water. Irrigation water pH values after ozonation were always lower than unozonated water, and the pH of the leach-water from the ozonated columns was lower in all cases (Table VI). This lower pH allowed for more dissolution of slightly soluble minerals (like calcite). Mineral dissolution reactions typically increase the Ca and Mg ion concentrations and lower the SAR. A decrease in the SAR results in cation exchange reactions with exchangeable Na. That is, the Ca and Mg released by mineral weathering compete for Na on cation exchange sites and the SAR is 'buffered' back towards its original value. The cation exchange capacity of the soil is a large reservoir for exchangeable cations that can strongly influence the composition of the soil solution. This possibly explains the increase in ion concentrations with only small changes in the solution SAR values.

The benefits of ozone in the irrigation water, when observed, might be attributed to the higher electrolyte concentration and lower pH of the soil water. Both of these factors can significantly increase the permeability and the saturated hydraulic conductivity of soils. Other studies on the effects of SAR and the total electrolyte concentration on the saturated hydraulic conductivity of the Milham soil suggest that the small changes we observed in K_{sat} could be attributed to this (unpublished data). Other studies with the Ramona soil, by Lebron and Suarez (1992), showed that decreases in pH increased clay flocculation and increased K_{sat}. As a follow-up to this study, it would be interesting to compare the effects of ozone with small additions of acid to the irrigation water.

Additionally, the concentration of oxygen and the oxidation/reduction potential (E_h) of soil solutions were expected to increase as a result of ozonation, and these expected changes did occur. The aged tap water without ozone had an average E_h of 510 mV and the ozonated water at 10 mg L^{-1} had an E_h of 1200 mV. At an ozone concentration of 1.4 mg L^{-1}, the E_h was over 1000 mV. These high E_h values gradually decreased as the ozone decomposed. The dissolved oxygen concentration rose to ~12 mg L^{-1}. Improved crop vigor, which has been reported with ozonated irrigation water, may be attributed at least in part, to these two factors.

There was little effect on the hydraulic conductivity with varying initial concentration of ozone. A concentration of 10 mg L^{-1} was the highest

concentration we tested, and this concentration showed the greatest effect on K_{sat}. In the pretreatment of drinking water to improve flocculation of suspended colloids, it has been found that a concentration of 0.5-1.5 mg L^{-1} is the optimal range for flocculation (Maier, 1984). In this case, the water to solid ratio is very large, while in a soil the opposite is true. The ozone was rapidly lost once the water entered the soil columns, and we were unable to detect ozone in the leachate, even from very thin soil columns or in sand columns. We speculate that the higher concentrations of ozone were needed for ozone to move into the soil.

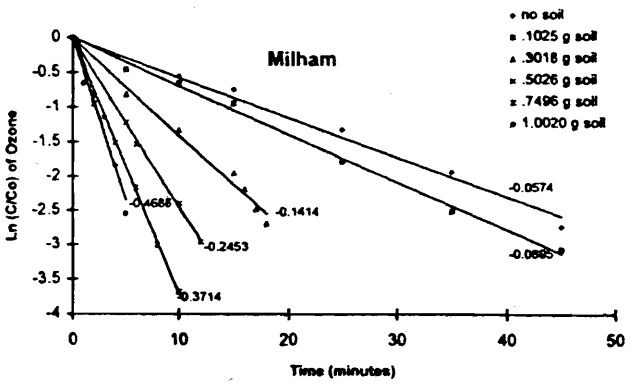


Figure 2. First-order plot for the loss of ozone in well-mixed soil suspensions of the Milham soil. The numbers next to the lines are the slopes and are the "apparent rate constants" which were then corrected for suspension density.

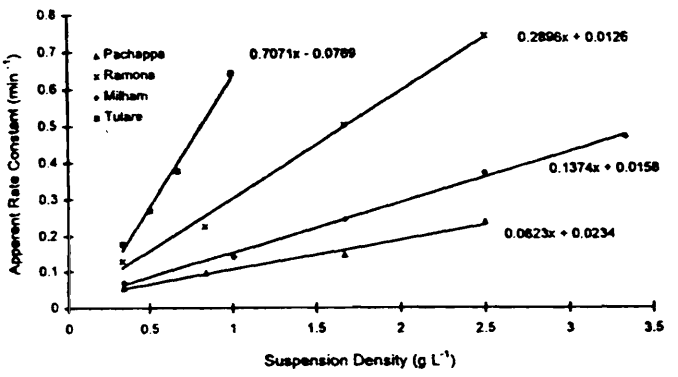


Figure 3. Relationship between the "apparent rate constants" for ozone loss and the suspension densities for four soils. The numbers next to the lines are the first-order rate constants, normalized to unit suspension density. The units on the normalized rate constants are L g^{-1} min^{-1}.

Experiment 2: Batch Suspension Study

To identify the factors that affect the rate of ozone loss from irrigation water and soil, we set-up a series of well-mixed batch suspension studies and monitored the loss of ozone from the system. It was found that the rate of ozone loss followed first order kinetics with respect to the concentration of ozone in solution. The reaction is first order because a plot of the natural log of the concentration of ozone versus time gave a straight line (Figure 2). The rate constant is also dependent on the suspension density.

A plot of the rate constants versus the soil/water ratios indicates that a linear relationship existed between the rate loss constant of ozone and the suspension density (Figure 3). A total of 13 soils were tested in this study and Figure 3 is representative of the data obtained. This figure shows that the rate constants were proportional to the mass of soil in contact with the solution, and that each soil had its own unique rate constant. The measured rate constants for all 13 soils were normalized for the suspension density and are reported in Table VII. The variability in the rate constants among the soils led us to examine the chemical and physical properties of the soils that might have affected the rate of ozone consumption.

TABLE VII. THE RATE CONSTANTS "k" AND AVERAGE pH VALUES DURING REACTION IN EXPERIMENT II

Soil Type	Rate Constant k ($L\ g^{-1}\ min^{-1}$)	pH
Buffalo >7"	0.240	6.64
Buffalo 0-2"	0.358	6.81
Holtville	0.071	6.56
Hanford	0.016	6.75
Imperial	0.054	6.43
Etiwanda	0.027	6.77
Grangeville	0.087	6.63
Ramona	0.290	6.70
Pachappa	0.082	6.60
Milham	0.137	6.70
Tulare	0.707	6.40
Coachella	0.016	6.77
Merriam	0.055	6.56

Modeling

Yurteri and Gurol (1988) proposed a mathematical model to predict the rate constant for ozone degradation in wastewater. Their model took into

account the pH, total organic carbon (TOC), and alkalinity of the water. We attempted to formulate a model based on these parameters and other properties of the soil. The model initially included parameters for the specific surface area of the soil, which is related to clay content and texture, and the water-soluble alkalinity of the soil. Those terms did not add substantially to the model and were removed. The properties that we found which had a significant impact on the rate were the pH of the solution, total organic carbon in the soil (as measured by the "Loss-On-Ignition" method), and the soil to water ratio, or suspension density.

This model was calibrated using the rate data from 13 different soils and the pH values measured in the batch reactors (Table VII). The following equations were developed to predict the loss of ozone in soil/water suspensions:

$$d[O_3]/dt = -k(m/v)[O_3] - k_w[O_3] \quad [3]$$

$$\log k = 6.68 - 1.23\ pH + 1.27 \log(TOC) \quad [4]$$

where k is the normalized first-order rate constant ($L\ g^{-1}\ min^{-1}$) that is calculated from pH and TOC of the soil using Eq. [4]; (m/v) is the ratio of soil mass to water volume ($g\ L^{-1}$); and k_w is the rate constant for ozone loss in our irrigation water without soil ($0.05\ min^{-1}$). The constants in Eq. [4] were calibrated for pH values between 6.7 and 8.7 and TOC values between 0.8 and 12%.

Figure 4 is a plot comparing the measured and predicted rate constants for ozone degradation using the above equations. The trend line drawn depicts a one-to-one relationship between measured and predicted rate constants. The variability in the model indicates that there are still unknown parameters, which were not identified, that have a significant effect on the rate of ozone loss. It is likely that a chemical oxidation method for determining "ozone reactive organic matter" would give a better prediction than the loss-on-ignition method.

Using the measured rate constants, the saturated water content, the initial infiltration rate and the steady state hydraulic conductivity, we calculated that irrigation water containing 10 mg L^{-1} of ozone, would penetrate <2 mm into the Milham soil (see Appendix for calculations). This suggests that the benefits of ozone in irrigation water may be largely restricted to the very surface of the soil. However, macropore flow and the slower reaction kinetics during laminar flow could move ozonated water deeper into the soil.

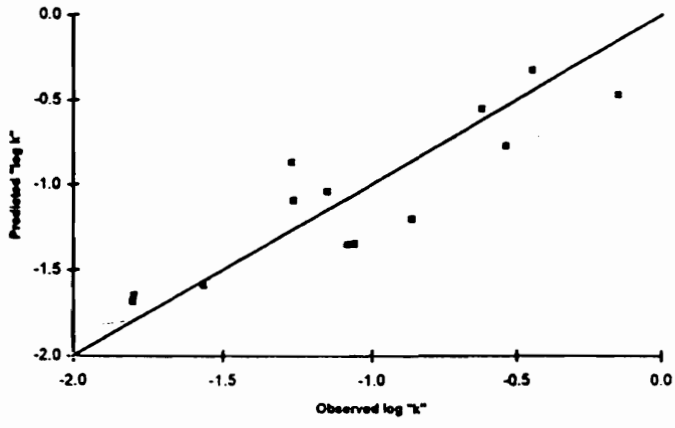


Figure 4. Observed rate-loss constants for ozone in a soil/water suspensions versus predicted constants using Equations [3] and [4].

Conclusions

There appeared to be some benefits to adding ozone to irrigation water in these short-term studies. Not all of the soils tested showed improvements in hydraulic conductivity, especially in the Milham soil samples with high exchangeable sodium (T-4 and T-5). The results were mixed for the Tulare soil and there was no significant improvement in the Ramona soil. The improvement in physical properties that was observed could be attributed to higher electrolyte concentrations in the soil water and lower pH values, both of which lead to improved clay flocculation and reduced dispersion. However, long-term studies are needed to determine if the gradual degradation of soil organic matter could lead to increased clay dispersion, surface crusting, and loss of structure. The surface of soils is typically very sensitive to changes in structure due to aggregate breakdown, which leads to crusting, sealing, and erosion. Organic matter is considered beneficial in holding soil aggregates together and binding clays. Destruction of this organic matter in the surface layer by ozonation might lead to long-term problems with infiltration and crusting.

References

Dexter, A.R. and Kroesbergen, B. "Methodology for Determination of Tensile Strength of Soil Aggregates", J. Agric. Engng. Res. 31:139-147 (1985).

Greenberg, A.E., Clesceri, L.S. and Eaton, A.D. (Ed.), 1992. *Standard Methods for the Examination of Water and Wastewater.* Iodometric Method I. (Washington D.C.: Am. Public Health Assoc., 1992), p. 4-36 to 4-40.

Klute, A. (Ed.), *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. Second Edition.* (Madison, WI: Am. Soc. Agron. and Soil Sci. Soc. Am., 1986), p. 416-421.

Langlais, B., Reckhow, D.A. and Brink, D.R. (Ed.), *Ozone in Water Treatment. Practical Application of Ozone: Principles and Case Studies. Chap. III.* (Boca Raton, FL: Lewis Publishers, 1991), p. 133-316.

Lebron, I. and Suarez, D.L. "Variations in Soil Stability Within and Among Soil Types", Soil Sci. Soc. Am. J. 56:1412-1421 (1992).

Maier, D. "Microflocculation by Ozone", *In* R.G. RIP and A. NETZER (Ed.) *Handbook of Ozone Technology and Applications, Volume II, Ozone for drinking water treatment.* (Boston, MA: Butterworth Publishers, 1984). p. 123-140.

Pedersen, L. and Redsun, H. "Ozone Application for Agricultural Crop Production: Survey of Selected Manufacturers and Farmers", (Report prepared for Pacific Gas and Electric Co., San Francisco, CA. by Dantec Engineering, Inc. 605 Thornhill Rd., Danville, CA 94526. September 1996), p. 111.

Shainberg, I. AND Letey, J. "Response of Soils to Sodic and Saline Conditions", Higardia 52 (2):1-57 (January 1984).

Sparks, D.L. (Ed.), *Methods of Soil Analysis: Part 3, Chemical Methods* (Madison, WI: Soil Sci. Soc. Am., 1996), p. 1002-1005.

Yurteri, C. and Gurol, M.D. "Ozone Consumption in Natural Water: Effect of Background Organic Matter, pH, and Carbonate Species", Ozone Sci. Eng. 7:1-11 (1988).

Zahow, M.F. and Amrhein, C. "Reclamation of Saline Sodic Soil Using Synthetic Polymers and Gypsum", Soil Sci. Soc. Am. J. 56:1257-1260 (1992).

Appendix

Calculating the depth of ozonated water movement into the Milham soil.

Two different hydraulic conductivity constants were used to calculate the depth of ozonated water movement into the T-1 Milham soil. The first hydraulic conductivity constant used was the saturated hydraulic conductivity constant, determined at steady state conditions to be 49.8 cm day^{-1} (from Table II). The second hydraulic conductivity constant used was an initial wetting rate conductivity constant determined to be 0.2 cm sec^{-1} by measuring the initial rate of water movement into an dry packed column of T-1 Milham soil. Darcy's law was then applied to determine the depth of water penetration into a soil column.

$$\text{Darcy's law is: } J = -K_{sat}[d\psi/dz]$$

where J is the flux in cm day^{-1}, $d\psi$ is the hydraulic head (cm), and dz is the depth of soil through which flow is occurring (cm). The negative flux indicates downward flow. For the measurement we assumed that Darcy's law was obeyed (piston displacement with no preferential flow or bypass). If the water is ponded in a thin layer at the soil surface, the term $d\psi/dz$ is 1. The rate of water penetration is approximately 12 cm min^{-1} during the initial wetting, but only 0.035 cm min^{-1} at steady state. This information is then coupled with the rate of ozone loss.

The rate of ozone loss can be described using a simplified version of Equation [3]. In this form, the loss of ozone without soil ($- k_w[O_3]$) can be ignored because it is small relative to the loss by soil. Equation [3] simplifies to:

$$d[O_3]/dt = -k(m/v)[O_3]$$

The integrated form of this equation is: $\ln[O_3] - \ln[O_{3\ initial}] = -k(m/v)t$.

For the Milham soil, the first-order rate constant (k) from Table 7 is 0.137 L $g^{-1}min^{-1}$. The soil mass to water volume ratio (m/v) in the column study was assumed to be the water holding capacity of the soil (Table 4), which is 2242 g L^{-1}. Solving the integrated equation for the time (t) for the initial ozone concentration to go from 10 mg L^{-1} to 0.1 mg L^{-1} gives:

$$t = (\ln[0.1] - \ln[10])/(0.137)(2242) = 0.015 \text{ min} = 0.9 \text{ sec.}$$

Thus, 99% of the ozone in the irrigation water is lost within 1 second of entering the soil. At this rate of ozone loss, the ozone would reach a depth of 1.8 mm during the rapid, initial infiltration. Following the initial wet-up, the rate of water infiltration decreases and the effective penetration depth is calculated to be <1 mm. At steady state, where K_{sat} was measured, the depth of ozone penetration would be <0.01 mm.

These calculations are based on kinetics constants that were determined in rapidly mixed soil/water suspensions at low soil/water ratios. Water flow into a soil is not a turbulently mixed system and there is a good chance that the reactions between ozone and the soil are slower than measured in the batch system. Even still, it is likely that ozone movement into a dry soil via irrigation water is restricted to the soil surface.

	Search	Results
#11	'2000 xvi' in SO	37
#10	'xvi'	3802
#9	'2000'	162936
#8	ozone and soil organic matter	8
#7	matter	72816
#6	organic	92135
#5	soil	492486
#4	ozone	5162
#3	ozone and soil	929
#2	soil	492486
#1	ozone	5162

Results of: ozone and soil organic matter

Record 1 of 2 in CAB Abstracts 1998/08-2000/07
TI: Climate change and global crop productivity.
AU: Reddy-KR (ed.); Hodges-HF
SO: 2000, xvi + 472 pp.; many ref.
PB: CABI Publishing; Wallingford; UK
LA: English
AB: Following an overview of climate change and global crop productivity, the book is divided into 4 sections: the problem-changing biosphere (climatic change and variability, and agricultural contributions to greenhouse gas emissions); crop ecosystem responses to climatic change (rice, maize and sorghum, soyabean, cotton, root and tuberous crops, vegetable crops, tree crops, productive grasslands, rangelands, crassulacean acid metabolism crops, crop-weed interactions, pests and population dynamics, **soil organic matter** dynamics, and interactive effects of **ozone**, ultraviolet-B radiation, sulfur dioxide and carbon dioxide); mitigation strategies (crop breeding strategies for the 21st century, and role of biotechnology in crop productivity in a changing environment); and economic and social impacts (global, regional and local food production and trade in a changing environment).
PT: Book
IB: 0-85199-439-3
AN: 20001909204

Record 2 of 2 in CAB Abstracts 1998/08-2000/07
TI: Effects of **ozone** treatment on the **soil organic matter** on contaminated sites.
AU: Ohlenbusch-G; Hesse-S; Frimmel-FH

Richardson M.L. 1994 The Dictionary of Substances and their Effects Royal Soc. Chemistry Cambridge UK

O59 Ozone

O_3

CAS Registry No. 10028-15-6
Synonyms triatomic oxygen
Mol. Formula O_3 **Mol. Wt.** 48.00
Uses Air and water disinfectant. Bleaching textiles, waxes and oils. In organic synthesis.
Occurrence In atmosphere (about 0.05 ppm at sea level). Air pollutant.

Physical properties

M. Pt. -193°C; **B. Pt.** -111.9°C; **Specific gravity** d^0 (gas) 2.144 g l^{-1}, $d^{-195.4}$ (liquid) 1.614 g l^{-1}.

Solubility

Water: 49 cm^3 100 cm^{-3} at 0°C. Organic solvent: oils (unspecified)

Occupational exposure

US TLV (TWA) 0.05 ppm (0.1 mg m^{-3}); **US TLV (STEL)** 0.2 ppm (0.4 mg m^{-3}); **UK Long-term limit** 0.1 ppm (0.2 mg m^{-3}) under review; **UK Short-term limit** 0.3 ppm (0.6 mg m^{-3}) under review.

Ecotoxicity

Fish toxicity

LC_{50} (96 hr) rainbow trout 9.3 µg l^{-1} (1).
LC_{50} (24 hr) bluegill sunfish 0.06 mg l^{-1} (2).
LC_{50} (24 hr) yellow perch embryo 0.21 mg l^{-1} (3).
LC_{50} rainbow trout embryo 0.19-0.31 mg l^{-1} (3).

Invertebrate toxicity

Mycobacterium kansasii, *Escherichia coli* and *Tetrahymena pyriformis* were subjected to 0.5-2 min aeration with 1 mg O_3 l^{-1}. Specific changes in phospholipid content occurred only at 30 sec ozonization. Longer periods of ozonization did not cause further changes in phospholipid content. Superoxide dismutase had a protective role (4).

On exposure *Euglena gracilis* suffered damage to the plasma membrane. Disruption to vitamin B_{12} and acetate uptake was seen within 15 min of treatment. Leakage of potassium was detected after 30 min of treatment. Recovery times were within 5 hr for vitamin B_{12} and potassium uptake and 20 hr for acetate uptake (5).

Inactivation of the cytosolic enzymes of *Saccharomyces cerevisiae* occurred following treatment in suspension. Of those enzymes studied, glyceraldehyde-3-phosphate dehydrogenase exhibited the greatest inactivation. Often affected to lesser extents were: NAD-glutamate dehydrogenase; 6-phosphofructokinase; NAD-alcohol dehydrogenase; and pyruvate decarboxylase activities, Levels of nucleoside triphosphates including ATP, were reduced to ≈50% of the pre-treatment amounts. ATP lost from the cells appeared in the medium along with NAD and protein suggesting that the cells had been permeabilized (6).

Mammalian and avian toxicity

Acute data

LC_{50} (3 hr) inhalation rabbit 36 ppm (7).
LC_{50} (4 hr) inhalation rat 4800 ppm (8).
LC_{50} (3 hr) inhalation mouse, guinea pig, cat 12,600-34,500 ppb (7,9,10).
LC_{50} (4 hr) inhalation hamster 10,500 ppb (8).
TC_{Lo} (3 hr) human 0.2 ppm (11).
Intrapleural or intra-arterial (135 min) rat 0.12, 0.25, 0.50 or 1.00 ppm caused increased frequency of breathing and tidal volume decrease as a function of both concentration and exposure duration. Cardiopulmonary measures and breathing mechanics were only marginally affected (12).
Inhalation (3 hr) rat 1 ppm caused heart rate and mean arterial blood pressure to decrease. Effects were more marked in 11 wk old rats than those aged 4 or 8 wk. There were no sex-related differences in the responses (13).
Rat exposed to 0.4 ppm (3, 6 or 12 hr) showed ≈15% decrease in Fc-receptor-mediated phagocytosis by alveolar macrophages. Recovery above control levels occurred within 12 hr of exposure. In mice alveolar macrophage function change was not seen until 12 hr of exposure (14).

Sub-acute data

Rats exposed to 0.4 ppm 12 hr day^{-1} for 3 or 7 days did not show altered alveolar functions with the exception of reduced superoxide production at 3 days of exposure. Mice given repeat treatments did not exhibit any further decrease in phagocytosis over the single dose, however superoxide production by alveolar macrophages was inhibited by ≈50%
In both rats and mice alveolar macrophage number increased (14).
Inhalation (1, 3, 13, 52 or 78 wk) rat at 0.06-0.25 ppm to mimic exposure patterns in high-pollution summer environments for 5 day wk^{-1}. Natural killer cell activity of spleen cells, T-cell mitogen responses of spleen cells and histopathology of spleen, femur, thymus and mandibular, mediastinal and peribronchial lymph nodes were unaffected (15).
BALB/c mice (1, 3, 7 or 14 day) continuously exposed to 0.8 ppm exhibited delayed hypersensitivity reaction to sheep red blood cells. Lymphocyte numbers were reduced in both thymus and blood. The percentage of T- and B-lymphocytes in blood was the same as in the controls (16).

Carcinogenicity and long-term effects

Carcinogenicity chronic studies inhalation rat and mouse; oral rat in feed under investigation by the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. Commenced at end fiscal year 1992 (17).

Metabolism and pharmacokinetics

Predicted lower respiratory tract uptake during exercise in human ranges from 87 to 93% compared to 84-88% for quiet breathing. The total quantity absorbed per minute increases with age. The largest tissue dose was predicted to occur in the centriacinar region, where often studies have shown maximal morphological damage (18).

CHEMISTRY LIBRARY

Sensitisation

May have a role in causing asthmatic attacks rather than enhancing allergic sensitisation in guinea pigs sensitised with ovalbumin (19).
Increased ambient air concentration significantly raised the frequency of asthma attacks amongst city dwellers (20).

Genotoxicity

Salmonella typhimurium TA100, TA102, TA104 with and without metabolic activation negative (in non-toxic dose range) (21).
Escherichia coli B DNA single- and double-strand breaks in wild type and the mutant MQ1844 (*ozr*B). Another type of DNA damage repaired only by the *ozr*B gene product may be responsible for the killing effect (22).

Any other adverse effects to man

Lung function was impaired in young adults following exposure (2 hr) to 0.15 ppm with intermittent light exercise. Smokers were affected more than non-smokers (23).
Exercising men suffered mild subjective respiratory irritation at 0.12-0.14 ppm. 0.20-0.30 ppm had no adverse effects on patients with chronic heart or lung disease (24).
In vitro (24 hr) human alveolar macrophage 0.1-1.0 ppm. There was a minimal effect on protein expression or synthesis, but responses to particulate immune complexes and to bacterial lipopolysaccharide were impaired. PGE_2 and arachidonic acid were released suggesting the cell membrane was damaged. Susceptibility to infections agents may be increased in the longer term (25).

Any other adverse effects

1.0-2.0 ppm decreased survival time of influenza-infected rats and mice and suppressed the capacity of lung macrophages to destroy *Listeria* (24).
Erythrocytes (species unspecified) exposed showed lesions in the proteins of the plasma membrane. Secondary oxidants were as damaging as ozone. Toxic effect consists of oxidative inactivation of membrane-bound enzymes and membrane-structure disturbance (26).
Airway hyperresponsiveness in guinea pigs was closely related to dose (27).

Legislation

Swedish threshold limit value in working environment, 0.1 ppm (21).

Any other comments

Dry weight of rice plants exposed to 0.10 ppm was reduced by 50% at 5 and 6 wk (administered from vegetative to early heading stages). Root/shoot ratio and nitrogen uptake rate were modified under long-term exposure (28).
Wild plants were exposed to levels in a daily pattern mimicking atmospheric occurrence in southwest Germany. (The little stinging nettle reacted differently after the same dose in steady fumigation compared to a 24 hr concentration curve). Yield was generally reduced, particularly in the roots. No resistance was seen in plants selected from sites of higher burden (29).
Mouse lung carcinogenesis reviewed (30).
Contamination of aircraft cabins and toxicity reviewed (24).
Health effects including carcinogenesis reviewed (31-35).

Experimental toxicology, epidemiology, human health effects, ecotoxicology (limited), environmental effects and workplace experience reviewed (36).

References

1. Wedemayer, G. A. et al *J. Fish. Res. Board Can.* 1979, **36**, 605
2. Paller, M. H. et al *J. Environ. Sci. Health*, Part A 1979, **A14**, 169
3. Coler, R. A. et al *Ozone Sci. Eng.* 1980, **2**, 177
4. Sukhareva-Nemakova, N. N. et al *Izv. Akad Nauk. SSSR, Ser. Biol.* 1987, (6), 871-880 (Russ.) (*Chem. Abstr.* **108**, 33285y)
5. Chevrier, N. et al *Plant Cell Physiol.* 1990, **31**(7), 987-992
6. Hinze, H. et al *Arch. Microbiol.* 1987, **14**(2), 105-108
7. *Ind. Med. Surg.* 1956, **25**, 301
8. *AMA Arch. Ind. Health* 1957, **15**, 181
9. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 1957, **16**, 22
10. *Ind. Med. Surg.* 1957, **26**, 63
11. Lewis, R. J. et al *Registry of toxic effects of chemical substances* 1984, National Institute for Occupational Safety and Health No. 83-107-4
12. Tepper. J. S. et al *J. Appl. Toxicol.* 1990, **10**(1), 7-15
13. Uchiyama, I. et al *Environ. Res.* 1986, **41**(2), 529-537
14. Oosting, R. S. et al *Toxicol. Appl. Pharmacol.* 1991, **110**(1), 170-178
15. Selgrade, M. K. et al *Inhalation Toxicol.* 1990, **2**(4), 375-389
16. Shiraishi, F. et al *Environ. Res.* 1987, **43**(1), 186-190
17. *National Toxicology Program Fiscal Yr 1993 Annual Report* Apr 1994, US Dept of Health and Human Services, US
18. Overton, J. H. et al *Health Phys.* 1989, **57**(Suppl. 1), 29-36
19. Sumitomo, M. et al *Int. Arch. Allergy Appl. Immunol.* 1990, **93**(2-3), 139-147
20. Marin, C. et al *Pollut. Atmos.* 1991, **130**, 177-192 (Fr.) (*Chem. Abstr.* **115**, 188913v)
21. Victorin, K. et al *Environ. Mol. Mutagen.* 1988, **11**(1), 65-77
22. Hamelin, C. et al *Mutat. Res.* 1989, **214**(2), 253-255
23. Kagawa, J. et al *Proc.-APCA Annu. Meet.* 1988, 81st(7), Paper 88/122.4
24. Melton, C. E. *Report* 1989, SOT/FAA/AM-89/13, Order No. AD-A219264, available NTIS
25. Becker, S. et al *Toxicol. Appl. Pharmacol.* 1991, **110**(3), 403-415
26. Kaler, G. V. et al *Biol. Membr.* 1989, **6**(11), 1164-1169 (Russ.) (*Chem. Abstr.* **112**, 31846r)
27. Suzuki, S. et al *J. Toxicol. Environ. Health* 1990, **30**(2), 123-134
28. Noiichi, I. et al *Environ. Pollut.* 1991, **74**(2), 149-164
29. Maier-Reiter, W. et al *Report* 1989, UBA-FB-89-158, available UB/TIB, Hannover (Ger.) (*Chem. Abstr.* **114**, 253062n)
30. Witschi, H. *Exp. Lung Res.* 1991, **117**(2), 473-483
31. Lippmann, M. *J. Air Pollut. Control Assoc.* 1989, **39**(5), 672-695
32. Bignon, J. *Pollut. Atmos.* 1990, **128**, 431-440 (Fr.) (*Chem. Abstr.* **115**, 55784g)
33. Mustafa, M. G. et al *Ann. N. Y. Acad. Sci.* 1988, **534**, 714-723
34. Borck, C. *Ann. N. Y. Acad. Sci.* 1988, **534**, 106-110
35. Hobbs, C. M. et al *J. Toxicol., Clin. Toxicol.* 1991, **29**(3), 375-384
36. *ECETOC Technical Report No. 30(4)* 1991, European Chemical Industry Ecology and Toxicology Centre, B-1160 Brussels

Annu. Rev. Phytopathol. 1996. 34:347-366.

OZONE AND PLANT HEALTH

Heinrich Sandermann Jr
GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Institut für Biochemische Pflanzenpathologie, Oberschleissheim, D-85764 Germany; email: sandermann@gsf.de

KEY WORDS: **ozone** phytotoxicity, gene induction, phytoalexins, memory effect, cross-induction, disease mechanisms

▶ ABSTRACT

▲ Top
▼ Next
▼ Literature

Phytotoxic effects of **ozone** are described with emphasis on secondary plant metabolism. Numerous **ozone**-induced genes, enzymes and stress metabolites of antioxidative and phytopathological defense reactions have been discovered for herbaceous plants and forest tree species. **Ozone** induces reactions normally elicited by viral and microbial pathogens. The molecular basis (receptors, signal chains) for induction by **ozone** remains to be elucidated. The induced stress reactions seem to change plant predisposition to either enhanced tolerance or susceptibility for a second stressor. The following topics are discussed: **ozone** and biotic disease, the role of **ozone** on field sites and **ozone** limit values.

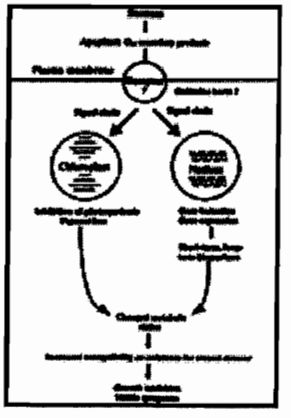
▶ INTRODUCTION

▲ Top
▲ Previous
▼ Next
▼ Literature

Ozone is a secondary air pollutant formed in the troposphere by the interaction of hydrocarbons, nitrogen oxides, and sunlight. Ambient **ozone** concentrations are typically in the range between 20 and 60 nL $\cdot L^{-1}$, with peak episodes of up to 250 nL $\cdot L^{-1}$. The following aspects of **ozone** phytotoxicity have been considered in earlier Annual Reviews: damage reactions (78, 89), plant pathology (22), influencing factors (49), affected metabolic processes

(25), interaction with plant parasites (44), initial events (47), effects on plant productivity (66), responses to pollutant mixtures (88), and crop loss (45). Several publications on **ozone** phytotoxicity are available (42, 95, 109, 117). Recent reviews cover **ozone** and plant defense systems (53), **ozone** and pathogen interactions (72), and **ozone** and forest decline (97).

After stomatal uptake, the intercellular tissue concentration of **ozone** rapidly declines (58, 61). Although this indicates detoxification, the **ozone** decomposition products, or second messenger molecules, can affect photosynthesis or gene expression in dose dependence on external **ozone**. It is not yet clear whether an oxidative burst occurs and whether visible lesions are caused by **ozone** through a program of local cell death (apoptosis) similar to that postulated for the hypersensitive response induced in incompatible plant-pathogen interactions (24, 39, 75, 113). Membrane lysis and chlorotic or necrotic symptoms usually occur as rather late **ozone** effects. Often a second stressor such as frost, drought, or a pathogen seems to participate. The overall effects of **ozone** on plants are summarized in Figure 1. Published experimental work on **ozone** phytotoxicity deals almost exclusively with visual symptoms and growth effects, as well as with the problem of leaf-internal **ozone** dose, effects on stomatal regulation, photosynthetic functions, and assimilate allocation. A number of reviews are available (21, 23, 47, 48, 85, 87). These articles and the above-cited reviews are mainly concerned with primary plant metabolism, whose inhibition by **ozone** leads to a general reduction of growth and competitive fitness of the plant. On the other hand, the probability of disease in plants is determined in large part by defensive and antioxidative reactions of secondary metabolism (6, 12, 24, 26, 43, 56, 100, 110). These reactions include a local oxidative burst, cell wall reinforcement (lignin, callose, extensins), and the induction of phytoalexins, antioxidative systems, and pathogenesis-related (PR)-proteins. The present review[1] focuses on these types of reaction, which have only recently been studied with regard to **ozone** phytotoxicity. The main new finding is that **ozone** can act as a powerful and ubiquitous abiotic elicitor, which raises new questions on the role of ambient **ozone** in plant disease.



View larger version (20K):
[in this window]
[in a new window]

Figure 1. Overview of cellular **ozone** effects. After stomatal uptake, most of the **ozone** is decomposed in the apoplast. Well-documented **ozone** effects on chloroplast functions and nuclear gene expression occur by unknown mechanisms, possibly involving an **ozone** receptor, an oxidative burst, and signal chains. These effects are often linked to a second stressor and can finally lead to visible effects.

CASE STUDIES

▲ Top
▲ Previous
▼ Next
▼ Literature

Controlled Ozone Exposure: Technical Remarks

Many case studies described below rest on controlled exposure experiments that may be criticized with regard to the plant's genetic, developmental, or nutritional status. A common criticism is aimed at the higher than ambient **ozone** concentrations often needed to obtain biochemical and ecophysiological effects in short-term fumigation experiments. **Ozone** effects usually are twofold (42, 48): An initial phase of stress reactions and lowered photosynthesis may or may not be followed by a second phase of visible symptom development (chlorotic or necrotic lesions). Symptom development may be fast in herbaceous plants, or may be delayed by many months in conifers. The important point is to assign observed effects to the first (chronic) or the second (acute) phase. According to Paracelsus, toxic effects are dependent on dose rather than on concentration. A simple measure of external **ozone** dose is obtained by multiplying mean ambient **ozone** concentration by the total hours of exposure, leading to units of $\mu L \cdot L^{-1} \cdot h$. More sophisticated external dose definitions have been given (68, 87). Typical short-term laboratory experiments at elevated **ozone** concentrations apply external **ozone** doses of a few $\mu L \cdot L^{-1} \cdot h$. Field **ozone** doses per growing season are much higher (50 to 400 $\mu L \cdot L^{-1} \cdot h$; 68, 87). Elevated **ozone** concentrations thus appear permissible in laboratory experiments, provided that the chronic and acute effect ranges are differentiated.

Tobacco: Differential Ozone Sensitivity

The tobacco cultivars Bel W3 (sensitive) and Bel B (tolerant) have been widely used as differential biomonitors for **ozone** (50). The difference in **ozone** sensitivity has been attributed to stomatal conductance, intercellular leaf volume, and soluble sugar or ascorbate contents (25, 49, 50). However, under standardized conditions, these parameters were not significantly different between the two cultivars (50, 65, 102). Upon a standard **ozone** exposure (5 h, 150 $nL \cdot L^{-1}$), only Bel W3 developed "water-logging" and subsequent necrotic lesions, with local production of lignin-like material and callose (65, 101, 102). In addition, only Bel W3 developed an early burst of ethylene and its precursor, ACC (65, 102, 127). Other phytohormones (ABA, cytokinins) were not significantly affected (127). Bel B showed no lesions, but showed a rapid increase of putrescine and its biosynthetic enzyme, arginine decarboxylase, at similar external **ozone** concentrations. Hydroxycinnamoyl conjugates of putrescine were rapidly formed and were recovered from the intercellular wash fluid (apoplast extract) of Bel B (65). Pulse radiolysis showed the conjugates, but not the free polyamines, to be efficient radical scavengers, and root application of polyamines largely protected Bel W3 against **ozone** (15).

Because polyamine and ethylene biosynthesis both proceed from *S*-adenosyl methionine, the induction of either pathway was proposed to decide for or against **ozone** tolerance (65). Two stress enzymes commonly induced by fungal elicitors and viral infections, phenylalanine ammonia-lyase (PAL) and basic ß-1,3-glucanase, was preferentially induced by **ozone** in Bel W3 (101, 102). Two days of ambient **ozone** were enough to induce ß-1,3-glucanase activity sixfold in Bel W3 (104). NO_2 and SO_2 had no inductive effect, but NO_2 was a strong synergist when tested in combination with **ozone** for ß-1,3-glucanase induction (102). An acidic chitinase (PR-3b) was induced by **ozone** specifically in the

apoplast of Bel W3 (114). In contrast, the stress-related tyramine hydroxycinnamoyl transferases were induced by **ozone** in both cultivars. The tyramine conjugates were rapidly incorporated into cell wall material, most likely lignin (102, 104).

At the transcript level, gene expression of basic ß-1,3-glucanase, basic and acidic chitinases, and of the pathogenesis-related protein, PR-1b, was preferentially induced in Bel W3 (34, 101). The nature and induction of plant PR proteins have been reviewed (12). The basic glycosidase transcripts are also induced by ethylene (12, 29), so **ozone** may have acted in part via stress ethylene (34, 65). The fastest **ozone** response of Bel W3 (at 1 h) was the induction of ethylene and ACC and of in situ ACC oxidase activity (65, 127). Several transcripts were also induced by a combination of automobile exhaust and **ozone** and, to a lower degree, by **ozone** alone, when tested at a fixed time of 48 h (8). **Ozone** generally represses the expression of numerous genes (7, 27, 40, 86), in particular those encoding chloroplast proteins. The induction of basic ß-1,3-glucanase transcripts was confined to leaf parts directly exposed to **ozone** and thus was nonsystemic (33). Other **ozone** responses (e.g. the acidic isoenzymes) are likely to be systemic, but this has so far not been examined. **Ozone**-induced gene expression in tobacco and other plant species is summarized in Table 1 and has in part been reviewed earlier (53, 93). Studies with *Nicotiana plumbaginifolia* L. have further documented the antioxidative response to **ozone** and UV-B, and also to a lesser degree to SO_2 (123). A number of antioxidative genes, including those for Fe-, CuZn- and Mn-dependent superoxide dismutases, were largely nonresponsive. The transcript amounts of catalase 2 and lipid-peroxide–dependent glutathione peroxidase gave strong increases. Catalase is a postulated receptor for salicylic acid, an inducer of SAR (56). **Ozone** also induces PR proteins 1a and 1b, as well as the biosynthesis of salicylic acid in tobacco cv Xanthi-nc (126). Tobacco cv PBD6, which was engineered to overexpress Mn-SOD in the mitochondria or the chloroplast, showed a fourfold higher **ozone** tolerance only upon overexpression in the chloroplast (119B). This finding pointed to a role of chloroplast reactive oxygen species in **ozone** phytotoxicity, even though **ozone** itself hardly penetrates into the chloroplast (58, 61). An earlier 15-fold overexpression of CuZn-SOD in tobacco chloroplasts had not led to tolerance for high **ozone** (83), possibly owing to excess generated hydrogen peroxide (83, 119B).

Table 1. Ozone-induced gene expression‡

View this table:
[in this window]
[in a new window]

In summary, the differential **ozone** effect on tobacco can be rationalized by oxidant amplification and induction of HR-like processes occurring in Bel W3 and oxidant scavenging occurring in Bel B (15, 65, 102). **Ozone** sensitivity appears to be determined by the metabolic disposition of the two tobacco lines. A standard **ozone** pulse (150 $\mu L \cdot L^{-1}$, 5 h) induced an apoplastic oxidant peak within 2–3 h in both tobacco cultivars, a second higher peak at 8 h occurring only in Bel W3. Fatty acid hydroperoxides were isolated and were active in the induction of tyramine hydroxycinnamoyl transferases (103).

Parsley: Cross-Induction of Defensive Pathways by Ozone

Parsley activates the genes and enzymes of flavone glycoside biosynthesis upon UV-exposure and those of furanocoumarin phytoalexins upon treatment with fungal spores or fungal elicitor (43). **Ozone** acts as a cross inducer and simultaneously activates both pathways at the metabolite, enzyme, and transcript levels (30, 31, 32). Early genes responded to **ozone** within 3 h (PR-1, PR-2, elicitor-induced protein 16), intermediate genes (for the biosynthetic enzymes) within 6 h, and late genes within 12 h (extensin, peroxidase). Actin mRNA did not respond to **ozone**.

Apoplastic ascorbate of parsley plants was increased, but total ascorbate was decreased by **ozone** (32). Necrotic development started at 24 h, with renewed gene inductions as well as formation of callose and of fluorescent cell wall material (30). In vitro translation of poly(A) + RNA revealed about 20 induced and 10 repressed proteins (31). Differential cDNA screening and in vitro translation has led to identification of one of the **ozone**-induced proteins as PR-1-1, a protein induced by fungal elicitor (31). The rather close temporal linkage between the induced biochemical reactions and necrotic development was reminiscent of the linkage between **ozone**-induced necrosis and phytoalexin induction in soybean (55) and ethylene induction in a number of herbaceous plant species (65, 77, 116).

Further Herbaceous Plant Species

As in tobacco (101) and parsley (30), **ozone** induces cell wall autofluorescence in soybean, probably owing to impregnation of the cell wall with phenolic compounds (14). A similar reaction is known for pathogen-induced HR (24, 39, 43). Soybean ubiquitin (93) and lipoxygenase (71) transcripts were also induced by **ozone**. Soybean (57) and barley (91) increased polyamine levels and arginine decarboxylase activity, respectively, in response to **ozone**. In potato, **ozone** increased both ethylene and polyamines, and it decreased the ribulose bis-phosphate carboxylase subunits at the protein and transcript levels (27, 86). The transcript for ACC synthase in potato increased 1 h after onset of **ozone** exposure (96). ACC oxidase mRNA in tomato was already strongly induced after 30 min (119), followed by increases in SAM-synthase and ACC-synthase mRNAs. Only one of four ACC synthase genes (ACS 2) and one of three SAM synthetase genes were affected. The levels of ACC and ethylene strongly increased (119), thereby matching the results described above for tobacco Bel W3.

In *Arabidopsis*, **ozone** induced mRNA levels of a glutathione S-transferase (GST) and of phenylalanine ammonia-lyase (PAL) within 3 h, and of a neutral peroxidase and a cytosolic CuZn-superoxide dismutase 12 h after **ozone** onset (106). The induction of the *Arabidopsis* GST has also been correlated with pathogen-induced HR as well as SAR (39). In a subsequent study, *Arabidopsis* transcripts for cytosolic ascorbate peroxidase, CuZn-SOD, and glutathione S-transferase were increased by **ozone**. Transcripts for chloroplast proteins including rubisco were decreased (20). **Ozone** also induced an *Arabidopsis* mRNA for a novel 8.3-kDa protein (107).

Conifer Species: Early Stress Reactions

Conifers represent the most **ozone**-tolerant plant group, mainly because of low stomatal conductance (18, 61, 87). Subacute **ozone** nevertheless led to a rapid induction of putrescine and of the lignin-biosynthetic enzyme cinnamyl alcohol dehydrogenase in Norway spruce [*Picea abies* (L.) Karsten] needles (37, 62, 94). Photosynthesis and monoterpenes, as well as chloroplast pigments, were largely unaffected (62, 63, 94). In Scots pine needles, stilbenes as well as the biosynthetic enzymes, stilbene

synthase, and pinosylvin O-methyltransferase, were all strongly induced (90, 94). These parameters were undetectable in control needles. Stilbenes normally occur constitutively in pine heartwood and are inducible phytoalexins in pine sapwood. The needle induction of stilbenes by **ozone** appeared to occur outside of the normal spatial and temporal control. Ponderosa pine (*Pinus ponderosa* Lawson) needles showed increased stilbene contents as an **ozone** response in the field (San Bernardino Mountains), as well as upon controlled exposure (H Sandermann & W Heller, unpublished results). Induced stilbenes in cultured pine cells (59) became rapidly cell-wall associated, which was reminiscent of the cell-wall binding of tyramine- conjugates in tobacco (102, 104). An elicitor-induced spruce stress lignin has been characterized as an extensin/lignin complex with great structural similarity to early developmental lignins (60). The **ozone**-induced stress lignin is so far elusive.

The flavan, catechin, was strongly induced by **ozone** in both Norway spruce and Scots pine needles and, like the stilbenes in pine needles, remained elevated for several months after the end of **ozone** exposure (63, 64). Norway spruce seedlings responded to **ozone** by rapid development of ethylene and ACC; malonyl-ACC increased only in long-term experiments (63, 108). Detached needles from ponderosa pine and Jeffrey pine (*Pinus jeffreyi* Balf) also showed increased ethylene development in relation to **ozone** exposure and visible injury (112). With regard to antioxidative defense, **ozone** and SO_2 caused an increase of various antioxidants in needles of spruce and fir (76). Apoplastic ascorbate in spruce needles also increased under **ozone** (84). It is not known whether apoplastic ascorbate can undergo long-distance transport, so experiments in which **ozone** is applied by branch cuvette may be invalidated by a source-sink flux of ascorbate. The antioxidants, putrescine and catechin, were also induced by **ozone** in spruce needles (62, 64, 94).

Unidentified **ozone**-induced stress proteins were detected in both Norway spruce (98) and Scots pine (*Pinus sylvestris* L.) needles (40). At the transcript level, the genes of CAD, of a short-chain alcohol dehydrogenase and of extensin, appeared to be induced by **ozone** in Norway spruce (Table 1). In Scots pine, transcript amounts for extensin and stilbene synthase were induced by **ozone** (Table 1). Recently, **ozone** was found to induce transcripts for ubiquitin and 3-hydroxymethylglutaryl-CoA synthase in pine (Table 1). These proteins are involved in protein turnover and in terpene biosynthesis, respectively, and are stress related. For example, induced terpene (oleoresinol) biosynthesis is important in defense against bark beetles and their associated fungi (111). **Ozone** induces pine and spruce CAD at the enzyme and transcript levels (36, 37, 52, 64, 94). Cultured spruce cells contained only a single gene and isoenzyme of CAD. The latter was identical to spruce cambial CAD, so **ozone** stress appeared to interfere with the normally strict developmental control of wood lignin formation (36, 37). The cDNA clone isolated for stress CAD (36) appeared also to represent the first known gene of conifer wood formation. Pathogenesis-related proteins in Norway spruce needles (Western blotting) and ß-1,3-glucanase enzyme activity were induced by **ozone** (54). Chitinase and ß-1,3-glucanase from various tree species had earlier been shown to lyse fungal cell walls (120). Conifer roots or embryos infected with fungal pathogens accumulated chitinase or ß-1,3-glucanases (80, 105).

In summary, conifers resembled herbaceous plants with respect to the **ozone** induction of antioxidative and phytopathological defense reactions at the levels of transcription, translation, and stress metabolites.

The required concentrations of **ozone** generally were two- to threefold higher, probably owing to the low stomatal conductance of conifers (61, 87).

Conifer Species: Memory Effects

When Norway spruce and Scots pine trees that had shown early **ozone**-induced reactions were cultivated for another year under normal nursery conditions, the following delayed effects occurred: Norway spruce developed pigment loss, banding, chlorosis, and a decline in photosynthetic capacity in the older needles only at the time (May) of the flush of new needle formation (64, 63, 93). The flush of new needles constitutes an endogeneous stress that also triggers needle yellowing, caused by overall Mg^{++} deficiency (61), and increased susceptibility to bark beetles (111). The delayed **ozone** effects were strictly dose dependent, with the onset **ozone** doses about 100 $\mu l \cdot L^{-1} \cdot h$ (63). The specificity for the older needles and for the time of the new flush agreed with field observations of spruce decline (61, 119A), but the specific visible symptoms have not been observed in the field. Scots pine showed a premature loss of the **ozone**-treated needles in the year following **ozone** treatment, at the normal time (late August) of shedding of old needles (64, 63, 93). A similar premature needle shedding in late summer occurs as an **ozone** response of ponderosa pine in the field (42, 79, 109). The normal endogeneous senescence stress seemed to trigger premature needle shedding as a consequence of exposure to **ozone** doses above 40 $\mu L \cdot L^{-1} \cdot h$ administered in the year before (63). In summary, the induced early stress reactions seemed to imprint a "memory" for past **ozone** exposure into conifer needles. Several features of conifer decline could be reproduced, but one component of the visible symptoms of spruce needles (banding pattern) has not been described as a decline symptom. Recent long-term fumigation studies of spruce and pine have shown that visible **ozone** symptoms are variable and depend on the exact experimental conditions (J Schnitzler & C Langebartels, unpublished results).

Deciduous Tree Species

Young beech (*Fagus sylvatica* L.) trees originating from 800 meters altitude have been treated with **ozone** in closed chamber and in parallel open-top experiments. An increase to 1.5–2.0 times ambient **ozone** was enough to inhibit dark reactions of photosynthesis and to cause visible leaf symptoms (70, 108). In addition, beech showed delayed "memory" effects because bud break and growth were inhibited in response to the subacute **ozone** treatment in the previous year (69, 82). With regard to stress metabolites, lasting increases of ethylene, ACC, and N-malonyl-ACC were induced by **ozone** (108). In spite of the apparent **ozone** sensitivity, beech trees grow well at elevations up to 1500 m in high-**ozone** areas of the Black Forest and the Alps. The apparent contradiction may be due to the existence of sensitive phases (4) or of adapted ecotypes (4, 9, 108). However, genotypes and mechanisms of stress adaptation of beech have not yet been characterized. The influence of **ozone** on deciduous trees in the field has apparently so far not been clearly differentiated from detrimental effects of drought and of pathogens [see (109, 119A)]. Good ecophysiological knowledge exists for **ozone**-sensitive and **ozone**-tolerant varieties or clones of certain deciduous tree species such as birch and poplar (4, 18, 42, 73, 85, 87). A population shift from **ozone**-sensitive pine species to **ozone**-tolerant oak and shrub species (as well as incense cedar and white fir) has been described for Southern California (9, 79, 109).

OZONE AND BIOTIC DISEASE: EMPIRICAL OBSERVATIONS

In early studies (89), wheat seedlings under **ozone** remained free of mold, whereas those without **ozone** became moldy. Subsequent studies indicated that near-ambient **ozone** exhibited a marked influence on plant-pathogen interaction by changing host plant predisposition. **Ozone** effects on pathogens were less pronounced (44, 100). These observations have been corroborated by many subsequent studies in numerous plant-pathogen systems (4, 51, 72). **Ozone** enhanced susceptibility or tolerance toward pathogens in a poorly predictable way. It was proposed that necrotrophic pathogens would more easily colonize **ozone**-weakened plants, whereas biotrophic infections might be lessened (44, 72). In model studies with pine seedlings, **ozone** enhanced root attack by *Heterobasidion annosum* because the presence of mycorrhiza suppressed this effect (13). The fungus elicited a number of defense reactions in conifer seedlings (5, 13).

In the classical field studies in the San Bernardino Mountains, California, **ozone** predisposed ponderosa pine and Jeffrey pine for enhanced attack by *Heterobasidion annosum* and by bark beetles (42, 51, 74, 79, 109). Generally, **ozone** facilitates the attack of a second abiotic or biotic stressor (4, 18, 74, 94, 109, 117). With regard to herbivores and insects, **ozone** often had significant effects that may have been due to changed plant metabolic status (45, 51, 122). Again, only poor predictions with respect to increased tolerance or susceptibility through **ozone** were possible. The positive or negative effects of ethylene on disease incidences were also largely nonpredictable (1). Recent empirical observations include increased fungal susceptibility of poplar (125) and wheat (119C). Spider mites were favored on **ozone**-treated clover and peanut (46).

OUTLOOK

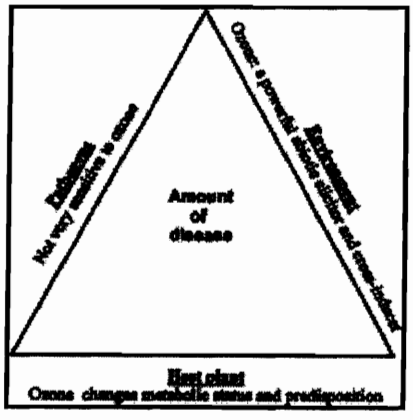
▲ Top
▲ Previous
▼ Next
▼ Literature

Field and Controlled Exposure Studies: Ozone as a Plant Disease Agent

The role of **ozone** as a disease-causing agent in agriculture has been extensively studied in crop loss–assessment programs (4, 35, 45). Because of the routine use of pesticides, the abiotic effects of **ozone** have mainly been considered. The possible predisposition for biotic disease may in fact not be of much agricultural importance because of the general use of pesticides. The predispositional effects of **ozone** may be more important for wild plant species. The resulting selection and gene pool effects have recently been summarized under the aspect of biodiversity (9).

Current **ozone** limit values generally do not include effects on plant secondary metabolism, interactions with second stressors, or predisposition for biotic disease. These values are generally based exclusively on ecophysiological and growth parameters. For example, gas exchange data have led to the conclusion that adult giant sequoia are not sensitive to atmospheric **ozone** (41). With regard to specific critical values, older studies determined that acceptable **ozone** concentrations for a single 4 h exposure were as follows: 50 nL $\cdot L^{-1}$ **ozone** for sensitive plant species, 100 nL $\cdot L^{-1}$ **ozone** for intermediate plant species, and 180 nL $\cdot L^{-1}$ **ozone** for tolerant plant species. Multiple exposures would presumably lower these **ozone** limit values (42, 68). All these critical **ozone** concentration values are in the range of ambient **ozone** (see Introduction). Onset **ozone** dose values for effects on growth or photosynthesis were subsequently estimated as 25–100 $\mu L \cdot L^{-1} \cdot h$ for conifers, 10–20 $\mu L \cdot L^{-1} \cdot h$ for hardwood trees, and 3–

7 $\mu L \cdot L^{-1} \cdot h$ for agricultural crop plants (87). Again, these critical dose values are in the range of ambient **ozone** (68, 87). Species differences in **ozone** sensitivity were related to inherent differences in leaf conductance and **ozone** uptake (87). When **ozone** uptake was used as the basis of comparison, responses in all plant species became comparable despite possible differences in the antioxidant status. This finding suggested a common biochemical response mechanism for **ozone** (87). More recently, critical exposures have been defined as those sufficient to cause a 10% reduction in growth or yield (35). AOT40 was defined as the sum of hourly average **ozone** concentrations above 40 $nL \cdot L^{-1}$ for a defined time period. A limit AOT40 value of 10 $\mu L \cdot L^{-1} \cdot h$ was proposed for forest trees, calculated over 6 months and 24 h per day (35). An AOT40 value of 5.3 $\mu L \cdot L^{-1} \cdot h$ has been proposed for agricultural plants, calculated only for daylight hours over 3 months (May to July) (35). The memory effect of spruce occurred after pretreatment with an external **ozone** dose of 100 $\mu L \cdot L^{-1} \cdot h$ (63). Growth effects in many tree species also occurred at or below this dose value (4, 18, 85, 87, 117), while agricultural yield losses occurred at significantly lower external **ozone** doses (45, 68, 87, 117). Seasonal external **ozone** dose values of 100 $\mu L \cdot L^{-1} \cdot h$ or AOT40 values of 10 $\mu L \cdot L^{-1} \cdot h$ are often exceeded in Europe and in the United States (35, 67, 68, 70, 87). In addition, it has been proposed that perennial plants such as conifers can integrate **ozone** exposure, so that lifetime doses should be considered (66, 87). Overviews of predispositional **ozone** effects are available for low temperature and moisture (19), pathogens (72), insects (51), as well as general stress and nutritional processes (74, 87, 109). These predispositional effects may lower the limit values mentioned above and, on the basis of lifetime **ozone** doses, may be of particular importance for long-lived tree species. Recent results on **ozone** effects can be summarized by incorporation into the classical plant disease triangle, as shown in Figure 2. The concept of host predisposition by environmental factors and of stress interaction was originally developed by pioneers of phytopathology such as R Hartig, P Sorauer, and E. Gäumann (100).



View larger version (23K):
[in this window]
[in a new window]

Figure 2. Role of **ozone** in plant disease. The classical triangle (2) depicting the amount of plant disease as dependent on the host plant, the pathogen, and environmental factors is modified to include the **ozone** effects described in the text.

In summary, it may appear that tree species (and even more so, herbaceous plant species) suffer from chronic damage by ambient **ozone** values. Inhibition of photosynthesis and growth parameters have

indeed been observed at field sites (85, 87), but these effects are nonspecific. The specific visible symptoms caused by **ozone** alone in controlled experiments (mottling, banding patterns) have apparently been observed in the field only in special cases, such as in certain crop plants (35, 42, 50, 117), certain deciduous trees [e.g. *Prunus serotina* (4)], as well as ponderosa pine and Jeffrey pine in the San Bernardino Mountains (79, 81, 109, 117). An **ozone**-dominated 500-km stretch in California and Nevada, USA, remains the only field area where a role of **ozone** has been clearly established in forest decline (79, 81, 95). Field studies in the San Bernardino Mountains (42, 74, 79, 109) are consistent with Figure 1. Inhibition of photosynthesis and growth has led to a loss of competitiveness and fitness. This effect, as well as the observed unregulated induction of plant defense systems (ethylene, stilbenes), and inhibition of oleoresinol defense, explains the predisposition for attack by bark beetles and root rot fungi.

Even though the level of tree damage is significant in the Eastern USA (18, 74, 109) and in Central Europe (119A), a causal linkage between forest decline and **ozone** has not yet been conclusively established (74, 95, 97). The specific visible symptoms (banding, mottling, chlorosis) caused on older conifer needles by **ozone** directly or through the memory effect have apparently never been clearly identified outside California. The discrepancy between symptoms observed in controlled **ozone** exposure and symptoms observed in the field could be resolved by the following scenario. In field sites not strongly dominated by **ozone**, this pollutant may only imprint a predisposition. A second stressor such as frost, drought, pathogens, or nutritional deficiencies may then trigger visual symptoms. The combined stress may produce the nonspecific symptoms often observed in the field (42, 45, 87), e.g. pigment loss and premature leaf or needle fall, rather than the pure **ozone** symptoms. This scenario links **ozone** to the most commonly observed symptoms of forest decline (42, 74, 119A), and it is in agreement with frequent reports of senescence-like **ozone** symptoms (18, 42, 78, 89). Careful long-term exposure experiments with combined stressors are clearly needed to test this scenario. Serious effects of **ozone** on crop plants and forest trees are expected in the future if the predicted tripling of tropospheric **ozone** concentrations occurs by the year 2025 (17).

Molecular Aspects: Ozone as an Elicitor and Cross-Inducer

As noted above, **ozone** effects on gene expressions were transient upon short-term exposure, while stress enzymes and metabolites in several cases remained elevated for many months (63). Long-term exposures have led to more persistent transcript increases (128). Some of the **ozone** effects resembled those characteristic for SAR (39, 57) and HR (24, 39, 75, 113), as well as for ethylene (28, 29), wounding, and other stimuli. Several examples for cross-induction of viral, fungal, or UV-B-defense pathways have been described above. In fact, **ozone** induced all three reaction types distinguished for plant defense against pathogens (12), glycosidases, localized barriers, and antioxidative enzymes. Plants seem to mistake **ozone** for a viral or microbial pathogen, so **ozone** should be a useful experimental tool to elucidate induced defense pathways. The exact relationship between **ozone** effects and SAR, HR, and other general defense reactions remains to be elucidated, because the role of an oxidative burst and of second messengers (radicals, peroxides, jasmonic acid, salicylic acid, oligosaccharides, systemin, traditional phytohormones, redox state, ion gradients, etc.) is not yet clear. The phytohormone, ethylene, its precursor, ACC, and in particular the enzyme ACC-oxidase and its mRNA generally respond rapidly

to **ozone**. An early burst of ethylene has been correlated with visible **ozone** lesion development in many herbaceous plant species (65, 77, 116). The reaction between **ozone** and ethylene could produce toxic agents (77). However, plants rapidly detoxified one such product (formaldehyde); the detoxifying formaldehyde dehydrogenase is widely distributed in plant species (38). Conflicting data exist for another possible mechanism, namely a relationship of ethylene biosynthesis to HR-like programmed cell death (28, 39). Elevated ubiquitin (Table 1) has been correlated to the formation of HR-like lesions in tobacco (11, 113).

It is not clear whether **ozone** can give rise to a higher than stoichiometric oxidative burst and whether there is an **ozone** receptor that can amplify **ozone** effects. An attractive, but as yet untested possibility is that fungal elicitor receptors (26), other membrane receptors, or the proteins encoded by plant resistance genes (110) can act as **ozone** receptors. Most **ozone** responses have as yet not been localized in the plant tissue. Probably some responses are localized in the areas of subsequent HR-like lesions, while SAR-like responses take place in the adjacent tissue. The promoter structures of **ozone** responsive genes remain to be characterized for regulatory elements. In terms of the induced gene-for-gene scenario for incompatible plant-pathogen interactions (23A), the strict temporal and spatial regulation of plant defense genes may be disturbed by the unregulated gene induction caused by **ozone**. This uncoupling effect may convert incompatible to compatible interactions, so that the induced defense reactions may lead to increased susceptibility rather than pathogen tolerance.

ACKNOWLEDGMENTS

▲ Top
▲ Previous
▼ Next
▼ Literature

Work from this Institute was performed in the laboratories of Drs. D Ernst, W Heller, and C Langebartels. Their excellent contributions over the past years and their improvements to this manuscript are gratefully acknowledged. Valuable suggestions also came from Professor J Ebel, Botanisches Institut, LMU München. Our work has been supported by EUROSILVA (BMBF), by Bayerisches Staatsministerium für Landesentwicklung und Umweltfragen (PBWU, BayForKlim), by Deutsche Forschungsgemeinschaft (Sa 180/18), by Limagrain (Chappes, France), and by Fonds der Chemischen Industrie.

FOOTNOTES

▲ Top
▲ Previous
▼ Literature

[1] [1]Abbreviations: AOT40, accumulated hourly **ozone** concentrations over a threshold of 40 nL • L^{-1}; CAD, cinnamylalcohol dehydrogenase; GST, glutathione S-transferase; HR, hypersensitive response; PR, pathogenesis-related; SAR, systemic acquired resistance; SOD, superoxide dismutase. ▲

Annu. Rev. Phytopathol. 1996. 34:347-366

0066-4286/96/0901-0347

Full-text links to some of the citing articles in other journals:

- Riehl Koch, J., Scherzer, A. J., Eshita, S. M., Davis, K. R. (1998). **Ozone** Sensitivity in Hybrid Poplar Is Correlated with a Lack of Defense-Gene Activation. *Plant Physiol.* 118: 1243-1252 [Abstract] [Full Text]
- Coakley, S. M., Scherm, H., Chakraborty, S. (1999). CLIMATE CHANGE AND PLANT DISEASE MANAGEMENT. *Annu. Rev. Phytopathol.* 37: 399-426 [Abstract] [Full Text]
- Abarca, D., Roldan, M., Martin, M., Sabater, B. (2001). Arabidopsis thaliana ecotype Cvi shows an increased tolerance to photo-oxidative stress and contains a new chloroplastic copper/zinc superoxide dismutase isoenzyme. *J. Exp. Bot.* 52: 1417-1425 [Abstract] [Full Text]

Annu. Rev. Phytopathol. 1996. 34:347-366.

LITERATURE CITED

1. Abeles FB, Morgan PW, Saltveit ME. 1992. *Ethylene in Plant Biology*,pp. 114–19. New York: Academic. 2nd ed.
2. Agrios GN. 1988. *Plant Pathology*,p. 43. New York: Academic. 3rd ed.
3. Deleted in proof
4. Ashmore MR. 1992. Critical levels for ozone. In *Workshop on Critical Levels of Air Pollutants for Europe, Egham*, ed. MR Ashmore, RB Wilson,pp. 20–47. London, UK: Dep. Environ.
5. Asiegbu FO, Daniel G, Johansson M. 1994. Defence related reactions of seedling roots of Norway spruce to infection by *Heterobasidion annosum* (Fr.) Bref. *Physiol. Mol. Plant Pathol.* 45:1–19 [ISI]
6. Ayres PG. 1984. The interaction between environmental stress injury and biotic disease physiology. *Annu. Rev. Phytopathol.* 22:53–75 [ISI]
7. Bahl A, Kahl G. 1995. Air pollutant stress changes the steady-state transcript levels of three photosynthesis genes. *Environ. Pollut.* 88:57–65 [ISI]
8. Bahl A, Loitsch SM, Kahl G. 1995. Transcriptional activation of plant defence genes by short-term air pollution stress. *Environ. Pollut.* 89:221–27 [ISI]
9. Barker JR, Tingey DT, eds.1992. *Air Pollution Effects on Biodiversity*. New York: Van Nostrand Reinhold
10. Bauer S, Galliano H, Pfeiffer F, Messner B, Sandermann H, Ernst D. 1993. Isolation and characterization of a cDNA clone encoding a novel short-chain alcohol dehydrogenase from Norway spruce (*Picea abies* [L.] Karst). *Plant Physiol.* 103:1479–80 [CrossRef][ISI][Medline]
11. Becker F, Buschfeld E, Schell J, Bachmair A. 1993. Altered response to viral infection by tobacco plants perturbed in ubiquitin system. *Plant J.* 3:875–81 [ISI]
12. Bol JF, Linthorst HJM, Cornellissen BJC. 1990. Plant pathogenesis-related proteins induced by virus infection. *Annu. Rev. Phytopathol.* 28:113–38 [Abstract]
13. Bonello P, Heller W, Sandermann H. 1993. Ozone effects on root-disease susceptibility and defence responses in mycorrhizal and non-mycorrhizal seedlings of Scots pine (*Pinus sylvestris* L.). *New Phytol.* 124:653–63 [ISI]
14. Booker FL, Fiscus EL, Miller JE. 1991. Ozone-induced changes in soybean cell wall physiology.

In *Active Oxygen/Oxidative Stress and Plant Metabolism*, ed. EJ Pell, K Steffen,pp. 229–32. Rockville, MD: Am. Soc. Plant Physiol.

15. Bors W, Langebartels C, Michel C, Sandermann H. 1989. Polyamines as radical scavengers and protectants against ozone damage. *Phytochemistry* 28:1589–95 [CrossRef][ISI]
16. Deleted in proof
17. Chameides WL, Kasibhatla PS, Yienger J, Levi H II.1994. Growth of continental-scale metro-agro-plexes, regional ozone pollution, and world food production. *Science* 264:74–77 [ISI]
18. Chappelka AH, Chevone BI. 1992. Tree responses to ozone. In *Surface Level Ozone Exposures and their Effects on Vegetation*, ed. AS Lefohn,pp. 271–324. Chelsea, MI: Lewis
19. Chappelka AH, Freer-Smith PH. 1995. Predisposition of trees by air pollutants to low temperatures and moisture stress. *Environ. Pollut.* 87:105–17 [ISI]
20. Conklin PL, Last RL. 1995. Differential accumulation of antioxidant mRNAs in *Arabidopsis thaliana* exposed to ozone. *Plant Physiol.* 109:203–12 [Abstract]
21. Cooley DR, Manning WJ. 1987. The impact of ozone on assimilate partitioning in plants: a review. *Environ. Pollut.* 47:95–113 [ISI]
22. Darley EF, Middleton JT. 1966. Problems of air pollution in plant pathology. *Annu. Rev. Phytopathol.* 4:103–18
23. Darrall NM. 1989. The effect of air pollutants on physiological processes in plants. *Plant Cell Environ.* 12:1–30 [ISI]

23A. Wit PJGM. 1992. Molecular characterization of gene-for-gene systems in plant-fungus interactions and the application of avirulence genes in control of plant pathogens. *Annu. Rev. Phytopathol.* 30:391–418 [Abstract]

24. Dixon RA, Harrison MJ, Lamb CJ. 1994. Early events in the activation of plant defense responses. *Annu. Rev. Phytopathol.* 32:479–501 [ISI]
25. Dugger WM, Ting IP. 1970. Air pollution oxidants—their effects on metabolic processes in plants. *Annu. Rev. Plant Physiol.* 21:215–34 [ISI]
26. Ebel J, Cosio EG. 1994. Elicitors of plant defense reactions. *Int. Rev. Cytol.* 148:1–36 [ISI]
27. Eckardt NA, Pell EJ. 1994. O_3-induced degradation of Rubisco protein and loss of Rubisco mRNA in relation to leaf age in *Solanum tuberosum* L. *New Phytol.* 127:741–48 [ISI]
28. Ecker JR. 1995. The ethylene signal transduction pathways in plants. *Science* 268:667–75 [ISI] [Medline]
29. Ecker JR, Davis RW. 1987. Plant defense genes are regulated by ethylene. *Proc. Natl. Acad. Sci. USA* 84:5202–6 [ISI]
30. Eckey-Kaltenbach H, Ernst D, Heller W, Sandermann H. 1994. Biochemical plant responses to ozone. IV. Cross-induction of defensive pathways in parsley (*Petroselinum crispum L.*) plants. *Plant Physiol.* 104:67–74 [ISI]
31. Eckey-Kaltenbach H, Grosskopf E, Sandermann H, Ernst D. 1994. Induction of pathogen defense genes in parsley (*Petroselinum crispum* L.) plants by ozone. *Proc. R. Soc. Edinburgh B* 102:63–74 [ISI]
32. Eckey-Kaltenbach H, Heller W, Sonnenbichler J, Zetl I, Schäfer W, et al.1993. Oxidative stress and plant secondary metabolism: 6"-O-Malonylapiin in parsley. *Phytochemistry* 34:687–91 [CrossRef][ISI]
33. Ernst D, Bodemann A, Schmelzer E, Langebartels C, Sandermann H. 1996. ß-1,3-Glucanase mRNA is locally, but not systemically induced in *Nicotiana tabacum* cv. Bel W3 after ozone fumigation. *J. Plant Physiol.* In press
34. Ernst D, Schraudner M, Langebartels C, Sandermann H. 1992. Ozone-induced changes of mRNA levels of ß-1,3-glucanase, chitinase and "pathogenesis-related" protein 1b in tobacco plants. *Plant Mol. Biol.* 20:673–82 [ISI][Medline]
35. Fuhrer J, Achermann B, ed.1994. Critical levels for ozone. A UN-ECE Workshop Rep., Fed. Res. Stn. Agric. Chem. Environ. Hyg., CH-3097 Liebefeld-Bern, Switz.
36. Galliano H, Cabané M, Eckerskorn C, Lottspeich F, Sandermann H, Ernst D. 1993. Molecular

cloning, sequence analysis and elicitor-/ozone-induced accumulation of cinnamyl alcohol dehydrogenase from Norway spruce (*Picea abies* L.). *Plant Mol. Biol.* 23:145–56 [ISI][Medline]
37. Galliano H, Heller W, Sandermann H. 1993. Ozone induction and purification of spruce cinnamyl alcohol dehydrogenase. *Phytochemistry* 32:557–63 [CrossRef][ISI]
38. Giese M, Bauer-Doranth U, Langebartels C, Sandermann H. 1994. Detoxification of formaldehyde by the spider plant (*Chlorophytum comosum* L.) and by soybean (*Glycine max* L) cell suspension cultures. *Plant Physiol.* 104:1301–9 [ISI]
39. Greenberg JT, Guo A, Klessig DF, Ausubel FM. 1994. Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. *Cell* 77:551–63 [ISI][Medline]
40. Grosskopf E, Wegener-Strake A, Sandermann H, Ernst D. 1994. Ozone-induced metabolic changes in Scots pine: mRNA isolation and analysis of in vitro translated proteins. *Can. J. For. Res.* 24:2030–33 [ISI]
41. Grulke NE, Miller PR. 1994. Changes in gas exchange characteristic during the life span of giant sequoia: implications for response to current and future concentrations of atmospheric ozone. *Tree Physiol.* 14:659–68 [ISI]
42. Guderian R, ed.1985. *Air Pollution by Photochemical Oxidants. Formation, Transport, Control and Effects on Plants*. Ecol. Stud. 52. Berlin: Springer-Verlag
43. Hahlbrock K, Scheel D. 1989. Physiology and molecular biology of phenylpropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:347–69 [ISI]
44. Heagle AS. 1973. Interactions between air pollutants and plant parasites. *Annu. Rev. Phytopathol.* 11:365–488 [ISI]
45. Heagle AS. 1989. Ozone and crop yield. *Annu. Rev. Phytopathol.* 27:397–423 [ISI]
46. Heagle AS, Brandenburg RL, Burn JC, Miller JE. 1994. Ozone and carbon dioxide effects on spider mites in white clover and peanut. *J. Environ. Qual.* 23:1168–76 [ISI]
47. Heath RL. 1980. Initial events in injury to plants by air pollutants. *Annu. Rev. Plant Physiol.* 31:395–431 [ISI]
48. Heath RL. 1994. Alterations of plant metabolism by ozone exposure. In *Plant Responses to the Gaseous Environment. Molecular, Metabolic and Physiological Aspects*, ed. RG Alscher, AR Wellburn,pp. 121–45. London: Chapman & Hall
49. Heck WW. 1968. Factors influencing expression of oxidant damage to plants. *Annu. Rev. Phytopathol.* 6:165–88 [ISI]
50. Heggestadt HE. 1991. Origin of Bel-W3, Bel-C and Bel-B tobacco varieties and their use as indicators of ozone. *Environ. Pollut.* 74:264–91 [ISI]
51. Heliövaara K, Väisänen R. 1993. *Insects and Pollution*. Boca Raton, FL: CRC Press
52. Heller W, Rosemann D, Osswald WF, Benz B, Schönwitz R, et al.1990. Biochemical response of Norway spruce (*Picea abies* [L.] Karst.) towards 14-month exposure to ozone and acid mist. Part I. Effects on polyphenol and monoterpene metabolism. *Environ. Pollut.* 64:353–66 [ISI]
53. Kangasjärvi J, Talvinen J, Utriainen M, Karjalainen R. 1994. Plant defence systems induced by ozone. *Plant Cell Environ.* 17:783–94 [ISI]
54. Kärenlampi SO, Airaksinen K, Miettinen ATE, Kokko HI, Holopainen JK, et al.1994. Pathogenesis-related proteins in ozone-exposed Norway spruce (*Picea abies* (Karst) L.). *New Phytol.* 126:81–89
55. Keen NT, Taylor OC. 1975. Ozone injury in soybeans. Isoflavonoid accumulation is related to necrosis. *Plant Physiol.* 55:731–33 [ISI]
56. Kessmann H, Staub T, Hofmann C, Maetzke JH, Herzog J, et al.1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annu. Rev. Phytopathol.* 32:439–59 [ISI]
57. Kramer GF, Lee EH, Rowl RA, Mulchi CL. 1991. Effects of elevated CO2 concentration on the polyamine levels of field-grown soybean at three O3 regimes. *Environ. Pollut.* 73:137–52 [ISI]
58. Laisk A, Kull O, Moldau H. 1989. Ozone concentration in leaf intercellular air spaces is close to zero. *Plant Physiol.* 90:1163–67 [ISI]

59. Lange BM, Lapierre C, Sandermann H. 1995. Elicitor-induced spruce stress lignin: Structural similarity to early developmental lignins. *Plant Physiol.* 108:1277–87 [ISI]
60. Lange BM, Trost M, Heller W, Langebartels C, Sandermann H. 1994. Elicitor-induced formation of free and cell-wall-bound stilbenes in cell suspension cultures of Scots pine *Pinus sylvestris* L.). *Planta* 194:143–48 [ISI]
61. Lange OL, Heber U, Schulze E-D, Ziegler H. 1989. Atmospheric pollutants and plant metabolism. In *Forest Decline and Air Pollution. A Study of Spruce (Picea abies) on Acid Soils*, ed. E-D Schulze, OL Lange, R Oren, Ecol. Stud.77:238–73. Berlin: Springer-Verlag
62. Langebartels C, Führer G, Häckl B, Heller W, Kloos M, et al.1989. Dose-dependent reactions of Norway spruce to ozone fumigation. In *Air Pollution and Forest Decline*, ed. JB Bucher, I Bucher-Wallin.*Proc. 14. IUFRO Meet., Birmensdorf,* pp. 446–69
63. Langebartels C, Heller W, Ernst D, Führer G, Lippert M, et al.1996. Memory effects in the action of ozone on conifers. Submitted
64. Langebartels C, Heller W, Kerner K, Leonardi S, Rosemann D, et al.1990. Ozone-induced defense reactions in plants. In *Environmental Research with Plants in Closed Chambers*, ed. HD Payer, T Pfirrmann, P Mathy, Air Pollut. Res. Rep. EC 26, Brussels,pp. 358–68
65. Langebartels C, Kerner K, Leonardi S, Schraudner M, Trost M, et al.1991. Biochemical plant responses to ozone. I. Differential induction of polyamine and ethylene biosynthesis in tobacco. *Plant Physiol.* 95:882–89 [ISI]
66. Laurence JA, Weinstein LH. 1981. Effects of air pollutants on plant productivity. *Annu. Rev. Phytopathol.* 19:257–71 [ISI]
67. Laurila T, Lättilä H. 1994. Surface ozone exposures measured in Finland. *Atmos. Environ.* 28:103–14 [ISI]
68. Lefohn AS. 1992. Ozone standards and their relevance for protecting vegetation. In *Surface Level Ozone Exposures and their Effects on Vegetation*, ed. AS Lefohn,pp. 325–59. Chelsea, MI: Lewis
69. Leonardi S, Langebartels C, Sandermann H. 1990. Fall exposure of beech trees (*Fagus sylvatica* L.) to ozone and simulated acidic mist: Immediate and post-treatment effects on whole plant physiology. In *Environmental Research with Plants in Closed Chambers*, ed. HD Payer, T Pfirrmann, P Mathy, Air Pollut. Res. Rep. EC 26, Brussels,pp. 369–80
70. Lippert M, Steiner K, Payer HD, Simons S, Langebartels C, Sandermann H. 1995. Assessing the impact of ozone on photosynthesis of European beech (*Fagus sylvatica* L.) in environmental chambers *Trees.* In press
71. Maccarrone M, Veldink GA, Vliegenthart JFG. 1992. Thermal injury and ozone stress affect soybean lipoxygenase expression. *FEBS Lett.* 309:225–30 [ISI][Medline]
72. Manning WJ, von Tiedemann A. 1995. Climate change: Potential effects of increased atmospheric carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. *Environ. Pollut.* 88:219–45 [ISI]
73. Matyssek R, Gunthardt-Goerg MS, Keller TH, Scheidegger CH. 1990. Impairment of gas exchange and structure in birch leaves (*Betula pendula*) caused by low ozone concentrations. *Trees* 5:5–13
74. McLaughlin SB. 1994. Forest declines: Some perspectives on linking processes and patterns. In *Plant Responses to the Gaseous Environment. Molecular, Metabolic and Physiological Aspects*, ed. RG Alscher, AR Wellburn,pp. 315–38. London: Chapman & Hall
75. Mehdy MC. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiol.* 105:467–72 [ISI]
76. Mehlhorn H, Seufert G, Schmidt A, Kunert KJ. 1986. Effect of SO_2 and O_3 on production of antioxidants in conifers. *Plant Physiol.* 82:336–38 [ISI]
77. Mehlhorn H, Wellburn AR. 1987. Stress ethylene formation determines plant sensitivity to ozone. *Nature* 327:417–18 [ISI]
78. Middleton JT. 1961. Photochemical air pollution damage to plants. *Annu. Rev. Phytopathol.*

12:431–48 [ISI]
79. Miller PR. 1992. Mixed conifer forests of the San Bernardino mountains, California. In *The Response of Western Forests to Air Pollution*, ed. RK Olson, D Binkley, M Böhm, Ecol. Stud.97:462–97. New York: Springer-Verlag
80. Nsolomo VR, Woodward S. 1994. Glucanohydrolase enzyme activity in embryos of Scots, Corsican and lodgepole pines infected in vitro with *Heterobasidion annosum*. *Eur. J. For. Pathol.* 24:144–53 [ISI]
81. Olson RK, Peterson DL, Böhm M. 1992. Summary, projections and recommendations. In *The Responses of Western Forests to Air Pollution*, ed. RK Olson, D Binkley, M Böhm, Ecol. Stud.97:501–21. Berlin: Springer-Verlag
82. Pearson M, Mansfield TA. 1994. Effects of exposure to ozone and water stress on the following season's growth of beech (*Fagus sylvatica* L.). *New Phytol.* 126:511–15 [ISI]
83. Pitcher LH, Brennan E, Hurley A, Dunsmuir P, Tepperman JM, Zilinskas BA. 1991. Overproduction of petunia chloroplastic copper zinc superoxide dismutase does not confer ozone tolerance in transgenic tobacco. *Plant Physiol.* 97:452–55 [ISI]
84. Polle A, Wieser G, Havranek WM. 1995. Quantification of ozone influx and apoplastic ascorbate content in needles of Norway spruce trees (*Picea abies* L., Karst) at high altitude. *Plant Cell Environ.* 18:681–88 [ISI]
85. Pye JM. 1988. Impact of ozone on the growth and yield of trees: A review. *J. Environ. Qual.* 17:347–60 [ISI]
86. Reddy GN, Arteca RN, Dai Y-R, Flores HE, Negm FB, Pell EJ. 1993. Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose biphosphate carboxylase/oxygenase in ozone-stressed potato foliage. *Plant Cell Environ.* 16:819–26 [ISI]
87. Reich PB. 1987. Quantifying plant response to ozone: a unifying theory. *Tree Physiol.* 3:63–91
88. Reinert RA. 1984. Plant response to air pollutant mixtures. *Annu. Rev. Phytopathol.* 22:421–42 [ISI]
89. Rich S. 1964. Ozone damage to plants. *Annu. Rev. Phytopathol.* 2:253–66
90. Rosemann D, Heller W, Sandermann H. 1991. Biochemical plant responses to ozone. II. Induction of stilbene biosynthesis in Scots pine (*Pinus sylvestris* L.) seedlings. *Plant Physiol.* 97:1280–86 [ISI]
91. Rowland-Bamford A, Borl AM, Lea PJ, Mansfield TA. 1989. The role of arginine decarboxylase in modulating the sensitivity of barley to ozone. *Environ. Pollut.* 61:95–106 [ISI]
92. Sandermann H, Heller W, Langebartels C. 1989. Early biochemical effects of air pollutants: detection and possible significance for forest trees. In *Proc. Int. Congr. For. Decline Res: State Knowledge Perspectives*, ed. B Ulrich, Friedrichshafen; Kernforschungszentrum Karlsruhe,pp. 517–25
93. Sandermann H, Langebartels C, Heller W. 1990. Ozon bei Pflanzen. Frühe und "Memory"-Effekte von Ozonstressbei Nadelbäumen. *UWSF-Z. Umweltchem. Oükotox.* 2:14–15
94. Sandermann H, Schmitt R, Heller W, Rosemann D, Langebartels C. 1989. Ozone-induced early biochemical reactions in conifers. In *Acid Deposition, Sources, Effects and Controls*, ed. JWS Longhurst,pp. 243–54. London: Br. Library
95. Sandermann H, Wellburn AS, Heath RL, ed.1996. *Ozone and Forest Decline: A Comparison of Controlled Chamber and Field Experiments*. Ecol. Stud. Berlin: Springer-Verlag. In press
96. Schlagnhaufer CD, Glick RE, Arteca RN, Pell EJ. 1995. Molecular cloning of an ozone-induced 1-aminocyclopropane-1-carboxylate synthase cDNA and its relationship with a loss of rbcS in potato *Solanum tuberosum* L) plants. *Plant Mol. Biol.* 28:93–103 [ISI][Medline]
97. Schmieden U, Wild A. 1995. The contribution of ozone to forest decline. *Physiol. Plant.* 94:371–78 [CrossRef][ISI]
98. Schmitt R, Sandermann H. 1990. Biochemical response of Norway spruce (*Picea abies* [L.] Karst.) towards 14-month exposure to ozone and acid mist. Part II. Effects on protein biosynthesis. *Environ. Pollut.* 64:367–73 [ISI]

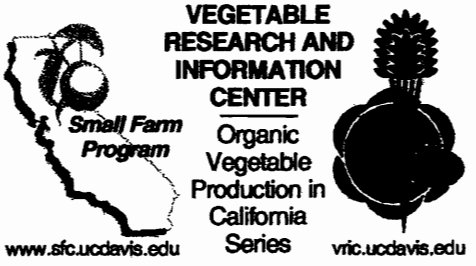
99. Schneiderbauer A, Back E, Sandermann H, Ernst D. 1995. Ozone induction of extensin mRNA in Scots pine, Norway spruce and European beech. *New Phytol.* 130:225–30 [ISI]
100. Schoeneweiss DF. 1975. Predisposition, stress and plant disease. *Annu. Rev. Phytopathol.* 13:193–211 [ISI]
101. Schraudner M, Ernst D, Langebartels C, Sandermann, H. 1992. Biochemical plant responses to ozone. III. Activation of the defense-related proteins ß-1,3-glucanase and chitinase in tobacco leaves. *Plant Physiol.* 99:1321–28 [ISI]
102. Schraudner M, Graf U, Langebartels C, Sandermann H. 1994. Ambient ozone can induce plant defense reactions in tobacco. *Proc. R. Soc. Edinburgh B* 102:55–61 [ISI]
103. Schraudner M, Langebartels C, Heinmöller P, Gäb S, Kettrup A, Sandermann H. 1995. Lipid oxidation products as potential signal molecules for ozone-induced plant responses? In *10th Int. Workshop Plant Membr. Biol., Regensburg, Abstr. S03*
104. Schraudner M, Langebartels C, Negrel J, Sandermann H. 1993. Plant defense reactions induced in tobacco by the air pollutant ozone. In *Mechanisms of Plant Defense Responses*, ed. B Fritig, M Legrand,pp. 286–90. Dordrecht: Kluwer
105. Sharma P, Borja D, Stougaard P, Lönneborg A. 1993. PR-proteins accumulating in spruce roots infected with a pathogenic *Phythium* sp. Isolate include chitinases, chitosanases and ß-1,3-glucanases. *Physiol. Mol. Plant Pathol.* 43:57–67 [ISI]
106. Sharma YK, Davis KR. 1994. Ozone-induced expression of stress-related genes in *Arabidopsis thaliana. Plant Physiol.* 105:1089–96 [ISI]
107. Sharma YK, Davis KR. 1995. Isolation of a novel *Arabidopsis* ozone-induced cDNA by differential display. *Plant Mol. Biol.* 29:91–98 [ISI][Medline]
108. Simons S. 1993. *Biochemische Effekte und Symptomentwicklung bei Buchen (Fagus sylvatica L.) und Nadelgehölzen unter realen und proportional erhöhten Ozonkonzentrationen.* PhD thesis. Univ. München
109. Smith WH. 1990. Air pollution and forests. In *Interactions Between Air Contaminants and Forest Ecosystems* Berlin: Springer-Verlag. 2nd ed.
110. Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG. 1995. Molecular genetics of plant disease resistance. *Science* 268:661–67 [ISI][Medline]
111. Steele CL, Lewinsohn E, Croteau R. 1995. Induced oleoresin biosynthesis in grand fir as a defense against bark beetles. *Proc. Natl. Acad. Sci. USA* 92:4164–68 [Abstract]
112. Telewski FW. 1992. Ethylene production by different age class Ponderosa and Jeffrey pine needles as related to ozone exposure and visible injury. *Trees* 6:195–98 [ISI]
113. Tenhaken R, Levine A, Brisson LF, Dixon RA, Lamb C. 1995. Function of the oxidative burst in hypersensitive disease resistance. *Proc. Natl. Acad. Sci. USA* 92:4158–63 [Abstract]
114. Thalmair M, Bauw G, Thiel S, Döhring T, Langebartels C, Sandermann H. 1996. Ozone and ultraviolet B effects on the defense-related proteins ß-1,3-glucanase and chitinase in tobacco. *J. Plant Physiol.* In press
115. Deleted in proof
116. Tingey DT, Standley C, Field RW. 1976. Stress ethylene evolution: A measure of ozone effects on plants. *Atmos. Environ.* 10:969–74 [ISI][Medline]
117. Treshow M, Anderson FK. 1989. *Plant Stress from Air Pollutants.* Chichester, UK: Wiley
118. Trost M. 1994. *Pinosylvin-3-O-methyl-transferase aus Kiefer (Pinus sylvestris L.): Reinigung, Charakterisierung und Induktion durch Ozon und Elicitor.* PhD thesis. Univ. München
119. Tuomainen J, Kangasjärvi J, Betz C, Ernst D, Langebartels C, Sandermann H. 1995. Ozone activation of ethylene biosynthesis proceeds via differential accumulation of transcripts for 1-aminocyclopropane-1-carboxylate synthase genes. In *Plant Growth Regulator Conference, Pittsburgh.* Abstr. No. 362
119A. United Nations Economic Comm. Eur.1995. Forest condition in Europe. Brussels: UN Econ. Comm.
119B. van Camp W, Willekens H, Bowler C, van Montagu M, Inzé D, et al.1994. Elevated levels of

superoxide dismutase protect transgenic plants against ozone damage. *Bio-Technology* 12:165–68
119C. von Tiedemann A, Pfähler B. 1994. Growth stage-dependent effects of ozone on the permeability for ions and non-electrolytes of wheat leaves in relation to the susceptibility to Septoria nodorum Berk. *Physiol. Mol. Plant Pathol.* 45:153–67
120. Wargo PM. 1975. Lysis of the cell wall of *Armillaria mellea* by enzymes from forest trees. *Physiol. Plant Pathol.* 5:99–105 [ISI]
121. Wegener A. 1995. *Ozon-induzierte Änderungen im Transkriptmuster von Kiefernkeimlingen (Pinus sylvestris L.): Charakterisierung beteiligter Gene*. PhD thesis. Univ. München
122. Whittaker JB. 1994. Interactions between insects and air pollutants. In *Plant Responses to the Gaseous Environment. Molecular, Metabolic and Physiological Aspects*, ed. RG Alscher, AR Wellburn,pp. 365–84. London: Chapman & Hall
123. Willekens H, van Camp W, Van Montagu M, Inzé D, Langebartels C, Sandermann H. 1994. Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* (L.). *Plant Physiol.* 106:1007–14 [ISI]
124. Deleted in proof
125. Woodbury PB, Laurence JA, Hudler GW. 1994. Chronic ozone exposure increases the susceptibility of hybrid *Populus* to disease caused by *Septoria musiva. Environ. Pollut.* 86:109–14 [ISI]
126. Yalpani N, Enyedi AJ, León J, Raskin J. 1994. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. *Planta* 193:372–76 [ISI]
127. Yin Z-H, Langebartels C, Sandermann H. 1994. Specific induction of ethylene biosynthesis in tobacco plants by the air pollutant, ozone. *Proc. R. Soc. Edinburgh B* 102:127–30 [ISI]
128. Zinser C. 1996. *Induktion der Gene der Zimtalkohol-Dehydrogenase und der Stilbensynthase durch Ozon und UV-B in der Kiefer (Pinus sylvestris L.)* PhD thesis. Univ. München

Full-text links to some of the citing articles in other journals:

- Riehl Koch, J., Scherzer, A. J., Eshita, S. M., Davis, K. R. (1998). Ozone Sensitivity in Hybrid Poplar Is Correlated with a Lack of Defense-Gene Activation. *Plant Physiol.* 118: 1243-1252 [Abstract] [Full Text]
- Coakley, S. M., Scherm, H., Chakraborty, S. (1999). CLIMATE CHANGE AND PLANT DISEASE MANAGEMENT. *Annu. Rev. Phytopathol.* 37: 399-426 [Abstract] [Full Text]
- Abarca, D., Roldan, M., Martin, M., Sabater, B. (2001). Arabidopsis thaliana ecotype Cvi shows an increased tolerance to photo-oxidative stress and contains a new chloroplastic copper/zinc superoxide dismutase isoenzyme. *J. Exp. Bot.* 52: 1417-1425 [Abstract] [Full Text]

WEED MANAGEMENT FOR ORGANIC CROPS



RICHARD SMITH, UC Cooperative Extension Farm Advisor, Monterey and Santa Cruz Counties; W. THOMAS LANINI, UCCE Weed Ecologist, UC Davis; MARK GASKELL, UCCE Farm Advisor, Santa Barbara and San Luis Obispo Counties; JEFF MITCHELL, UCCE Vegetable Crops Specialist, Kearney Agricultural Center, Parlier; STEVEN T. KOIKE, UCCE Farm Advisor, Monterey and Santa Cruz Counties; and CALVIN FOUCHE, UCCE Farm Advisor, San Joaquin County

Specific information on organic vegetable production practices in California is scarce, and growers need sound information to guide their management decisions. The Organic Vegetable Production in California Series is made up of publications written by Farm Advisors and Specialists from the University of California's Division of Agriculture and Natural Resources. Each publication addresses a key aspect of organic production practices applicable to all vegetable crops.

Weed management in organic vegetable production systems must involve the use of many techniques and strategies, all with the goal of achieving economically acceptable weed control and crop yields. Weeds can always be pulled or cut, but the question is simply how much time and money can a grower expend to reduce weed pressure. The more a grower is able to reduce weed pressure, the more economical it is to produce crops.

Ideally, growers would like to achieve a level of zero weeds on the farm. In practice, this may not be achievable, but any reduction in weeds and in the amount of weed seed or perennial propagules reaching the soil will make subsequent weed control operations less expensive. An understanding of what resources weeds require and why weeds are present in the first place is useful when you begin to formulate a control strategy.

For weeds to grow, they must have access to water, nutrients, and light. The first or biggest plant to occupy a site has a competitive advantage over later plants. The cultural practices used in vegetable production (for instance, using transplants, pre-emergent flaming of weeds, pre-germination of weeds) often provide opportunities for the crop to gain that advantage. The goal is for the crop to outcompete the weeds, reducing the availability of resources to the weeds. If you can give the crop a competitive advantage through organically acceptable techniques, subsequent hand weeding operations and costs can be minimized. The following are common techniques available to organic growers to manage weeds in vegetable production operations.

CULTURAL PRACTICES

Water Management

Effective water management is key to controlling weeds in a vegetable operation. There are a number of ways that careful irrigation management can help you reduce weed pressure on your crops:

- *Pre-germination of weeds.* In pre-germination, irrigation or rainfall germinates weed seeds just before the cash crop is planted. The newly germinated weeds can be killed by light cultivation or flaming. Pre-germination should occur as close as possible to the date of planting to ensure that changes in weather conditions do not have an opportunity to change the spectrum of weeds (cool vs. warm season) in the field.
- *Planting to moisture.* Another technique similar to pre-germination is planting to moisture. After weeds are killed by cultivation, the top 2 to 3 inches of soil are allowed to dry and form a dust mulch. At planting, the dust mulch is pushed away and large-seeded vegetables such as corn or beans can be planted into the zone of soil moisture. These seeds can germinate, grow, and provide partial shading of the soil surface without supplemental irrigations that would otherwise provide for an early flush of weeds.
- *Buried drip irrigation.* Drip tape buried below the surface of the planting bed can provide moisture to the crop and minimize the amount of moisture that is available to weeds closer to the surface. If properly managed, this technique can provide significant weed control during periods without rain.

Crop Competition

Crops that grow vigorously can often outcompete weeds. Weeds grow best where competition is sparse; for instance, between rows or in gaps in a crop stand. Crops that are well adapted to their planted areas are often better competitors since they will tend to occupy a site rapidly. If you increase the density of the crop by decreasing the in-row spacing or by reducing the space

University of California • Division of Agriculture and Natural Resources
Publication 7250

between rows you will improve the crop's competitiveness. A close-planted crop will close the canopy more rapidly, reducing the weeds' ability to compete. Some crops (including tomato, bean, and sweet corn) compete effectively with weeds if given an early competitive advantage, while others (including onion and garlic) never establish a competitive canopy. The use of transplants give the crop an advantage over the weeds because transplants enter the field larger and more developed that the weeds. With help from subsequent cultivation or hand weeding operations, a transplanted crop can develop a full canopy and crowd out weeds.

Reducing the Weed Seed Bank

Practices that reduce the production of weed seed also reduce weed pressure and can help keep weeding costs down over time. In an ideal situation, no weed would be allowed to go to seed. Any that do go to seed can aggravate weed problems for many years to come. As an example, common purslane seed has been shown to remain viable for over 20 years in the soil, and black mustard seed survives for over 40 years. The longevity of weed seed, together with the large numbers of seed produced by individual plants (100,000 per plant for large purslane or barnyardgrass plants), can lead to the long-term build-up of enormous seed banks in the soil. If you make it a policy to remove weeds prior to seed production, you can reduce weed pressure in subsequent seasons.

Careful weed management during the season is important, but it must be followed up with off-season weed control as well. Short-season crops such as lettuce can provide opportunities for frequent cultivations and a rapid turnover of crops on the land, thus reducing some weeds' ability to mature and set seed. Highly competitive cover crops can also smother weeds. If you carry weeds with seed out of the field for disposal, you can also significantly reduce the seed bank. Each of these techniques can help growers minimize weed problems, and that translates to lower hoeing bills.

CULTIVATION

Cultivation is probably the most widely used weed control method in organic vegetable operations. Mechanical cultivation uproots or buries weeds. Burial works best on small weeds, while larger weeds are better controlled by destruction of the root-shoot connection or by slicing, cutting, or turning the soil to eliminate the root system's contact with the soil. Cultivation is effective against almost all weeds, with the exception of certain parasitic forms such as dodder. Effective cultivation must precisely and accurately target weed-growth areas, and so requires good land preparation and bed shaping. Shallow cultivation usually is best, since it brings fewer weed seeds to the surface. Level beds allow more precise depth of tillage. Cultivation requires relatively dry soil; subsequent irrigations should be delayed long enough to prevent the weeds from re-rooting. In addition, cultivations should be carried out early enough in the growth cycle to kill weeds such as burning nettle and purslane that set seed early in the growth cycle.

The goal of cultivation is to cut out weeds as close to the seed row as possible without disturbing the crop. In most cases, precision cultivation can take care of the weeds on over 80 percent of the bed. The remaining weeds must be removed from the seed row by hand or using other mechanical means. Here are some common cultivation implements:

- Various knives, L-shaped and crescent-shaped beet hoes, and sweeps can be used to cut and uproot weeds on bed tops within 1 to 3 inches of the crop row. These can sometimes be combined with reversed-disc hillers that cut vining weeds such as field bindweed and move soil away from the crop row. Disc hillers are often reversed as crops get larger so they will throw soil around the base of the crop plant to bury weeds. Rolling cultivators (Lillistons) have become common cultivating implements for a number of crops. A rolling cultivator's primary purpose is to uproot weeds, but it can also be adjusted to throw soil and bury weeds in the crop row.
- A new generation of cultivators has been developed to remove weeds from between the seed rows, and in some situations from the seed row itself. Spring-tine cultivators, torsion Bezzerides cultivators, Budding in-row weeders, and brush hoes all can be adjusted to take out weeds between seed rows or close to the seed row. Some of these cultivators can remove weeds from the seed row itself in fields planted to tough-stemmed crops like cotton. Computer-guided cultivators that can distinguish the crop from weeds are under development and may soon be able to remove weeds selectively from within the seed row.
- Cultivation implements are often mounted on sleds for accurate, close cultivation in row crops. Guide wheels, cone wheels, and other devices are also used, but in general these are less precise than sleds. Various implements can be attached to these guidance setups to remove weeds.

Even the best cultivators will not eliminate all weeds, so some hand weeding is often necessary. It is easier to remove weeds by hand while they are small. The proper timing of cultivations depends on the speed of weed growth: in spring a two- to three-week period

is about right; in the fall or winter, longer periods between cultivations may be appropriate. The practice and experience of the grower are important factors in effective cultivation.

Weeds that compete with the crop early in the crop cycle may be more damaging to crop yield than weeds that establish later in the season. Late-season weeding may disturb the crop's root system or knock off flowers or fruit, which may reduce yields. Obviously, late season cultivations to reduce weed seed production must be weighed against the potential for yield loss.

FLAMERS

Flamers are useful for weed control. Propane-fueled models are the most common. Flaming does not burn weeds to ashes; rather, the flame rapidly raises the temperature of the weeds to more than 130°F; The sudden increase in temperature causes the plants' cell sap to expand, rupturing the cell walls. For greatest flaming efficiency, weeds must have fewer than two true leaves. Grasses are difficult to impossible to kill by flaming because the growing point is protected underground. After flaming, weeds that have been killed rapidly change from a glossy appearance to a duller appearance.

Flaming can be used prior to crop emergence in slow-germinating vegetables such as peppers, carrots, onions, and parsley. In addition, flaming can be used postemergence on crops such as young onion and garlic or as a directed treatment to the base of tougher crops (such as sweet corn) when they are 12 or more inches tall. Postemergence flaming does adversely impact the yield of the crop, so its use must be weighed against the potential damage the weeds might cause. Typically, flaming can be applied at a speed of 3 to 5 mph through fields, although this depends on the heat output of the unit being used. Best results are obtained under windless conditions, as winds can prevent the heat from reaching the target weeds. The efficiency of flaming is greatly reduced if moisture from dew or rain is present on the plants. Early morning and early evening are the best times to observe the flame patterns and adjust the equipment.

STERILIZATION

Soil sterilization in organic agriculture involves the use of heat or naturally generated biocides to kill weeds. Heat is applied as steam or by soil solarization. In steam sterilization, the steam is injected into the soil to kill weed seeds. The large quantities of fuel and water required by this technique make it an expensive choice, so its use is limited to small acreages of high-value horticultural crops or landscaping. Ozone is a naturally occurring biocide that is being researched for use as a soil sterilant. The ozone is generated mechanically and then injected into the soil. Ozone injection shows promise as a weed-reduction tool, but it is unclear at this time whether this technique will be considered an organically acceptable practice.

Soil solarization involves placing a clear plastic mulch over a tilled, moist soil to allow the solar energy to heat the soil and kill germinating weed seeds. To be most effective, solarization should be performed during summer and fall periods of maximum solar radiation exposure. These are the steps involved in solarization:

1. Prepare the area as if for planting, making certain that the area is as level as possible.
2. Irrigate to field capacity and then place the clear plastic mulch over the area, covering the edges of the plastic with soil to hold them in place. Irrigation and pressing the plastic down for a tight fit against the soil reduces the amount of airspace and ensures good transfer of heat. Any tears in the plastic should be promptly repaired or they will allow heat to escape.
3. Allow the plastic to remain in place for a minimum of 4 weeks (length of treatment depends on the amount of solar radiation).
4. Remove the plastic mulch and immediately plant the crop. You can remove the plastic or use it as a plastic mulch for the subsequent crop by burning holes in the film and transplanting into those holes. Solarization generally yields successful results in the Central and Imperial Valleys, but is less reliable in coastal valleys.

MULCHES

Mulching is another weed control method. A mulch blocks light, preventing weed germination and growth. The materials that can be used as mulches are varied, and include plastics and organic materials such as municipal yard waste, wood chips, straw, hay, sawdust, and newspaper. To be effective, a mulch needs to block all light to the weeds, and some mulch materials require a thicker application layer that others to accomplish this.

Plastic mulches vary in thickness from 1.5 mil to about 4 mils. The most common color for weed-control plastic is black, since it completely blocks light. More recently, a clear, infrared-transmitting (IRT) plastic has been introduced. The IRT plastic blocks certain wavelengths of light but allows others to pass, and that heats the soil better for early-season crop growth. Plastic mulches are generally placed on the beds and their edges covered with dirt to keep them from blowing

away. Drip irrigation is needed to get moisture to the crop under the plastic mulch. Certain weeds, including nutsedge, are able to penetrate the plastic and so are not completely controlled by plastic mulches. Other weeds can grow in the openings provided for crops. Further problems with plastic mulches include difficulties keeping them in place under windy conditions, disposal after the crop is harvested (they are not as yet recyclable), and their cost (including the cost of needed drip irrigation).

Organic mulches such as municipal yard waste, straw, hay, and wood chips must be maintained in a layer 4 or more inches thick in order to block out light. Organic mulches break down over time, and the original thickness typically reduces by 60 percent after one year. Coarse green waste works better as a mulch. Organic mulches are mostly used for permanent crops, landscaping, and noncrop areas, although they are also very effective for transplanted vegetables.

Organic mulches can be grown in place. Plants used to produce organic mulches include cereals, clovers, vetches, and fava beans. These mulches (or living mulches, as they are sometimes termed) must die or be killed before or shortly after crop planting in order to avoid excessive competition with the crop. Living mulches were developed in the eastern United States, but are currently being tested on various fruiting vegetables in California (see UC ANR Publication 7248, Soil Management and Soil Quality for Organic Crops).

BENEFICIAL ORGANISMS

Weeds are subject to disease and insect attacks just as crops are. Most biological control of weeds occurs in range or noncrop areas. As a result, biological control has little relevance for vegetable growers.

Geese have been used for weed control in tree, vine, and certain row crops. Most types of geese will graze weeds, but Chinese weeder geese are considered the best for row crops. Chinese weeder geese are smaller than other types and tend to walk around delicate crop plants rather than over them. Geese prefer grass species and will eat other weeds and crops only if they are hungry and all of the grasses are gone. If confined, geese will even dig up and eat Johnsongrass and bermudagrass rhizomes. You must take care to avoid placing geese near any grass crops such as corn, sorghum, or small grains, as this is their preferred food. Fruiting vegetables, such as tomatoes when they begin to color, might also be vulnerable, so geese would have to be removed from tomato fields at certain times. Geese require drinking water, shade during hot weather, and protection from dogs and other predators.

CHEMICAL CONTROL

Herbicides are chemicals that kill or suppress plants by affecting their physiological processes. Only a limited number of herbicides are organically acceptable, and these include contact materials such as acetic acid (vinegar), citric acid, and solutions of sodium nitrate, as well as a preemergent material, corn gluten. Herbicides can be used for selective weed control by manipulating the timing of application or placement of material, or by exploiting differences in the chemical tolerances of the crop and the target weed. Weeds that emerge before the crop can be killed with contact herbicides (acetic acid, etc.). These herbicides kill plants that have emerged, but have no residual activity on those that emerge later. Corn gluten is a preemergence material that is applied to the soil to suppress weeds as they germinate. Currently, the efficacy of these organically acceptable herbicides is marginal at best.

OTHER PUBLICATIONS IN THIS SERIES

Organic Certification, Farm Production Planning, and Marketing, UC ANR Publication 7247

Soil Management and Soil Quality for Organic Crops, UC ANR Publication 7248

Soil Fertility Management for Organic Crops, UC ANR Publication 7249

Insect Pest Management for Organic Crops, UC ANR Publication 7251

Plant Disease Management for Organic Crops, UC ANR Publication 7252

RESOURCES

Books

Bowman, G. (ed.) 1997. *Steel in the fields: A farmer's guide to weed management tools.* Beltsville, MD: Sustainable Agriculture Network.

Crampton, B. 1974. *Grass in California.* Berkeley: University of California Press.

Whitson, T. D. (ed.) 1992. *Weeds of the West.* Newark, CA: Western Society of Weed Science.

Videos

Bellinder, R., and J. Colquhoun. 1998. *New Tools for Mechanical Weed Control in Vegetables.* Ithaca, NY: Cornell University.

Grubinger, V., and M. J. Else. 1997. *Vegetable Farmers and Their Weed-control Machines.* Burlington, VT: University of Vermont and University of Massachusetts Extension.

Lanini, T. 1993. *Cultural Weed Control in Vegetables.* Davis, CA: University of California Division of Agriculture and Natural Resources, Program V93-E.

Websites

California Weed Science Society:
http://www.cwss.org/

University of California Integrated Pest Management Program: An excellent source of information on weed control in specific crops:
http://www.ipm.ucdavis.edu/

Weed Science Program at UC Davis: an excellent source of photographs and biological information on California weeds, with many links to other sites:
http://veghome.ucdavis.edu/weedsci/www/welcome.html

Western Weed Science Society:
http://www.wsweedscience.org/

Equipment

Contact local farm equipment dealers and distributors of cultivation equipment. In addition, consult the annual *American Vegetable Grower Magazine Buyers' Guide.*

An electronic version of this publication is available on the University of California ANR Communication Services website at http://anrcatalog.ucdavis.edu.

Publication 7250

To simplify information, trade names of products have been used. No endorsement of named products is intended, nor is criticism implied of similar products that are not mentioned.

pr-4/00-WJC

Journal of Food Protection, Vol. 64, No. 6, 2001, Pages 777–782

Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Lactobacillus leichmannii* by Combinations of Ozone and Pulsed Electric Field

RAGIP UNAL, JIN-GAB KIM, AND AHMED E. YOUSEF*

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Parker Hall, Columbus, Ohio 43210, USA

MS 00-329: Received 19 September 2000/Accepted 4 December 2000

ABSTRACT

Pulsed electric field (PEF) and ozone technologies are nonthermal processing methods with potential applications in the food industry. This research was performed to explore the potential synergy between ozone and PEF treatments against selected foodborne bacteria. Cells of *Lactobacillus leichmannii* ATCC 4797, *Escherichia coli* O157:H7 ATCC 35150, and *Listeria monocytogenes* Scott A were suspended in 0.1% NaCl and treated with ozone, PEF, and ozone plus PEF. Cells were treated with 0.25 to 1.00 μg of ozone per ml of cell suspension, PEF at 10 to 30 kV/cm, and selected combinations of ozone and PEF. Synergy between ozone and PEF varied with the treatment level and the bacterium treated. *L. leichmannii* treated with PEF (20 kV/cm) after exposure to 0.75 and 1.00 μg/ml of ozone was inactivated by 7.1 and 7.2 $\log_{10}$ CFU/ml, respectively; however, ozone at 0.75 and 1.00 μg/ml and PEF at 20 kV/cm inactivated 2.2, 3.6, and 1.3 $\log_{10}$ CFU/ml, respectively. Similarly, ozone at 0.5 and 0.75 μg/ml inactivated 0.5 and 1.8 $\log_{10}$ CFU/ml of *E. coli*. PEF at 15 kV/cm inactivated 1.8 $\log_{10}$ CFU/ml, and ozone at 0.5 and 0.75 μg/ml followed by PEF (15 kV/cm) inactivated 2.9 and 3.6 $\log_{10}$ CFU/ml, respectively. Populations of *L. monocytogenes* decreased 0.1, 0.5, 3.0, 3.9, and 0.8 $\log_{10}$ CFU/ml when treated with 0.25, 0.5, 0.75, and 1.0 μg/ml of ozone and PEF (15 kV/cm), respectively; however, when the bacterium was treated with 15 kV/cm, after exposure to 0.25, 0.5, and 0.75 μg/ml of ozone, 1.7, 2.0, and 3.9 $\log_{10}$ CFU/ml were killed, respectively. In conclusion, exposure of *L. leichmannii*, *E. coli*, and *L. monocytogenes* to ozone followed by the PEF treatment showed a synergistic bactericidal effect. This synergy was most apparent with mild doses of ozone against *L. leichmannii*.

Consumer demand for minimally processed foods or freshlike products with reduced use of chemical additives has led to substantial developments in nonthermal food preservation technologies *(12)*. Pulsed electric field (PEF), high hydrostatic pressure, high voltage arc discharge, oscillating magnetic field pulses, intense pulsed light, UV light, ultrasound, X rays, and microwave processing are some of the emerging food processing technologies. The food industry is also interested in potent antimicrobial agents (e.g., ozone) as alternatives to conventional sanitizers.

PEF treatment is one of the emerging food processing technologies. PEF treatment effectively inactivates bacteria and yeast in orange juice, milk, skim milk, yogurt, liquid egg, and pea soup *(23)*; however, PEF is ineffective against bacterial and mold spores. Sale and Hamilton *(6, 18, 19)* demonstrated that microbial inactivation was nonthermal and PEF exerted its lethal effect by causing irreversible loss of membrane function.

Ozone is a very potent antimicrobial agent, capable of destroying bacteria, bacterial spores, protozoan cysts, and viruses at relatively low concentration and in short exposure time when applied to the pure cell suspensions *(5, 8, 21)*. Presence of organic matter (e.g., food components) increases the need for higher doses of ozone; these high doses are detrimental to the sensory attributes of foods. Depending on the commodities and the conditions of ozonation, more than 5 log of food microflora can be eliminated *(8, 9)*.

In today's food industry, the use of a combination of factors is becoming a popular food preservation technique. When liquid whole eggs are thermally pasteurized, addition of antimicrobials enhances the safety of the product *(4, 17)*. Combining selected hurdles in a food may provide an additive or a synergistic effect against pathogens of concern. Since PEF and ozone both act on cell membranes, synergistic effects might be expected. Therefore, the purpose of this research was to explore potential enhancement of inactivation of selected foodborne bacteria by PEF when they are pretreated with ozone.

MATERIALS AND METHODS

Microorganisms. *Lactobacillus leichmannii* ATCC 4797, *Escherichia coli* O157:H7 ATCC 35150, and *Listeria monocytogenes* Scott A were obtained from the culture collection at the Department of Food Science and Technology, The Ohio State University, Columbus, Ohio. Frozen stock cultures were transferred into Trypticase soy broth supplemented with 0.6% yeast extract (TSBYE) (Difco Laboratories, Detroit, Mich.) for *E. coli* O157:H7 and *L. monocytogenes*, and lactobacilli MRS broth (Difco) for *L. leichmannii*. After two successive transfers under similar conditions, bacterial cells were harvested by centrifugation at 5,000 × *g* for 10 min (Sorvall RC-5B Superspeed Centrifuge, DuPont Instruments, Wilmington, Del.). The cells were washed twice with

* Author for correspondence. Tel: 614-292-7814; Fax: 614-292-0218; E-mail: yousef.1@osu.edu.

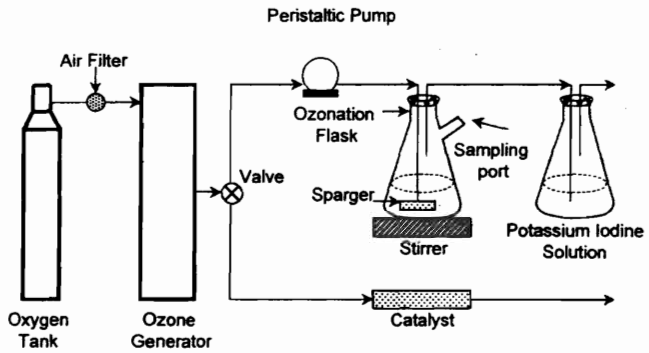


FIGURE 1. *Inactivation of microorganisms by ozone; experimental setup (modified from Kim (8)).*

0.85% NaCl and suspended in 0.1% NaCl solution before PEF and ozone treatments. The inoculum sizes were estimated by measuring absorbance at 600 nm with calculation of approximate counts from the standard curve for absorbance versus bacterial counts. The initial microbial inocula for use in the experiments were approximately 10^8 CFU/ml for *E. coli* O157:H7 and *L. monocytogenes* and 10^7 for *L. leichmannii.*

Preparation of ozone demand-free glassware and water. All glassware was washed with a mild detergent and thoroughly rinsed with hot tap water and deionized water. The glassware was then autoclaved and dried to remove volatile organic compounds. Ozone demand-free water was prepared by ozonating deionized water. The water was then autoclaved at 121°C for 15 min to remove the residual ozone and stored in sealed ozone demand-free glass containers until needed *(10).*

Ozone production. Ozone (1.1 mM, approximately 2.5% [vol/vol], approximately 3.65% [wt/wt]) was produced from purified, extra dry oxygen by an ozone generator (U.S. Filter/Polymetrics T-816, San Jose, Calif.). A stainless steel sparger with 10-μm pore size (Solvent Inlet Filter, Fisher Scientific, Fair Lawn, N.J.) was used for bubbling ozone in cell suspension. The amount of ozone produced from the generator was determined by the method described by Shechter *(22),* and the applied ozone dose for the reaction was calculated. All experimental work with ozone gas was done in a chemical hood. Excess ozone was destroyed by a heated catalyst or neutralized by diverting the gas stream into a reservoir containing 2% potassium iodine solution.

Inactivation by ozone. A batch-type reaction system (Fig. 1) was used for the ozone inactivation studies as follows. Cells suspended in 0.1% NaCl solution (200 ml) were treated with gaseous ozone (ozone concentration in the gas mixture, 52.8 mg/liter; flow rate, 11.4 ml/min) for 5, 10, 15, and 20 s; the resulting ozone doses (0.25, 0.5, 0.75, or 1.00 μg/ml of ozone cell suspension, respectively) were calculated as follows:

mg of ozone gas per ml of sample

= [ozone concentration (mg/liter) in the gas mixture

× flow rate (ml/min) × contact time (min)]

÷ [volume of cell suspension (ml) × 1,000]

All test solutions were continuously mixed during the treatment at 100 rpm with a stirrer. The temperature during the ozone treatment was 22°C.

PEF treatment. Cells suspended in 0.1% NaCl (electrical conductivity, 0.2 S/m) were treated with PEF. A PEF treatment

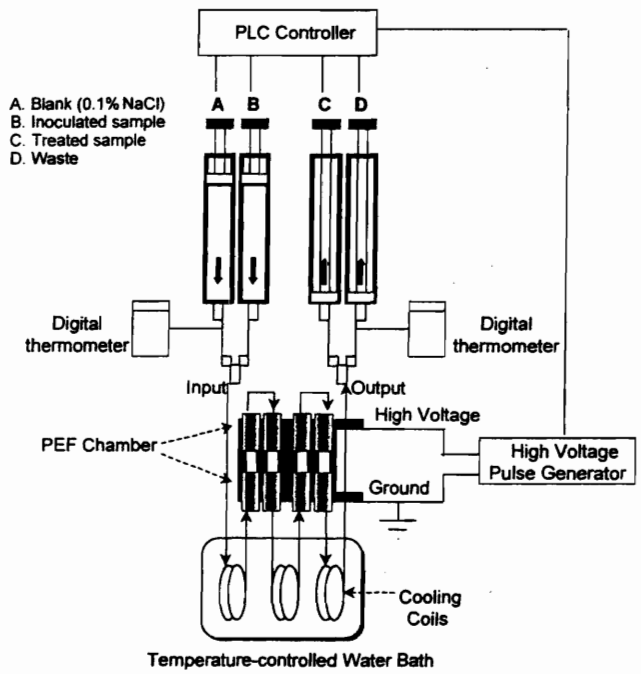


FIGURE 2. *PEF bench-scale processing unit and fluid-sample handling system, adapted from the* User's Manual of OSU-4C Pulsed Electric Field System and Fluid/Sample Handling Operation *(OSU-PEF Team, Food Science and Technology, The Ohio State University, Columbus). Figure is not drawn to scale.*

unit with four cofield flow electrodes in series (OSU-PEF Team, The Ohio State University) capable of handling 60-ml sample volumes (Fig. 2) was used throughout the study. Equipment and processing parameters were as follows: bipolar wave form; pulse frequency, 1,000 Hz; pulse duration time, 3 μs; number of chambers, 4; field strengths, 0 to 30 kV/cm; electrode diameter, 0.23 cm; electrodes gap, 0.292 cm; and product flow rate, 1 ml/s. The sample received approximately 12 pulses per chamber and total treatment time (145.6 μs) was calculated as follows:

total treatment time (μs)

= pulse duration (μs) × pulse frequency (pulse/s)

× number of chambers × (π/4)

× electrode diameter^2 (cm^2)

× gap distance (cm)/product flow rate (cm^3/s)

Input voltages and the pulse waveform were monitored with a two-channel digital oscilloscope (Tektronix TDS340A, Beaverton, Ore.). A programmable logic controller was used to control the flow rate of treated samples (Fig. 2). Temperature of the sample before the PEF treatment was 22°C, and it did not exceed 35°C after PEF treatments with 5, 10, 15, and 20 kV/cm; however, the temperature exceeded 35°C when electric field intensities were more than 20 kV/cm. A water bath, set at 22°C, was used to cool the PEF-treated samples initially and after the second and fourth treatment chamber. The temperature at the inlet of the first and outlet of the fourth treatment chamber was recorded with a dual channel digital thermometer (Tektronix DTM920). The cleaning and disinfecting of the system was done with a 500-ppm chlorine solution and rinsed with sterile 0.1% NaCl.

In combination treatments, cells were exposed to ozone, as indicated earlier, and treated immediately with PEF. The ozone-treated control (cell suspension remaining in reservoir B, Fig. 2)

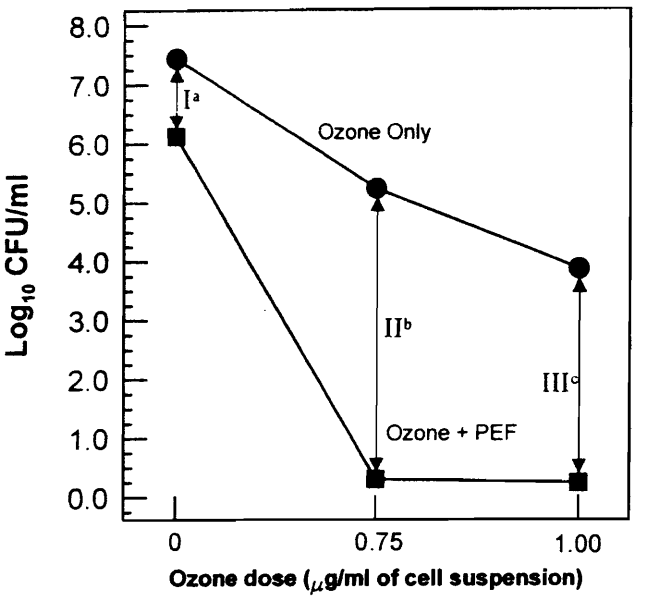


FIGURE 3. *Inactivation of* L. leichmannii *ATCC 4797 by combinations of ozone (0.75 and 1.00 μg/ml of cell suspension) and PEF (20 kV/cm). Results are averages of two independent trials. Differences between ozone and the combination treatment (segments I through III) with different superscripts are significantly different (P < 0.05).* —●—, *ozone only;* —■—, *ozone and PEF. Populations that survived the combined treatments were sometimes nondetectable by the plating procedure; therefore, estimated counts are reported.*

and ozone plus PEF–treated suspension (in reservoir C, Fig. 2) were sampled at the same time.

Enumeration of viable and injured cells. The cell suspension from each treatment was serially diluted with 0.85% NaCl. From the selected dilution, 100 μl in duplicate was surface plated simultaneously on nonselective and selective agar plates. The nonselective medium for *L. monocytogenes* and *E. coli* O157:H7 was Trypticase soy agar (Difco) supplemented with 0.6% yeast extract (TSAYE). The selective agar media were Trypticase soy agar supplemented with 5% NaCl (including 0.5% NaCl in the original formula) and violet red bile agar (VRBA; Difco) *(7)*, respectively. The plates were incubated at 37°C for 48 h and used to determine viable (CFU in TSAYE agar) and noninjured (corresponding CFU in selective agar) cells in the populations. The difference between the viable and noninjured cells was used to estimate injured survivors. *L. leichmannii* was enumerated on lactobacilli MRS (Difco) agar after incubation at 37°C for 48 h.

Statistical analysis. All experiments were run twice. Populations inactivated after the treatments were analyzed using the MINITAB statistical program (Minitab Inc., State College, Pa.). One-way analysis of variance was performed to compare the effect of the treatments. Synergistic effects between the ozone and the PEF treatments for the inactivation of *L. leichmannii, E. coli* O157:H7, and *L. monocytogenes* were evaluated (Figs. 3 through 5). The differences in inactivation between ozone-treated and ozone-PEF–treated cell suspensions (e.g., segments II or III, Fig. 3) were compared using Tukey's range test with those between the untreated and PEF-treated samples (segment I). If segment II or III was significantly greater than segment I, a significant synergistic effect was represented. If segment II or III was not significantly different from segment I, an additive effect was indicated.

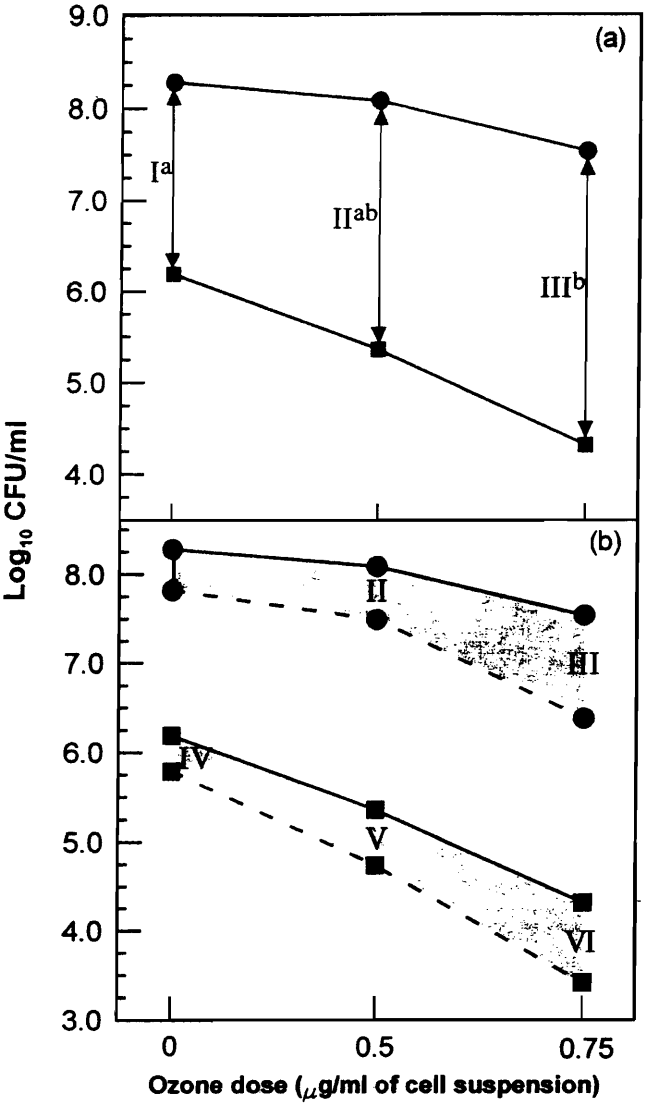


FIGURE 4. *Inactivation (a) and injury (b, shaded area) of* E. coli *O157:H7 by combinations of ozone (0.5 and 0.75 μg/ml of cell suspension) and PEF (15 kV/cm). Results are averages of two independent trials. Differences between ozone and the combination treatment (segments I through III, Fig. 4a) with different superscripts are significantly different (P < 0.05).* —●—, *ozone-treated cells plated on TSAYE;* —■—, *ozone and PEF–treated cells plated on TSAYE;* – – –●– – –, *ozone-treated cells plated on VRBA;* – – –■– – –, *ozone and PEF–treated cells plated on VRBA.*

RESULTS

According to an earlier study *(8)*, when bacterial cells are treated with different concentrations of ozone, mild lethal doses cause the greatest degree of injury. Therefore, we screened different ozone doses and electric field intensities and chose intermediate ozone doses and an electric field intensity for synergistic studies so that the combination would still result in countable cell populations and a measurable synergy (Table 1). Based on the screening study with ozone and PEF, ozone doses (0.75 and 1.00 μg of ozone per ml of cell suspension) and 20-kV/cm electric field intensity were chosen for the synergistic effects of ozone and PEF against *L. leichmannii* ATCC 4797. For *E.*

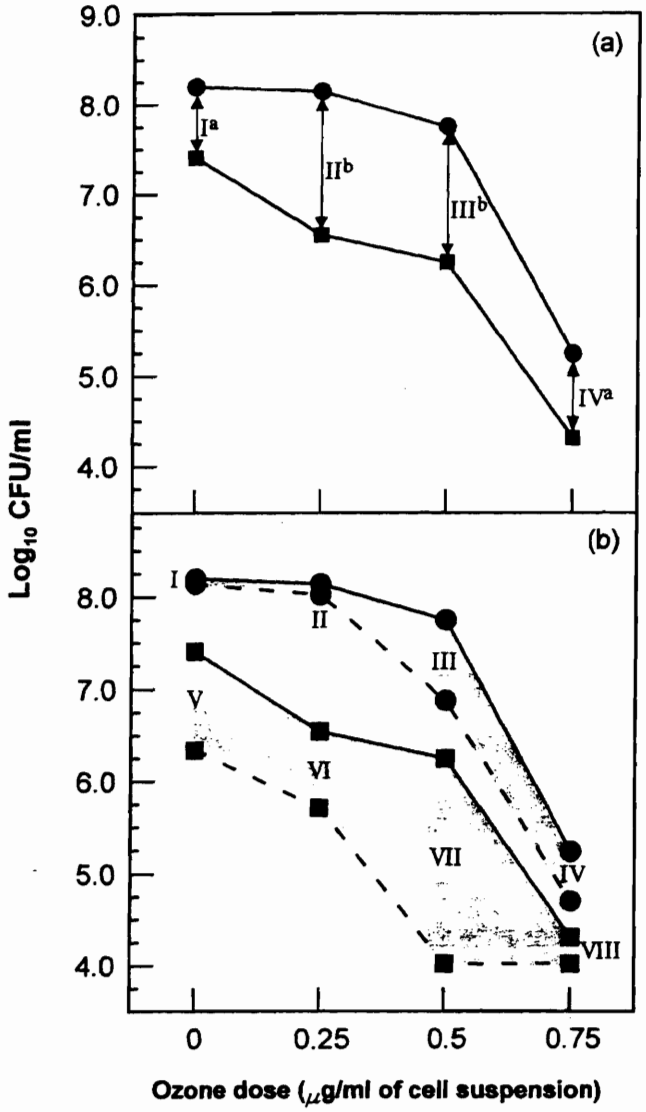


FIGURE 5. *Inactivation (a) and injury (b, shaded area) of* L. monocytogenes *Scott A by combinations of ozone (0.25, 0.50, and 0.75 μg/ml of cell suspension) and PEF (15 kV/cm). Results are averages of two independent trials. Differences between ozone and the combination treatment (segments I through IV, Fig. 5a) with different superscripts are significantly different (P < 0.05).* —●—, *ozone-treated cells plated on TSAYE;* —■—, *ozone and PEF–treated cells plated on TSAYE;* – – –●– – –, *ozone-treated cells plated on Trypticase soy agar plus 5% NaCl;* – – –■– – –, *ozone and PEF–treated cells plated on Trypticase soy agar plus 5% NaCl.*

coli O157:H7, 15-kV/cm electric field intensity and 0.5- and 0.75-μg/ml ozone cell suspension were selected. Ozone doses of 0.25, 0.5, and 0.75 μg/ml and a PEF of 15 kV/cm were selected for the synergistic study to inactivate *L. monocytogenes*.

Inactivation by ozone and PEF. Ozone treatment at 0.75 and 1.00 μg/ml significantly ($P < 0.05$) reduced the population of *L. leichmannii* (approximately 3×10^7) by 2.2 and 3.6 log, respectively (Table 1). PEF treatments (10 to 30 kV/cm) inactivated *L. leichmannii* by 0.7 to 4.8 log CFU/ml (Table 1), and all reductions in cell populations were statistically significant ($P < 0.05$). The temperature monitored during processing of samples by PEF (10, 15, and 20 kV/cm) did not exceed 35°C; however, the temperature exceeded 35°C when electric field intensities were higher than 20 kV/cm. Therefore, these high field intensities were not used in the synergistic study.

Ozone treatments at 0.25 and 0.5 μg/ml did not decrease the population of *E. coli* O157:H7 significantly ($P > 0.05$); however, significant population decreases were observed when 0.75 and 1.00 μg/ml of ozone were applied ($P < 0.05$). Cell inactivation increased as ozone dose increased. When *E. coli* O157:H7 was subjected to PEF treatments at 10 to 20 kV/cm, significant ($P < 0.05$) decreases in counts (0.5 to 4.5 $\log_{10}$ CFU/ml) were observed (Table 1).

The ozone dose at 0.25 μg/ml did not significantly decrease the population of *L. monocytogenes* ($P > 0.05$), but significantly greater inactivation was observed at an ozone cell suspension of 0.5 μg/ml or more ($P < 0.05$). As in the case of *L. leichmannii* and *E. coli* O157:H7, inactivation of *L. monocytogenes* also was enhanced as the applied ozone dose increased. Electric field intensities of 10 to 20 kV/cm significantly ($P < 0.05$) decreased the population of *L. monocytogenes* by 0.70 to 1.1 $\log_{10}$ CFU/ml (Table 1).

Synergistic effects of ozone and PEF. Treatment of *L. leichmannii* by PEF (20 kV/cm) after exposure to 0.75 and 1.0 μg/ml of ozone significantly ($P < 0.05$) decreased the counts by 7.1 and 7.2 $\log_{10}$ CFU/ml, respectively. In Figure 3, the difference of inactivation resulting from ozone only and ozone combined with PEF is indicated by segment I, II, or III. Segments II and III are significantly greater ($P < 0.05$) than segment I; therefore, the lethality of PEF treatment was synergistically enhanced by ozone treatment.

TABLE 1. *Inactivation of* L. leichmannii *ATCC 4797 (3×10^7 CFU/ml),* E. coli *O157:H7 (2×10^8 CFU/ml), and* L. monocytogenes *Scott A (2×10^8 CFU/ml) suspended in 0.1% NaCl after treatment with ozone and PEF*[a]

	Reduction in $\log_{10}$ CFU/ml								
	Ozone (μg/ml of cell suspension)				PEF (kV/cm)				
Microorganisms	0.25	0.50	0.75	1.00	10	15	20	25	30
L. leichmannii	ND	ND	2.2 (0.09)	3.6 (0.04)	0.7 (0.03)	1.1 (0.02)	1.3 (0.02)	2.8 (0.02)	4.8 (0.00)
E. coli	0.1 (0.28)	0.3 (0.08)	1.8 (0.06)	3.7 (0.29)	0.5 (0.09)	1.8 (0.03)	4.5 (0.06)	ND	ND
L. monocytogenes	0.1 (0.06)	0.5 (0.08)	3.0 (0.00)	3.9 (0.03)	0.7 (0.00)	0.8 (0.05)	1.1 (0.01)	ND	ND

[a] Data points are averages of two trials and are presented as mean (SD). ND, not determined.

However, synergy of PEF with ozone was greater at lower (II) than higher (III) ozone dose.

Combining PEF treatment (15 kV/cm) with a 0.5-μg/ml ozone cell suspension caused an additive but not synergistic effects against *E. coli* O157:H7, since segment II is not significantly greater than segment I ($P > 0.05$) (Fig. 4a); however, segment III was significantly ($P < 0.05$) greater than segment I, indicating that ozone at 0.75 μg/ml and PEF at 15 kV/cm synergistically decreased *E. coli* O157:H7 count by 3.6 $\log_{10}$ CFU/ml.

The PEF treatment (15 kV/cm) combined with 0.25 and 0.5 μg/ml of ozone cell suspension synergistically inactivated the population of *L. monocytogenes* Scott A by 1.7 and 2 $\log_{10}$ CFU/ml, since segments II and III were significantly greater than segment I ($P < 0.05$) (Fig. 5a). The combination of 0.75 μg/ml of ozone with 15 kV/cm of electric field strength inactivated 3.9 $\log_{10}$ CFU/ml; however, there is no evidence of additive or synergistic effect at this combination, since segment IV was significantly smaller than segment I ($P > 0.05$).

Cell injury by ozone, PEF, and their combinations. Cell suspensions of *E. coli* O157:H7 were first subjected to one of two ozone concentrations (0.5 or 0.75 μg/ml of cell suspension), then treated with PEF (15 kV/cm) and enumerated for total survivors on a nonselective medium (TSAYE) and for noninjured survivors on a selective medium (VRBA) (Fig. 4b). In general, cell death increased with an increase in ozone dose (Fig. 4, solid lines), and an appreciable proportion of cell population was injured at 0.5 and 0.75 μg/ml of ozone cell suspension (Fig. 4b, shaded area). PEF treatment alone did not injure *E. coli* O157:H7 significantly, since segment IV was not significantly greater than segment I ($P > 0.05$) (Fig. 4b). Cell injury increased from 0.2 to 0.6 $\log_{10}$ CFU/ml as the applied ozone dose increased from 0 to 0.75 μg/ml of ozone as indicated by the shaded area in Fig. 4b. Combination of ozone at 0.5 and 0.75 μg/ml with PEF (15 kV/cm), compared with ozone only, did not significantly injure *E. coli* O157:H7, since segments V and VI were not significantly greater than segments II and III, respectively ($P > 0.05$) (Fig. 4b).

The pattern of cell injury of *L. monocytogenes* was somewhat different from that of *E. coli* O157:H7. Very small portions of the cell population were injured by low-ozone dose (0.25 μg/ml of cell suspension); however, PEF treatment injured 90% of the cell population (Fig. 5b). As cell inactivation increased with increase of ozone dose, the degree of injury also increased. The maximum cell injury was obtained at 0.5 μg/ml of ozone cell suspension (Fig. 5b). Treatment with 0.5 μg/ml of ozone followed by PEF injured about 99% of the population (approximately 2 $\log_{10}$ CFU/ml); however, combination of a high dose of ozone (0.75 μg/ml) with PEF did not result in measurable cell injury.

DISCUSSION

We demonstrated that ozone and PEF are effective against *L. leichmannii* ATCC 4797, *E. coli* O157:H7 ATCC 35150, and *L. monocytogenes* Scott A. Combinations of ozone and PEF treatments caused lethality that was greater than the sum of lethalities of ozone and PEF applied individually. These synergistic effects between ozone and PEF were substantial under most of the experimental conditions. Synergy between ozone and PEF may have resulted from cell injury during the ozone treatment and rapid inactivation of injured cells when they were subsequently treated with PEF. Therefore, cells treated with ozone, PEF, and ozone and PEF were plated onto selective and nonselective agar media. Results (Figs. 4 and 5) show that *E. coli* O157:H7 was injured minimally at 0.5 μg/ml of ozone, but appreciable injury was observed at 0.75 μg/ml of ozone (Fig. 4b). This may explain the synergistic effect of 0.75 μg/ml of ozone in combination with PEF and lack of synergy when the combination included a treatment with 0.5 μg/ml of ozone. PEF alone did not cause appreciable cell injury.

Injury of *L. monocytogenes* was most prominent at 0.5 μg/ml of ozone (Fig. 5b, segment III). Interestingly, synergy between ozone and PEF in inactivating this pathogen also was most apparent at the intermediate concentrations of ozone (0.25 and 0.5 μg/ml of ozone) (Fig. 5a). Therefore, injury by ozone may have sensitized *L. monocytogenes* to PEF, resulting in a synergistic effect during the combined treatment. *L. monocytogenes* that was treated with PEF only exhibited cell injury (segment V compared with segment I; Fig. 5b). Additionally, the combination of ozone (0.5 μg/ml) and PEF synergistically enhanced cell injury. This may indicate that pretreatment of *L. monocytogenes* with ozone enhanced the ability of PEF to cause cell injury. These data may also indicate that ozone and PEF injured *Listeria* cells at different sites. Kim *(8)* evaluated injury of *Pseudomonas fluorescens*, *E. coli* O157:H7, *Leuconostoc mesenteroides*, and *L. monocytogenes* by ozone. He found that the degree of injury varied depending on the microorganism, ozone concentration, and exposure time. He concluded that maximum injury occurred at ozone concentrations producing limited cell inactivation.

Ohshima et al. *(14)* treated *E. coli* with combinations of PEF and ozone. They reported that simultaneous application of PEF and ozone synergistically inactivated *E. coli*; however, their data showed that ozone and PEF combinations had an additive rather than a synergistic effect.

PEF technology can be used as a terminal treatment or in combination with other hurdles such as nisin *(3, 15)*, benzoic and sorbic acids *(11)*, low pH *(24)*, and ozone *(14)*. For optimized food preservation systems by hurdle technology, the mode of action of each hurdle should be understood. According to Zimmermann *(25)*, an external electric field induces an additional transmembrane potential larger than the natural membrane potential. When the overall membrane potential reaches a critical value of approximately 1 V, dielectric rupture takes place. This rupture causes disorientation in the membrane structure, resulting in formation of pores and increasing the permeability of cell membrane. Depending on the electrical field strength, pulse duration, and pulse number, permeabilization can be either reversible or irreversible *(2)*.

In general, ozone molecules rapidly react with bacteria, leading to death. Ozone is believed to cause the oxidation

of lipids on the cell envelope of bacteria *(13, 21)*. Further oxidation leads to leakage of intracellular cell contents and damage of genetic material and death of microorganisms *(16, 20)*.

Although ozone is highly effective against microorganisms in pure cell suspensions, it is unlikely to be used directly in foods. Organic constituents of food compete with microorganisms for ozone, and thus high doses of this agent may be needed for effective elimination of microorganisms. These levels of ozone may alter sensory attributes and adversely affect the acceptability of foods; however, ozone may be conveniently used to disinfect the surfaces of fruits such as apples and oranges. Application of ozone on the surface of fruits can significantly reduce the microbial flora *(1)*. When juice extracted from ozone-washed fruits is treated with PEF, a synergistic effect may be observed.

In summary, combinations of ozone and PEF synergistically enhanced the inactivation of a spoilage microorganism, *L. leichmannii,* and two emerging foodborne pathogens, *E. coli* O157:H7 and *L. monocytogenes.* Our results may assist the recent efforts in developing PEF and other nonthermal pasteurization methods for liquid foods.

REFERENCES

1. Achen, M. 2000. Efficacy of ozone in inactivating *Escherichia coli* O157:H7 in pure cell suspensions and on apples. M.S. thesis. The Ohio State University, Columbus.
2. Benz, R., and U. Zimmermann. 1981. The sealing process of lipid bilayers after reversible electrical breakdown. Biochim. Biophys. Acta 640:169–178.
3. Calderon-Miranda, M. L., G. V. Barbosa-Canovas, and B. G. Swanson. 1999. Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin. Int. J. Food Microbiol. 51:19–30.
4. Delves-Broughton, J., G. C. Williams, and S. Wilkinson. 1992. The use of bacteriocin, nisin, as a preservative in pasteurized liquid whole eggs. Lett. Appl. Microbiol. 15:133–136.
5. Giese, A. C., and E. Christensen. 1954. Effects of ozone on organisms. Physiol. Zool. 27:101–115.
6. Hamilton, W. A., and A. J. H. Sale. 1967. Effects of high electric fields on microorganisms. II: mechanism of action of the lethal effect. Biochim. Biophys. Acta 148:789–800.
7. Kalchayanand, N., A. Sikes, C. P. Dunne, and B. Ray. 1998. Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. Food Microbiol. 15:207–214.
8. Kim, J. G. 1998. Ozone as an antimicrobial agent in minimally processed foods. Ph.D. thesis. The Ohio State University, Columbus.
9. Kim, J. G., A. E. Yousef, and G. W. Chism. 1999. Use of ozone to inactivate microorganisms on lettuce. J. Food Saf. 19:17–34.
10. Korich, D. G., J. R. Mead, M. S. Madore, N. A. Sinclair, and C. R Sterling. 1990. Effects of ozone, chlorine dioxide, chlorine and monochloramine on *Cryptosporidium parvum* oocyst viability Environ. Microbiol. 56:1423–1428.
11. Liu, X., A. E. Yousef, and G. W. Chism. 1997. Inactivation of *Escherichia coli* O157:H7 by the combination of organic acids and pulsed electric field. J. Food Saf. 16:287–299.
12. Mertens, B., and D. Knorr. 1992. Developments of nonthermal processes for food preservation. Food Technol. 46:124–133.
13. Murray, R. G., S. Pamela, and H. E. Elson. 1965. Location of mucopeptide of selection of the cell wall of *E. coli* and other gram-negative bacteria. Can. J. Microbiol. 11:547–560.
14. Ohshima, T., K. Sato, H. Terauchi, and M. Sato. 1997. Physical and chemical modifications of high-voltage pulse sterilization. J. Electrostatics 42:159–166.
15. Pol, I. E., H. C. Mastwijk, P. V. Bartels, and E. J. Smid. 2000. Pulsed-electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*. Appl. Environ. Microbiol. 66:428–430.
16. Prat, R., C. Nofre, and A. Cier. 1968. Effects de l'hypochloride de sodium, de l'ozone et des radiations ionisontes dur les constituants pyrimidiques d'*Escherichia coli*. Ann. Inst. Pasteur Paris 114:595–607.
17. Roberts, T. A. 1989. Combination of antimicrobials and processing methods. Food Technol. 1:156–163.
18. Sale, A. J. H., and W. A. Hamilton. 1967. Effects of high electric fields on microorganisms, I: killing of bacteria and yeasts. Biochim. Biophys. Acta 148:781–788.
19. Sale, A. J. H., and W. A. Hamilton. 1968. Effects of high electric fields on microorganisms. III: lysis of erythrocytes and protoplasts. Biochim. Biophys. Acta 163:37–43.
20. Scott, D. B. M. 1975. The effect of ozone on nucleic acids and their derivatives, p. 226–240. *In* W. J. Blogoslawski and R. G. Rice (ed.), Aquatic applications of ozone. International Ozone Institute, Syracuse, N.Y.
21. Scott, D. B. M., and E. C. Lesher. 1963. Effect of ozone on survival and permeability of *Escherichia coli*. J. Bacteriol. 85:567–576.
22. Shechter, H. 1973. Spectrophotometric method for determination of ozone in aqueous solutions. Water Res. 7:729–739.
23. Vega-Mercado, H., O. Martin-Belloso, B. L. Qin, F. J. Chang, M. M. Gongora-Nieto, G. V. Barbosa-Canovas, and B. G. Swanson. 1997. Non-thermal food preservation: pulsed electric fields. Trends Food Sci. 8:151–157.
24. Vega-Mercado, H., U. R. Pothakamury, F. J. Chang, G. V. Barbosa-Canovas, and B. G. Swanson. 1996. Inactivation of *Escherichia coli* by combining pH, ionic strength and pulsed electric fields hurdles. Food Res. Int. 29:117–121.
25. Zimmermann, U. 1986. Electrical breakdown, electropermeabilization and electrofusion. Rev. Physiol. Biochem. Pharmacol. 105:175–256.

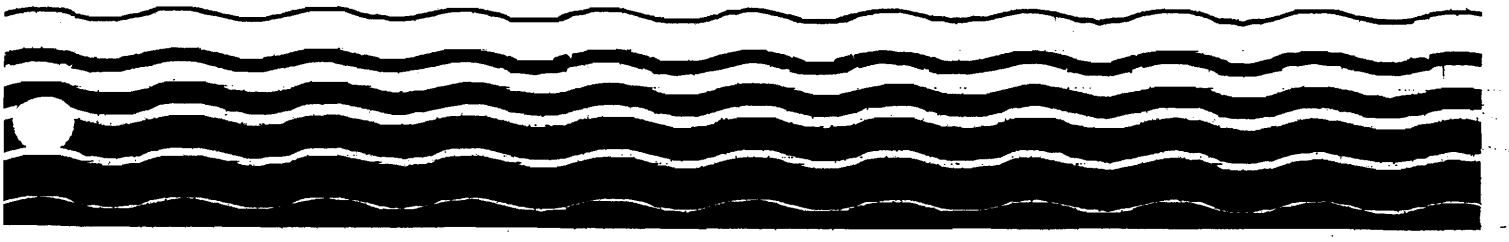
United States
Environmental Protection
Agency

Office of Water
(4607)

EPA 815-R-99-014
April 1999

EPA

Alternative Disinfectants and Oxidants Guidance Manual



#disinfect

Table of Contents Executive Summary 1. Introduction 2. Applicability of Disinfection Profiling and Benchmarking	
3. Creating a Profile: Data Requirements and Calculations	
4. Calculating the Benchmark 5. Using the Benchmark 6. Alternative Disinfection Benchmark 7. References	
Appendix A: History Appendix B: Log Inactivation Methods Appendis C: Ct Values for Inactivations Achieved by Various Disinfectants Appendix D: Determination of Contact Time Appendix E: Using the Regression Method	

Enhanced Coagulation and Enhanced Precipitative Softening Guidance Manual
EPA-815-R-99-012, May 1999

Manual	

Guidance Manual for Compliance with the Interim Enhanced Surface Water Treatment Rule: Turbidity Provisions
EPA-815-R-99-010, April 1999

Technical Guidances for Implementation of the Microbial and Disinfection Byproducts Rules

These guidance manuals support the Interim Enhanced Surface Water Treatment Rule and the Stage 1 Disinfectants/Disinfection Byproducts Rule. The manuals will aid EPA, State agencies and affected public water systems in implementing the two interrelated rules, and will help to ensure that implementation among these groups is consistent. Unless otherwise noted, files are less than 800k.

Alternative Disinfectants and Oxidants Guidance Manual
EPA 815-R-99-014, April 1999

Complete Document (1247 kB)			
	Cover, Table of Contents		
Chp. 1	Introduction		
Chp. 2	Disinfectant Use in Water Treatment		
Chp. 3	Ozone		
Chp. 4	Chlorine Dioxide		
Chp. 5	Potassium Permanganate		
Chp. 6	Chloramines		
Chp. 7	Peroxone (Ozone/Hydrogen Peroxide)		
Chp. 8	Ultraviolet Radiation		
Chp. 9	Combined Disinfectants		
App. A	Summary of Disinfectant Usage in the U.S.		
App. B	Selected Costs of Alternative Disinfection Systems		

Disinfection Profiling and Benchmarking Guidance Manual
EPA-815-R-99-013, August 1999

3. OZONE

Ozone was first used for drinking water treatment in 1893 in the Netherlands. While being used frequently in Europe for drinking water disinfection and oxidation, it was slow to transfer to the United States. In 1987, the Los Angeles Aqueduct Filtration Plant was placed in service and now treats up to 600 mgd of drinking water. In 1991, approximately 40 water treatment plants each serving more than 10,000 people in the United States utilized ozone (Langlais et al., 1991). This number has grown significantly, with Rice (in press) reporting that as of April 1998, 264 operating plants in the United States use ozone. Most of these facilities are small: 149 plants are below 1 mgd.

Ozone is used in water treatment for disinfection and oxidation. Early application of ozone in the United States was primarily for non-disinfection purposes such as color removal or taste and odor control. However, since the implementation of the SWTR and proposal of the DBP rule, ozone usage for primary disinfection has increased in the United States.

3.1 Ozone Chemistry

Ozone exists as a gas at room temperature. The gas is colorless with a pungent odor readily detectable at concentrations as low as 0.02 to 0.05 ppm (by volume), which is below concentrations of health concern. Ozone gas is highly corrosive and toxic.

Ozone is a powerful oxidant, second only to the hydroxyl free radical, among chemicals typically used in water treatment. Therefore, it is capable of oxidizing many organic and inorganic compounds in water. These reactions with organic and inorganic compounds cause an ozone demand in the water treated, which should be satisfied during water ozonation prior to developing a measurable residual.

Ozone is sparingly soluble in water. At 20°C, the solubility of 100 percent ozone is only 570 mg/L (Kinman, 1975). While ozone is more soluble than oxygen, chlorine is 12 times more soluble than ozone. Ozone concentrations used in water treatment are typically below 14 percent, which limits the mass transfer driving force of gaseous ozone into the water. Consequently, typical concentrations of ozone found during water treatment range from <0.1 to 1mg/L, although higher concentrations can be attained under optimum conditions.

Basic chemistry research (Hoigné and Bader, 1983a and 1983b; Glaze et al., 1987) has shown that ozone decomposes spontaneously during water treatment by a complex mechanism that involves the generation of hydroxyl free radicals. The hydroxyl free radicals are among the most reactive oxidizing agents in water, with reaction rates on the order of 10^{10} - 10^{13} M^{-1} s^{-1}, approaching the diffusion control rates for solutes such as aromatic hydrocarbons, unsaturated compounds, aliphatic alcohols, and formic acid (Hoigné and Bader, 1976). On the other hand, the half-life of hydroxyl free radicals is on the order of microseconds, therefore concentrations of hydroxyl free radicals can never reach levels above 10^{-12} M (Glaze and Kang, 1988).

As shown in Figure 3-1 ozone can react by either or both modes in aqueous solution (Hoigné and Bader, 1977):

- Direct oxidation of compounds by molecular ozone ($O_{3(aq)}$).
- Oxidation of compounds by hydroxyl free radicals produced during the decomposition of ozone.

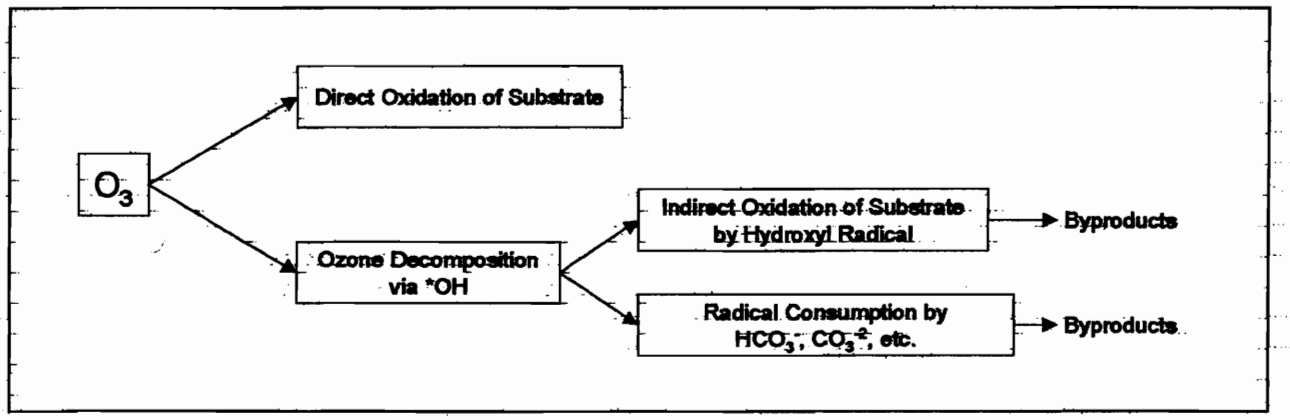


Figure 3-1. Oxidation Reactions of Compounds (Substrate) During Ozonation of Water

The two oxidation pathways compete for substrate (i.e., compounds to oxidize). The direct oxidation with aqueous ozone is relatively slow (compared to hydroxyl free radical oxidation) but the concentration of aqueous ozone is relatively high. On the other hand, the hydroxyl radical reaction is fast, but the concentration of hydroxyl radicals under normal ozonation conditions is relatively small. Hoigné and Bader (1977) found that:

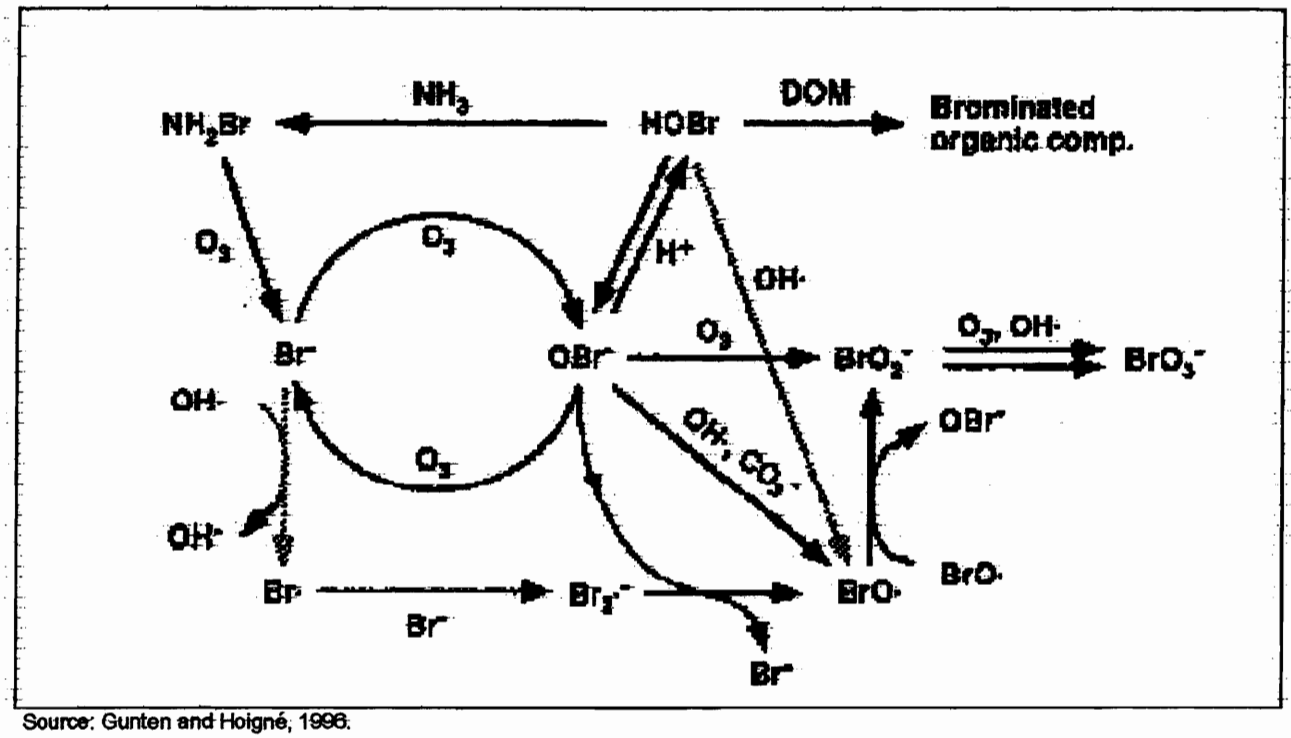
- Under acidic conditions, the direct oxidation with molecular ozone is of primary importance; and
- Under conditions favoring hydroxyl free radical production, such as high pH, exposure to UV, or addition of hydrogen peroxide, the hydroxyl oxidation starts to dominate.

This latter mechanism is used in advanced oxidation processes such as discussed in Chapter 7, Peroxone, to increase the oxidation rates of substrates.

The spontaneous decomposition of ozone occurs through a series of steps. The exact mechanism and reactions associated have not been established, but mechanistic models have been proposed (Hoigné and Bader, 1983a and 1983b; Glaze, 1987). It is believed that hydroxyl radicals forms as one of the intermediate products, and can react directly with compounds in the water. The decomposition of ozone in pure water proceeds with hydroxyl free radicals produced as an intermediate product of ozone decomposition, resulting in the net production of 1.5 mole hydroxyl free radicals per mole ozone.

In the presence of many compounds commonly encountered in water treatment, ozone decomposition forms hydroxyl free radicals. Ozone demands are associated with the following:

- Reactions with natural organic matter (NOM) in the water. The oxidation of NOM leads to the formation of aldehydes, organic acids, and aldo- and ketoacids (Singer, 1992).
- Organic oxidation byproducts. Organic oxidation byproducts are generally more amenable to biological degradation and can be measured as assimilable organic carbon (AOC) or biodegradable dissolved organic carbon (BDOC).
- Synthetic organic compounds (SOCs). Some SOCs can be oxidized and mineralized under favorable conditions. To achieve total mineralization, hydroxyl radical oxidation should usually be the dominant pathway, such as achieved in advanced oxidation processes.
- Oxidation of bromide ion. Oxidation of bromide ion leads to the formation of hypobromous acid, hypobromite ion, bromate ion, brominated organics, and bromamines (see Figure 3-2).
- Bicarbonate or carbonate ions, commonly measured as alkalinity, will scavenge the hydroxyl radicals and form carbonate radicals (Staehelin et al., 1984; Glaze and Kang, 1988). These reactions are of importance for advanced oxidation processes where the radical oxidation pathway is predominant.



Source: Gunten and Hoigné, 1996.

Figure 3-2. Reaction of Ozone and Bromide Ion Can Produce Bromate Ion and Brominated Organics

3.2 Ozone Generation

3.2.1 Ozone Production

Because ozone is an unstable molecule, it should be generated at the point of application for use in water treatment. It is generally formed by, combining an oxygen atom with an oxygen molecule (O_2):

$$3O_2 \Leftrightarrow 2O_3$$

This reaction is endothermic and requires a considerable input of energy.

Schönbein (Langlais et < biblio >) first discovered synthetic ozone through the electrolysis of sulfuric acid. Ozone can be produced several ways, although one method, corona discharge, predominates in the ozone generation industry. Ozone can also be produced by irradiating an oxygen-containing gas with ultraviolet light, electrolytic reaction and other emerging technologies as described by Rice (1996).

Corona discharge, also known as silent electrical discharge, consists of passing an oxygen-containing gas through two electrodes separated by a dielectric and a discharge gap. Voltage is applied to the electrodes, causing an electron flowthrough across the discharge gap. These electrons provide the energy to disassociate the oxygen molecules, leading to the formation of ozone. Figure 3-3 shows a basic ozone generator.

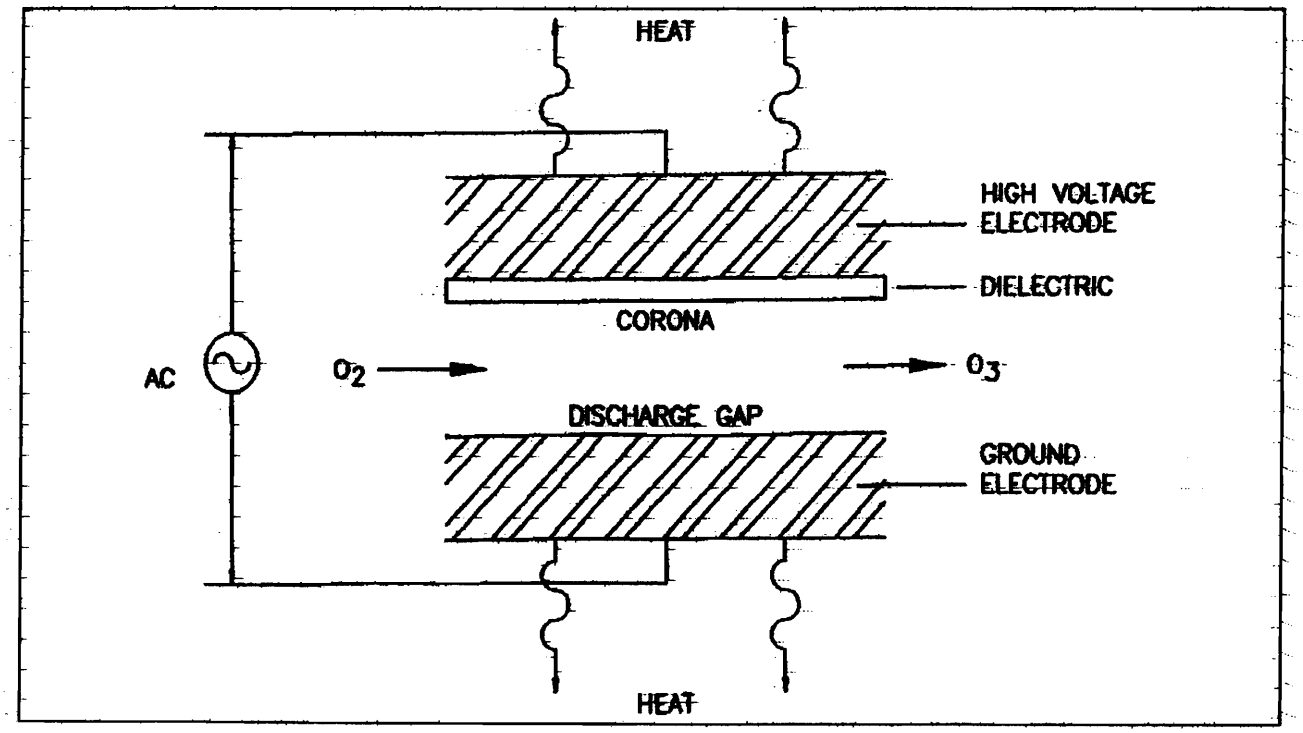


Figure 3-3. Basic Ozone Generator

3.2.2 System Components

As shown in Figure 3-4, ozone water treatment systems have four basic components: a gas feed system, an ozone generator, an ozone contactor, and an off-gas destruction system. The gas feed system provides a clean, dry source of oxygen to the generator. The ozone contactor transfers the ozone-rich gas into the water to be treated, and provides contact time for disinfection (or other reactions). The final process step, off-gas destruction, is required as ozone is toxic in the concentrations present in the off-gas. Some plants include an off-gas recycle system that returns the ozone-rich off-gas to the first contact chamber to reduce the ozone demand in the subsequent chambers. Some systems also include a quench chamber to remove ozone residual in solution.

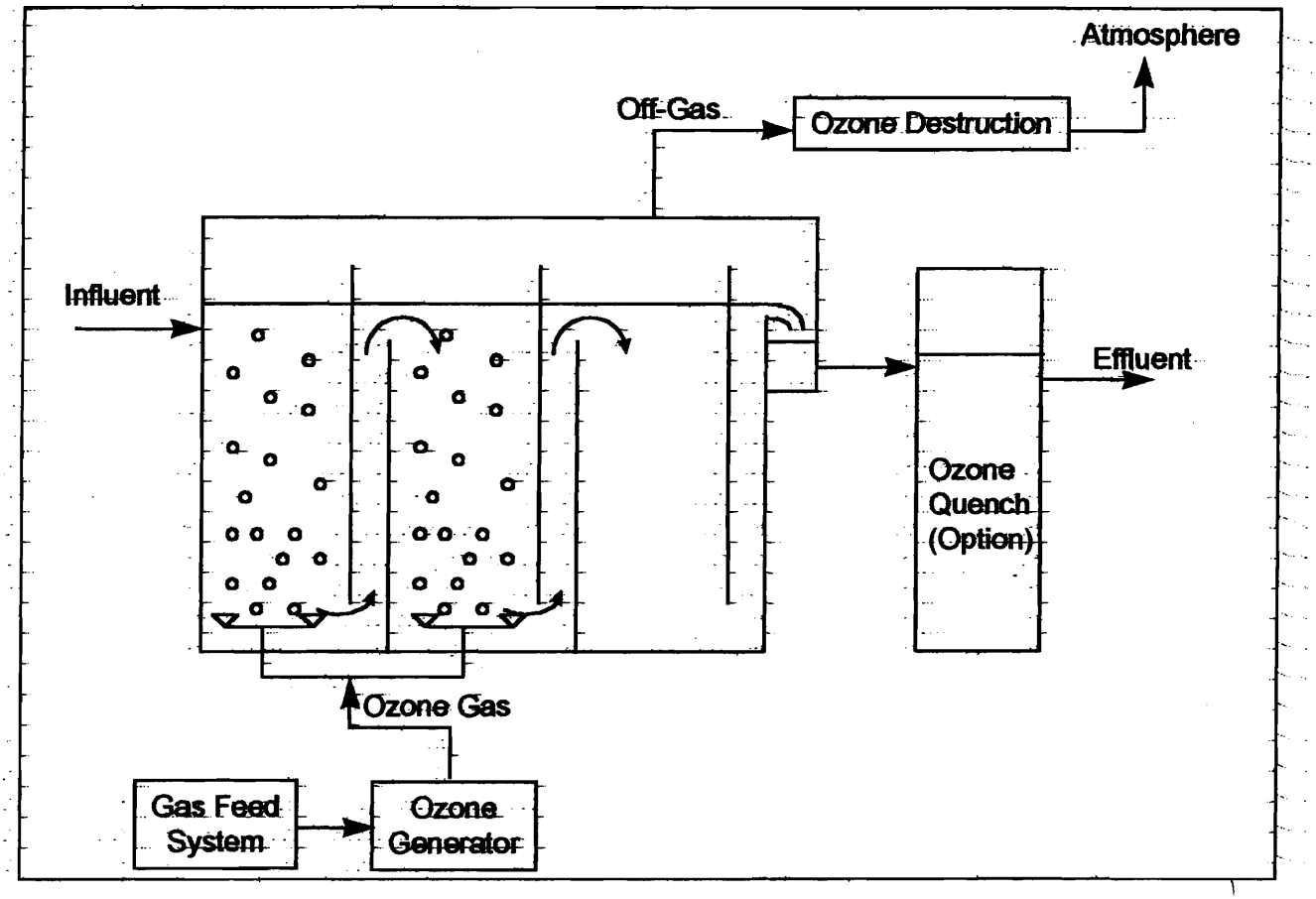


Figure 3-4. Simplified Ozone System Schematic

3.2.2.1 Gas Feed Systems

Ozone feed systems are classified as using air, high purity oxygen or mixture of the two. High purity oxygen can be purchased and stored as a liquid (LOX), or it can be generated on-site through either a cryogenic process, with vacuum swing adsorption (VSA), or with pressure swing adsorption (PSA). Cryogenic generation of oxygen is a complicated process and is feasible only in large systems. Pressure swing adsorption is a process whereby a special molecular sieve is used under pressure to

selectively remove nitrogen, carbon dioxide, water vapor, and hydrocarbons from air, producing an oxygen rich (80–95 percent O_2) feed gas. The components used in pressure swing adsorption systems are similar to high pressure air feed systems in that both use pressure swing molecular absorption equipment. Low pressure air feed systems use a heat reactivated desiccant dryer.

Oxygen Feed Systems - Liquid oxygen feed systems are relatively simple, consisting of a storage tank or tanks, evaporators to convert the liquid to a gas, filters to remove impurities, and pressure regulators to limit the gas pressure to the ozone generators.

Air Feed Systems - Air feed systems for ozone generators are fairly complicated as the air should be properly conditioned to prevent damage to the generator. Air should be clean and dry, with a maximum dew point of -60° C (-80° F) and free of contaminants. Air preparation systems typically consist of air compressors, filters, dryers, and pressure regulators. Figure 3-5 is a schematic of large scale air preparation system.

Particles greater than 1 μm and oil droplets greater than 0.05 μm should be removed by filtration (Langlais et al., 1991). If hydrocarbons are present in the feed gas, granular activated carbon filters should follow the particulate and oil filters. Moisture removal can be achieved by either compression or cooling (for large-scale system), which lowers the holding capacity of the air, and by desiccant drying, which strips the moisture from the air with a special medium. Desiccant dryers are required for all air preparation systems. Large or small particles and moisture cause arcing which damages generator dielectrics.

Typically, desiccant dryers are supplied with dual towers to allow regeneration of the saturated tower while the other is in service. Moisture is removed from the dryer by either an external heat source or by passing a fraction (10 to 30 percent) of the dried air through the saturated tower at reduced pressure. Formerly, small systems that require only intermittent use of ozone , a single desiccant tower is sufficient, provided that it is sized for regeneration during ozone decomposition time.

Air preparation systems can be classified by the operating pressure: ambient, low (less than 30 psig), medium, and high (greater than 60 psig) pressure. The distinguishing feature between low and high pressure systems is that high pressure systems can use a heatless dryer. A heatless dryer operates normally in the 100 psig range, rather than the 60 psig range. Rotary lobe, centrifugal, rotary screw, liquid ring, vane, and reciprocating compressors can be used in air preparation systems. Table 3-1 lists the characteristics of many of these types of compressors.

Reciprocating and liquid ring compressors are the most common type used in the United States, particularly in small systems, the former because the technology is so prevalent and the latter because liquid ring compressors do not need aftercoolers. Air receivers are commonly used to provide variable air flow from constant volume compressors. Oil-less compressors are used in modern systems to avoid hydrocarbons in the feed gas (Dimitriou, 1990).

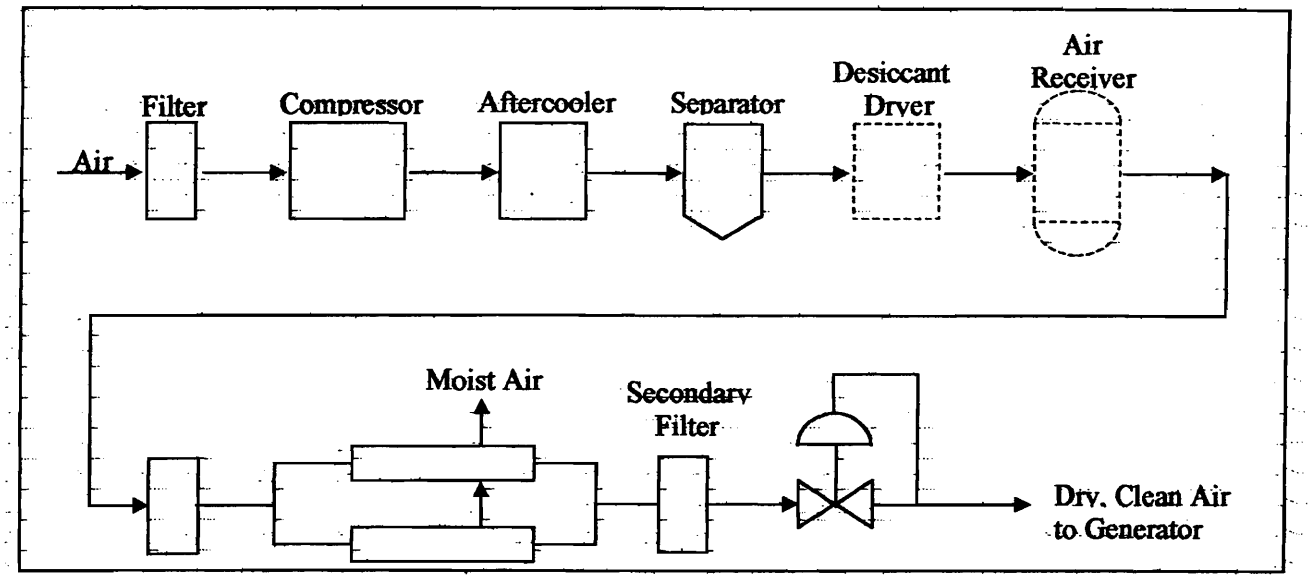


Figure 3-5. Schematic of an Air Preparation System

Table 3-1. Types of Compressors Used in Air Preparation Systems

Compressor Type	Pressure	Volume	Comments
Rotary lobe	Low – 15 psi	Constant or variable with unloading	Common in Europe
Centrifugal	30 – 100 psi depending on no. of stages	Variable, high volume	Medium efficiency, cost effective in high volumes
Rotary Screw	50 psi (single stage) to 100 psi (2 stage)	Variable with unloading	Slightly more efficient than rotary lobe, draws approximately 40% of full load power in unloaded state, available in non-lubricated design for larger capacities.
Liquid Ring	10–80 psi	Constant volume	Does not require lubrication or aftercooler, relatively inefficient, common in United States.
Vane	High - to 100 psi	Constant or variable	Relatively inefficient, not common in U.S.

Table 3-2 presents a comparison of the advantages and disadvantages of each gas feed system.

Table 3-2. Comparison of Air and High Purity Oxygen Feed Systems

Source	Advantages	Disadvantages
Air	• Commonly used equipment • Proven technology • Suitable for small and large systems	• More energy consumed per ozone volume produced • Extensive gas handling equipment required • Maximum ozone concentration of 3-5%
Oxygen (general)	• Higher ozone concentration (8-14%) • Approximately doubles ozone concentration for same generator • Suitable for small and large systems	• Safety concerns • Oxygen resistant materials required
LOX	• Less equipment required • Simple to operate and maintain • Suitable for small and intermediate systems • Can store excess oxygen to meet peak demands	• Variable LOX costs • Storage of oxygen onsite (Fire Codes, i.e. safety concerns) • Loss of LOX in storage when not in use
Cryogenic Oxygen Generation	• Equipment similar to air preparation systems • Feasible for large systems • Can store excess oxygen to meet peak demands	• More complex than LOX • Extensive gas handling equipment required • Capital intensive • Complex systems to operate and maintain

3.2.2.2 *Ozone Generators*

The voltage required to produce ozone by corona discharge is proportional to the pressure of the source gas in the generator and the width of the discharge gap. Theoretically, the highest yield (ozone produced per unit area of dielectric) would result from a high voltage, a high frequency, a large dielectric constant, and a thin dielectric. However, there are practical limitations to these parameters. As the voltage increases, the electrodes and dielectric materials are more subject to failure. Operating at higher frequencies produces higher concentrations of ozone and more heat requiring increased cooling to prevent ozone decomposition. Thin dielectrics are more susceptible to puncturing during maintenance. The design of any commercial generator requires a balance of ozone yield with operational reliability and reduced maintenance.

Two different geometric configurations for the electrodes are used in commercial ozone generators: concentric cylinders and parallel plates. The parallel plate configuration is commonly used in small generators and can be air cooled. Figure 3-6 shows the basic arrangement for the cylindrical configuration. The glass dielectric/high voltage electrode in commercial generators resembles a fluorescent light bulb and is commonly referred to as a "generator tube."

Most of the electrical energy input to an ozone generator (about 85 percent) is lost as heat (Rice, 1996). Because of the adverse impact of temperature on the production of ozone, adequate cooling should be provided to maintain generator efficiency. Excess heat is removed usually by water

flowing around the stainless steel ground electrodes. The tubes are arranged in either a horizontal or vertical configuration in a stainless steel shell, with cooling water circulating through the shell.

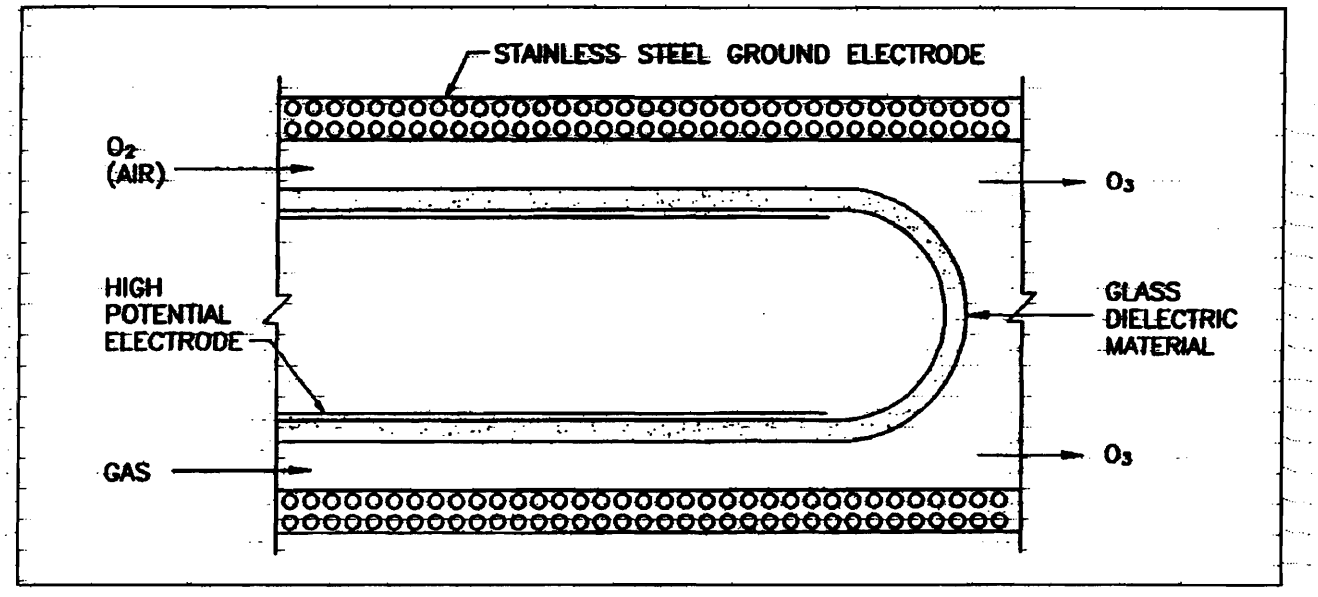


Figure 3-6. Cylindrical Electrode Schematic

Ozone generators are classified by the frequency of the power applied to the electrodes. Low frequency (50 or 60 Hz) and medium frequency (60 to 1,000 Hz) generators are the most common found in the water industry, however some high frequency generators are available. Table 3-3 presents a comparison of the three types of generators. Medium frequency generators are efficient and can produce ozone economically at high concentrations, but they generate more heat than low frequency generators and require a more complicated power supply to step up the frequency supplied by utility power. New installations tend to use medium or high frequency generators.

3.2.2.3 Ozone Contactors

Once ozone gas is transferred into water, the dissolved ozone reacts with the organic and inorganic constituents, including any pathogens. Ozone not transferred into the process water during contacting is released from the contactor as off-gas. Transfer efficiencies of greater than 80 percent typically are required for efficient ozone disinfection (DeMers and Renner, 1992).

Common ozone dissolution methods include:

- Bubble diffuser contactors;
- Injectors; and
- Turbine mixers.

Table 3-3. Comparison of Primary Characteristics of Low, Medium, and High Frequency Ozone Generators

Characteristic	Low Frequency (50 - 60 Hz)	Medium Frequency (up to 1,000 Hz)	High Frequency (> 1,000 Hz)
Degree of Electronics Sophistication	low	high	high
Peak Voltages	19.5	11.5	10
Turndown Ratio	5:1	10:1	10:1
Cooling Water Required (gal/lb of ozone produced)	0.5 to 1.0	0.5 to 1.5	0.25 to 1
Typical Application Range	< 500 lb/day	to 2,000 lb/day	to 2,000 lb/day
Operating Concentrations wt - % in air wt - % in oxygen	 0.5 to 1.5% 2.0 to 5.0%	 1.0 to 2.5%* 2 to 12%	 1.0 to 2.5%* 2 to 12%
Optimum Ozone Production (as a proportion of total generator capacity)	60 to 75%	90 to 95%	90 to 95%
Optimum Cooling Water Differential	8° to 10°F	5° to 8°F	5° to 8°F
Power Required (kW-h/lb O_3)	air feed: 8 to 12 O_2 feed: 4 to 6	air feed: 8 to 12 O_2 feed: 4 to 6	air feed: 8 to 12 O_2 feed: 4 to 6
Air Feed System Power Requirements (kW-h/lb O_3)	5 to 7	5 to 7	5 to 7

Source: Adapted from Rice, 1996, with modifications.

Bubble Diffuser Contactors

The bubble diffuser contactor is commonly used for ozone contacting in the United States and throughout the world (Langlais et al., 1991). This method offers the advantages of no additional energy requirements, high ozone transfer rates, process flexibility, operational simplicity, and no moving parts. Figure 3-7 illustrates a typical three stage ozone bubble diffuser contactor. This illustration shows a countercurrent flow configuration (ozone and water flowing in opposite directions), an alternating cocurrent/countercurrent arrangement, and a cocurrent flow configuration (ozone and water flowing in the same direction). Also, the number of stages can vary from two to six for ozone disinfection, with the majority of plants using two or three chambers for contacting and reaction (Langlais et al., 1991).

Bubble diffuser contactors are typically constructed with 18 to 22 ft water depths to achieve 85 to 95 percent ozone transfer efficiency. Since all the ozone is not transferred into the water, the contactor chambers are covered to contain the off-gas. Off-gas is routed to an ozone destruct unit, usually catalysts, thermal, or thermal/catalysts.

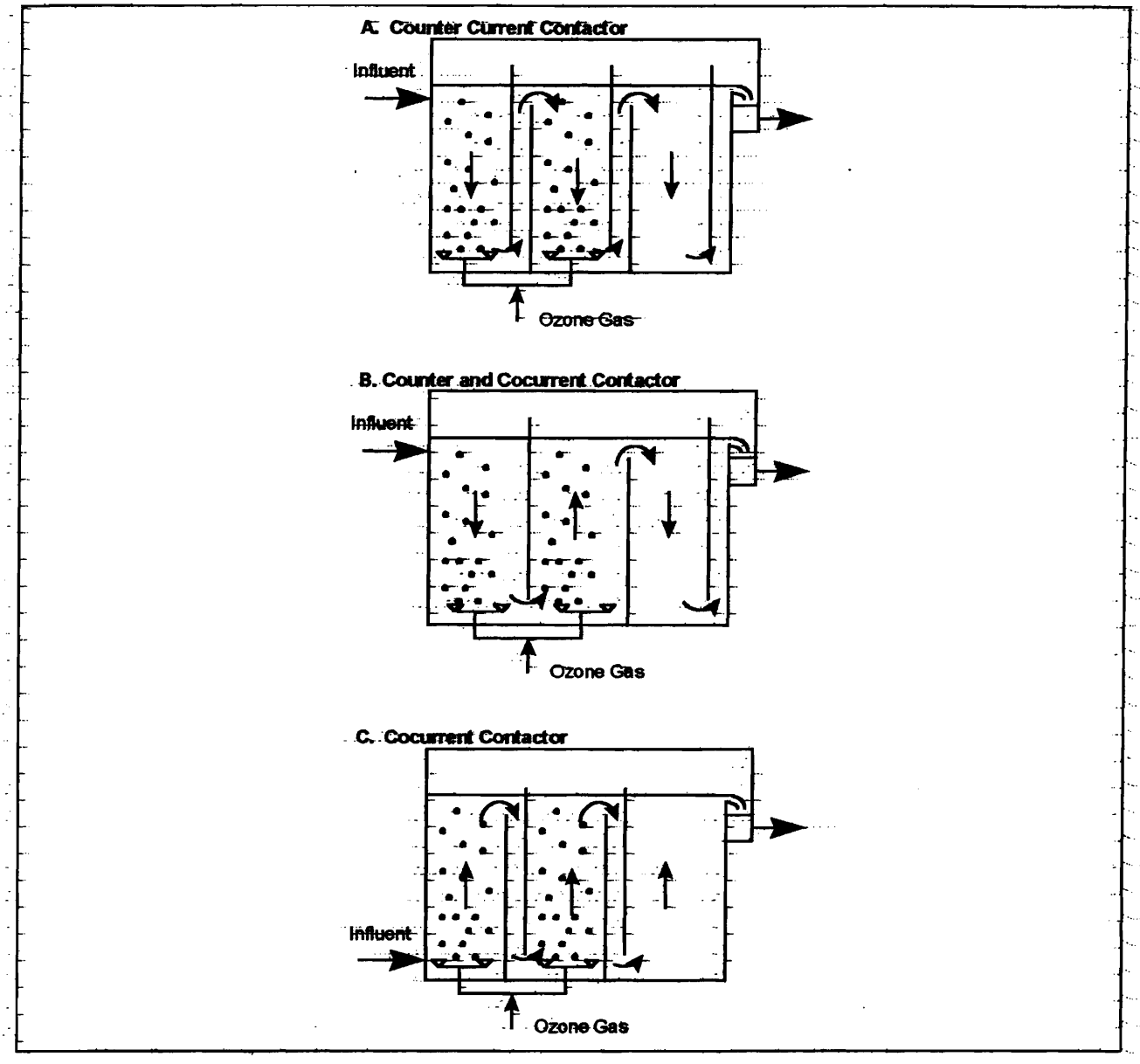


Figure 3-7. Ozone Bubble Contactor

Bubble diffuser contactors use ceramic or stainless steel diffusers that are either rod-type or disc-type to generate bubbles. Design considerations for these diffusers (Renner et al., 1988) include:

- Gas flow range of 0.5 to 4.0 scfm;
- Maximum headloss of 0.5 psig;
- Permeability of 2 to 15 cfm/ft^2/in of diffuser thickness; and porosity of 35 to 45 percent.

The configuration of the bubble diffuser contactor structure should best be designed to provide plug flow hydraulics. This configuration will minimize the overall volume of the contactor while still

meeting the CT requirements for the system. Contactor volume is determined in conjunction with the applied ozone dosage and estimated residual ozone concentration to satisfy the disinfection CT requirement.

Table 3-4 summarizes the advantages and disadvantages of the bubble diffuser contactor (Langlais et al., 1991). Also, diffuser pore clogging can be a problem when ozone dosages are intermittent and/ or when iron and manganese oxidation is required. Channeling of bubbles is dependent on the type of diffusers used and the spacing between diffusers.

Table 3-4. Bubble Diffuser Contactor Advantages and Disadvantages

Advantages	Disadvantages
No moving parts	Deep contact basins
Effective ozone transfer	Vertical channeling of bubbles
Low hydraulic headloss	Maintenance of gaskets and piping.
Operational simplicity	

Injector Dissolution

The injector contacting method is commonly used in Europe, Canada, and the United States (Langlais et al., 1991). Ozone is injected into a water stream under negative pressure, which is generated in a venturi section, pulling the ozone into the water stream. In many cases, a sidestream of the total flow is pumped to a higher pressure to increase the available vacuum for ozone injection. After the ozone is injected into this sidestream, the sidestream containing all the added ozone is combined with the remainder of the plant flow under high turbulence to enhance dispersion of ozone into the water. Figure 3-8 illustrates typical in-line and sidestream ozone injection systems.

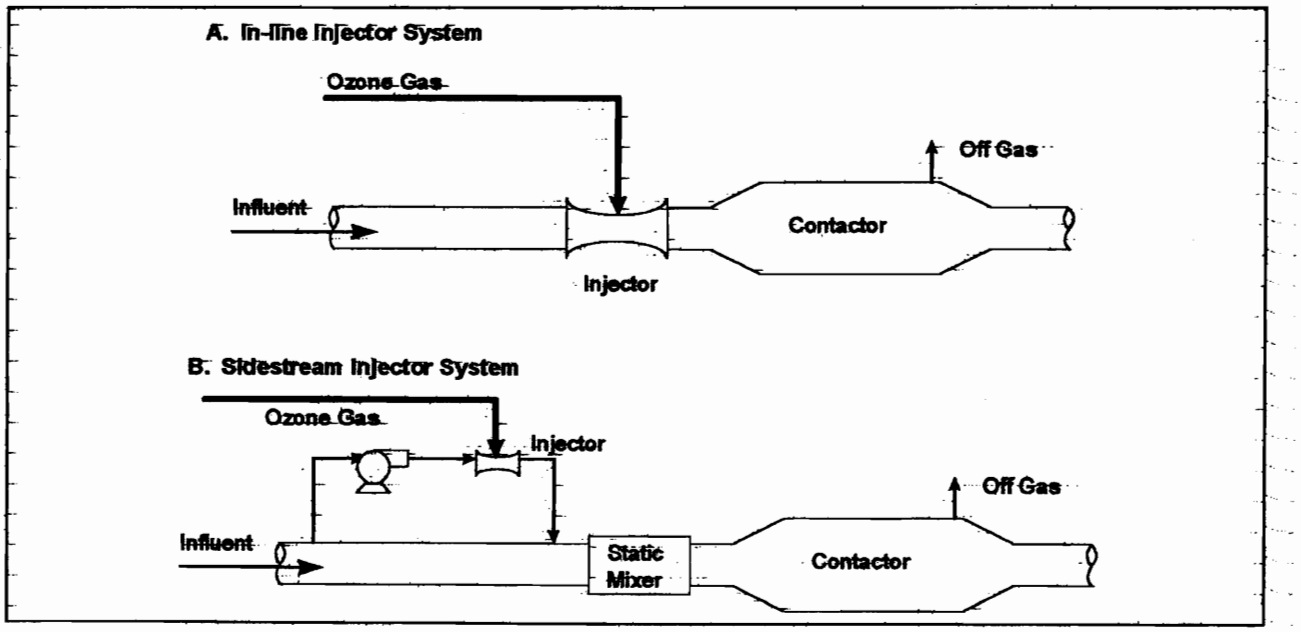


Figure 3-8. Sidestream Ozone Injection System

The gas to liquid ratio is a key parameter used in the design of injector contacting systems. This ratio should be less than 0.067 cfm/gpm to optimize ozone transfer efficiency (Langlais et al., 1991). Meeting this criterion typically requires relatively low ozone dosages and ozone gas concentrations greater than 6 percent by weight (DeMers and Renner, 1992). High concentration ozone gas can be generated using a medium-frequency generator and/or liquid oxygen as the feed gas.

To meet the CT disinfection requirements, additional contact time is required after the injector, typically in a plug flow reactor. The additional contact volume is determined in conjunction with the applied ozone dosage and estimated residual ozone concentration to satisfy the disinfection CT requirement.

Table 3-5 summarizes the advantages and disadvantages of injection contacting (Langlais et al., 1991).

Table 3-5. Injection Contacting Advantages and Disadvantages

Advantages	Disadvantages
Injection and static mixing have no moving parts	Additional headloss (energy usage) due to static mixers which may require pumping
Very effective ozone transfer	Turndown capability limited by injection system
Contactor depth less than bubble diffusion	More complex operation and high cost.

Turbine Mixer Contactors

Turbine mixers are used to feed ozone gas into a contactor and mix the ozone with the water in the contactor. Figure 3-9 illustrates a typical turbine contactor. The illustrated turbine mixer design shows the motor located outside the basin, allowing for maintenance access. Other designs use a submerged turbine.

Ozone transfer efficiency for turbine mixers can be in excess of 90 percent. However, the power required to achieve this efficiency is 2.2 to 2.7 kW-hr of energy per lb of ozone transferred (Dimitriou, 1990).

Turbine mixing basins vary in water depth from 6 to 15 ft, and dispersion areas vary from 5 to 15 ft (Dimitriou, 1990). Again, as with injector contacting, sufficient contact time may not be available in the turbine basin to meet disinfection CT requirements; consequently additional contact volume may be required.

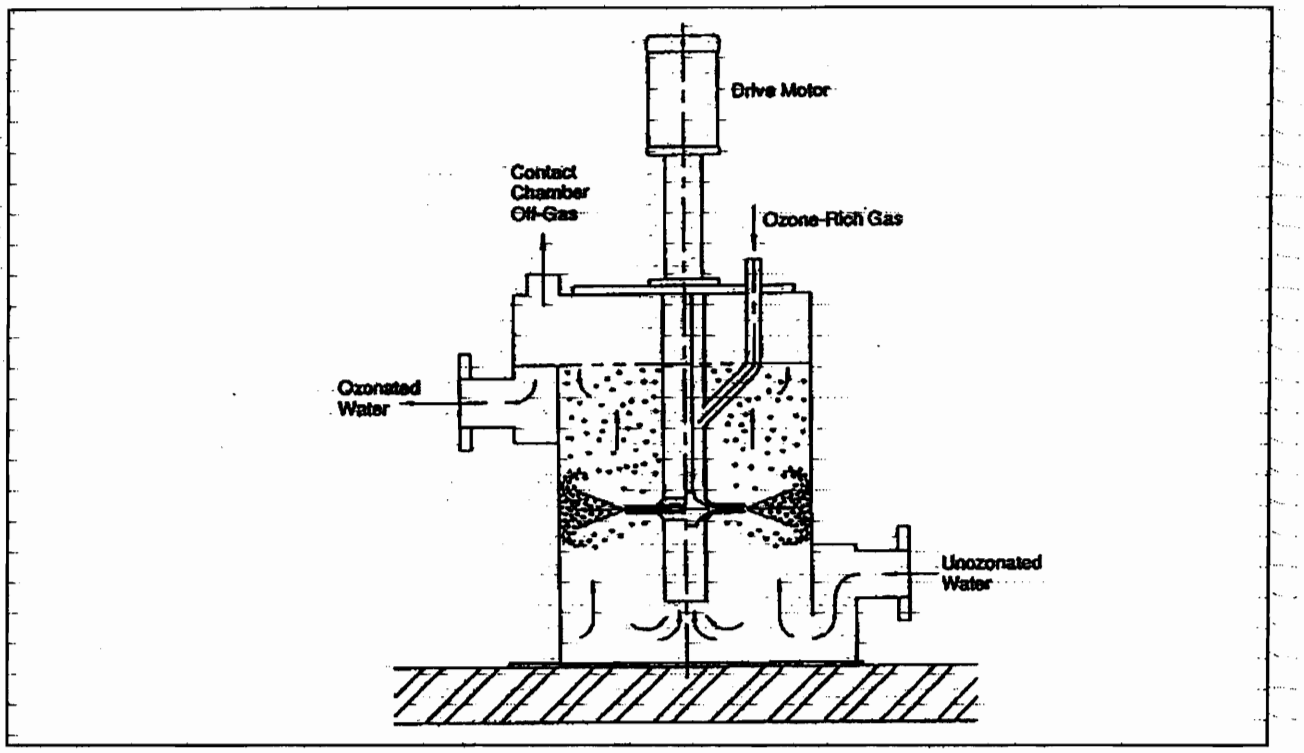


Figure 3-9. Turbine Mixer Ozone Contactor

Table 3-6 summarizes advantages and disadvantages for the turbine mixer contactor (Langlais et al., 1991).

Table 3-6. Turbine Mixer Contactor Advantages and Disadvantages

Advantages	Disadvantages
Ozone transfer is enhanced by high turbulence resulting in small bubble size	Require energy input
Contactor depth less than bubble diffusion	Constant gas flow rate should be maintained, reducing ozone transfer efficiency
Aspirating turbines can draw off-gas from other chambers for reuse	Maintenance requirements for turbine and motor
Eliminates diffuser clogging concerns	

3.2.2.4 *Off-gas Destruction Systems*

The concentration of ozone in the off-gas from a contactor is usually well above the fatal concentration. For example, at 90 percent transfer efficiency, a 3 percent ozone feed stream will still contain 3,000 ppm of ozone in the off-gas. Off-gas is collected and the ozone converted back to oxygen prior to release to the atmosphere. Ozone is readily destroyed at high temperature (> 350° C or by a catalyst operating above 100° C) to prevent moisture buildup. The off-gas destruct unit is designed to reduce the concentration to 0.1 ppm of ozone by volume, the current limit set by OSHA

for worker exposure in an eight hour shift. A blower is used on the discharge side of the destruct unit to pull the air from the contactor, placing the contactor under a slight vacuum to ensure that no ozone escapes.

3.2.2.5 Instrumentation

Instrumentation should be provided for ozone systems to protect both personnel and the equipment. Gas phase ozone detectors should be provided in spaces such as generator rooms where ozone gas may be and personnel are routinely present. An ozone detector is also needed on the outlet from the off-gas destruct unit to ensure the unit is working properly. These units should be interlocked with the ozone generator controls to shut down the ozone generation system should excess ozone be detected. A dew point detector on the feed gas supply just upstream of an ozone generator is required to protect the generator from moisture in the feed gas (when air is the feed gas). Flow switches on the cooling water supply are needed to protect the generator from overheating and a pressure switch to prevent over pressurization.

Other instrumentation can be used to monitor and control the ozone process, although manual control is adequate for small systems, but most small systems are designed to operate automatically, particularly in remote areas. Ozone monitors can be used in conjunction with process flow meters to match ozone dose to process demands and control ozone generation. Sophisticated control schemes can be implemented to minimize the cost of dosing with ozone and reduce operator attention requirements. Many systems include residual monitoring at various points in the contactor to maintain a desired ozone residual and prevent energy-wasting overdosing.

3.2.3 Operation and Maintenance

Even though ozone systems are complex, using highly technical instruments, the process is highly automated and very reliable, requiring only a modest degree of operator skill and time to operate an ozone system. Maintenance on generators requires skilled technicians. If trained maintenance staff are not available at the plant, this work can be done by the equipment manufacturer. Some facilities, such as the 600 mgd Los Angeles Aqueduct Filtration Plant, use plant mechanics to perform generator and facilities maintenance. Therefore, backup units are usually installed. Generators should be checked daily when in operation. After a shutdown, dry air or oxygen should be allowed to flow through the generator to ensure that any moisture has been purged prior to energizing the electrodes. At initial start up and after long down times, this process may take up to 12 hours and usually longer when air is the feed gas. As an alternative, a small flow of dry air can be passed through the generator continuously when it is in standby mode to maintain the dry condition.

Filters and desiccant in air preparation systems should be changed periodically, with the frequency depending on the quality of the inlet air and the number of hours in operation. Compressors require periodic service, depending on the type and operating time. LOX tanks should be periodically pressure tested. Piping and contact chambers should be inspected periodically to check for leaks and corrosion.

Dielectric tubes should be periodically cleaned. This operation should be performed when the generator efficiency drops 10-15 percent. Cleaning the tubes is a delicate operation as the tubes are fragile and expensive. Adequate space should be provided for the cleaning operation and for storage of spare tubes.

3.3 Primary Uses and Points of Application of Ozone

3.3.1 Primary Uses of Ozone

Ozone is used in drinking water treatment for a variety of purposes including:

- Disinfection;
- Inorganic pollutant oxidation, including iron, manganese, and sulfide;
- Organic micropollutant oxidation, including taste and odor compounds, phenolic pollutants, and some pesticides; and
- Organic macropollutant oxidation, including color removal, increasing the biodegradability of organic compounds, DBP precursor control, and reduction of chlorine demand.

3.3.1.1 Disinfection

Ozone is a powerful oxidant able to achieve disinfection with less contact time and concentration than all weaker disinfectants, such as chlorine, chlorine dioxide, and monochloramine (Demers and Renner, 1992). However, ozone can only be used as a primary disinfectant since it cannot maintain a residual in the distribution system. Thus, ozone disinfection should be coupled with a secondary disinfectant, such as chlorine, chloramine, or chlorine dioxide for a complete disinfection system.

3.3.1.2 Iron and Manganese Oxidation

Ozone will oxidize iron and manganese, converting ferrous (2+) iron into the ferric (3+) state and 2+ manganese to the 4+ state. The oxidized forms will precipitate as ferric hydroxide and manganese hydroxide (AWWA, 1990). The precise chemical composition of the precipitate will depend on the nature of the water, temperature, and pH. The ozone dose required for oxidation is 0.43 mg/mg iron and 0.88 mg/mg manganese (Langlais et al., 1991). Iron oxidizes at a pH of 6-9 but manganese is more effective at a pH of around 8. Also, over-ozonation has no effect on iron, but will resolubilize manganese, which then should be reduced to manganese dioxide downstream.

3.3.1.3 Oxidation of Taste and Odor Compounds

Ozone is used to oxidize/destroy taste and odor-causing compounds because many of these compounds are very resistant to oxidation. Suffet et al. (1986) confirmed that ozone is an effective oxidant for use in taste and odor treatment. They found ozone doses of 2.5 to 2.7 mg/L and 10 minutes of contact time (ozone residual of 0.2 mg/L) significantly reduced taste and odors in the

specific waters they tested. Most early U.S. water plants (i.e., 1940-1986) installed ozonation specifically for taste and odor removal.

3.3.1.4 DBP Precursor Control

Early work on oxidation of DBP precursors seemed to indicate that the effects of ozonation, prior to chlorination, were quite site-specific and unpredictable (Umphries et al., 1979). The key variables that seem to determine ozone's effect are dose, pH, alkalinity, and, above all, the nature of the organic material. At low pH levels, precursor destruction by ozone is quite effective; however, above some critical pH, ozone actually is less effective and in fact sometimes increases the amount of chlorination byproduct precursors. For most humic substances this critical pH is 7.5, which is the approximate level at which decomposition of ozone to hydroxyl free radicals increases rapidly, thus increasing organic oxidation rates. Therefore, the implications that at lower pH (approximately 6-7), at which molecular ozone predominates over the hydroxyl free radical, the initial THM precursor by-products are different in nature than those formed by the hydroxyl free radicals oxidized at higher pH levels. This is logical in light of the greater oxidation potential of the hydroxyl free radical over that of ozone.

As alkalinity increases, it has a beneficial effect on THM formation potential (THMFP) (Langlais et al., 1991). This is because alkalinity scavenges any hydroxyl free radicals formed during ozonation, leaving molecular ozone as the sole oxidant, which is only capable of oxidizing organic precursors to a lower oxidation sequence than does the hydroxyl free radical. Given neutral pH and moderate levels of bicarbonate alkalinity, THMFP level reductions of 3 to 20 percent have been shown at ozone doses ranging from 0.2 to 1.6 mg ozone per mg carbon (Singer et al., 1989; Georgeson and Karimi, 1988).

3.3.1.5 Increase Organic Biodegradation

Ozone can be effective in partially oxidizing organics in the water to biodegradable compounds that can be removed by biological filtration (Demers and Renner, 1992). This partial oxidation gives rise to lower molecular weight organics that are more easily biodegradable. This increase in the biodegradable fraction of organic carbon occurs as a result of moderate to high levels of ozonation. These ozone levels are typical of the doses commonly applied for disinfection.

3.3.1.6 Coagulation and Filtration Improvement

Ozone has been reported by some to improve coagulation and filtration efficiency (Gurol and Pidatella, 1993; Farvardin and Collins, 1990; Reckhow et al., 1993; Stolarik and Christie, 1997). However, others have found no improvement in filter effluent turbidity due to ozonation (Tobiason et al., 1992; Hiltebrand et al., 1986). Prendiville (1986) collected data from a large water treatment plant showing that pre-ozonation was more effective than pre-chlorination to reduce filter effluent turbidities. The cause of the improved coagulation is not clear, but several possibilities have been offered (Reckhow et al., 1986), including:

- Oxidation of organic compounds into more polar forms; and
- Oxidation of metals ions to yield insoluble complexes, such as ferric iron complexes.

3.3.2 Points of Application

The typical locations for feeding ozone in a water treatment plant are at the head of the treatment plant (raw water) pre-ozonation and after sedimentation.

Raw water quality and turbidity and ozone demand (the amount of ozone required for all oxidation requirements of the water) can be used to assess how to use ozone in the treatment process. Table 3-7 lists the criteria for selecting ozone feed points based on these two parameters. By moving the ozonation process further downstream after sedimentation, the ozone demand and production of byproducts are reduced. The advantage of placing ozone ahead of filtration is that biodegradable organics produced during ozonation can be removed by subsequent biological activity in the filters.

Table 3-7. Criteria for Selecting Ozone Feed Points for Small Systems

Raw Water Quality	Ozone Feed Point(s)	Special Considerations
Category I		Low ozone demand.
Turbidity < 10 NTU	Raw Water or After Sedimentation	Low disinfection byproducts.
Ozone Demand < 1mg/L		Low biodegradable organics.
Category II		Low ozone demand.
Turbidity > 10 NTU	After Sedimentation	High inorganic particulate.
Ozone Demand < 1mg/L		Low biodegradable organics.
Category III		High ozone demand
Turbidity < 10 NTU	Raw Water and/or After Sedimentation	Disinfection byproducts
Ozone Demand > 1mg/L		Biodegradable organics formation
Category IV		High ozone demand
Turbidity > 10 NTU	After Sedimentation and After First	Disinfection byproducts
Ozone Demand > 1mg/L	Stage Filtration, if necessary	Biodegradable organics formation

Source: DeMers and Renner, 1992.

For high quality water with direct filtration, the only practical ozone feed point is the raw water.

Category II (Table 3-7) water is characterized by low ozone demand and high turbidity. This water quality indicates the presence of inorganic material, such as clay or silt particles. For ozone to be most effective for Category II water disinfection, it should be added after either pre-sedimentation or conventional sedimentation.

Raw water with low turbidity and high ozone demand (Category III, Table 3-7) contains dissolved constituents, not suspended, that contribute to a high ozone demand. An example of this type of water is a ground water containing bromide ion, iron, manganese, color, or organics. For this water quality, ozone can be added to either the raw water or after sedimentation. If the water contains organic constituents that become more biodegradable by ozonation, a biological treatment step (see

Section 3.3.4) may be required. The presence of oxidizable organic constituents or bromide ion will generate disinfection byproducts upon ozonation.

Category IV (Table 3-7) water would be considered the most difficult water to treat with ozone due to its high turbidity and high ozone demand. An example of this water quality is surface water containing high concentrations of organic material and inorganic particles. The most effective use of ozone for this water quality is after sedimentation and possibly after filtration. If the water has an extremely high ozone demand, dual ozone feed points may be required to achieve disinfection goals, because the presence of large amounts of organic material may require a biological treatment step and may generate disinfection byproducts.

3.3.3 Impact on Other Treatment Processes

Ozonation does have an impact on other processes at the water treatment facility. The impacts of ozone addition include the following:

- The use of ozone generates biodegradable organic matter (BOM) that can result in biological growth which may also increase corrosion rates in distribution systems if not removed by biologically active filtration. When ozonation is placed before biological filters, it can impact the filters by increasing biological growth and increasing backwash frequency.
- Ozone is a strong oxidant that reacts with other oxidants, such as chlorine, chlorine dioxide, and monochloramine.
- Ozone oxidation of iron and manganese generates insoluble oxides that should be removed by sedimentation or filtration. These insoluble oxides also impact the filters by increasing load on the filters and increasing backwash frequency.
- Using pre- and/or internal ozone on most raw waters reduces the subsequent chlorine, chlorine dioxide, or monochloramine demand of the finished water so as to allow a stable chlorine-compound residual to be maintained at a much lower level.

The reader is referred to EPA's *Simultaneous Compliance Guidance Document* (expected to be available in 1999) for additional information regarding the interaction between oxidants and other treatment processes.

3.3.4 Biologically Active Filtration

Ozonation typically increases the biodegradability of NOM in water because many large organic molecules are converted into smaller organic molecules that are readily biodegradable. This increase in biodegradable dissolved organic carbon (BDOC) can lead to accelerated bacterial growth and regrowth in the distribution system if not removed in the treatment plant. LeChevallier et al. (1992) found that AOC levels less than 100 ppb may be necessary to control excessive bacterial regrowth in the distribution system if not removed in the treatment plant.

When ozonation is placed upstream of filtration, and environmental conditions such as dissolved oxygen, pH, and temperature are favorable, microbiological activity is increased in the filter and BDOC/AOC removal is enhanced. Ozone addition not only increases the biodegradability of the

dissolved organics, but also introduces large amounts of oxygen to the water, thus, creating an excellent environment for biological growth on the filter media. The advantages of biologically active filtration (Price, 1994) include the following which are all being met in most U.S. plants using ozone:

- Production of a biologically stable water that does not promote excessive bacterial growth and regrowth in the distribution system.
- Removal of NOM that can serve as precursors to byproduct formation as a result of residual disinfection with free or combined chlorine.
- Ozone oxidation as a primary disinfectant prior to biologically active filtration reduces the BDOC concentration in finished water, thus reducing chances of regrowth.
- Reduction of the residual disinfectant demand of the product water so that proposed regulations limiting the maximum disinfectant residual can be met.
- Removal or control of ozonation byproducts.

Biological activity can be supported on slow sand, rapid rate, and GAC media because these media provide a surface for bacteria to attach. Factors such as available surface area, hydraulic loading rate, contact time, availability of nutrients, temperature, and others will determine the performance and BDOC removal efficiency. Biomass develops to higher levels on GAC because of the rougher surface characteristics than on anthracite and sand.

3.3.4.1 Slow Sand Filters

Ozone addition prior to slow sand filtration can increase the efficiency of TOC removal by about 35 percent (Rachwal et al., 1988; Zabel, 1985). Ozone addition can also increase the efficiency of BDOC removal with slow sand filters (Eighmy et al., 1991; Malley et al., 1993).

3.3.4.2 Rapid Rate Filters

Research in the area of biologically active rapid rate filters has focused on the reduction of assimilable organic carbon (AOC) instead of BDOC. While studies have shown rapid rate filtration, employing either sand or dual media lowers AOC levels following ozonation, AOC does not measure all the BDOC. AOC measures only that portion of the BDOC that is more easily assimilable or more easily biodegradable under specific laboratory conditions by two specific microorganisms. Research data shows that biodegradation of AOC can occur in rapid rate filters. The data should be viewed with caution, since the more slowly biodegradable DOC, not measured by AOC, may be passing through rapid rate filters.

3.3.4.3 Granular Activated Carbon

GAC is made biologically active by the deliberate introduction of sufficient dissolved oxygen to water just before passing through GAC columns (Katz, 1980). The high surface area and long retention time in GAC provide an ideal environment to enhance biological growth.

Although ozone actually increases the amount of BDOC, the efficiency of subsequent biodegradation on GAC can be such that the BDOC in GAC effluent is lower than the BDOC in the ozone influent (Langlais et al., 1991). The degree to which biodegradable DOC is removed by ozone/GAC depends upon the process conditions of temperature, amount of BDOC, and the GAC column loading rate, measured by empty bed contact time (EBCT). For example, with an influent BDOC of 0.65 mg C/L and a 10 minute EBCT, one would expect an effluent BDOC of 0.25 mg C/L. The effluent BDOC then could be lowered by either:

Adding ozone, which would increase the GAC influent BDOC and, therefore, lower the effluent BDOC; or

Adding more GAC or decreasing the loading rate, which would extend the EBCT and lower the effluent BDOC (Billen et al., 1985, as cited in Langlais et al., 1991).

Huck et al. (1991) reported results from AOC profiles measured in a pilot treatment plant. The plant treated Saskatchewan River water and included coagulation, flocculation, and sedimentation prior to ozonation. Following ozonation, the water was filtered through a dual media (anthracite-sand) filter followed by GAC adsorption. The results demonstrate:

Variable AOC removal through coagulation, flocculation, and sedimentation (80 percent to zero);

Increased AOC after ozonation;

AOC removal through dual media filtration improving at lower hydraulic loading rates and filtered effluent AOC often less than raw water AOC, but highly variable; and

AOC levels after GAC were low, almost always below raw water AOC concentrations and adsorption appears to contribute to some immediate AOC removal.

3.3.5 Pathogen Inactivation and Disinfection Efficacy

Ozone has a high germicidal effectiveness against a wide range of pathogenic organisms including bacteria, protozoa, and viruses. Because of its high germicidal efficiency, ozone can be used to meet high inactivation required by water treatment systems with or without filters. However, ozone cannot be used as a secondary disinfectant because the ozone residual decays too rapidly. The ozone disinfection efficiency is not affected by pH (Morris, 1975), although because of hydroxyl free radicals and rapid decay, efficiency is the same but more ozone should be applied at high pH to maintain "C".

3.3.6 Inactivation Mechanisms

Inactivation of bacteria by ozone is attributed to an oxidation reaction (Bringmann, 1954; Chang, 1971). The first site to be attacked appears to be the bacterial membrane (Giese and Christensen, 1954) either through the glycoproteins or glycolipids (Scott and Lesher, 1963) or through certain amino acids such as typtophan (Goldstein and McDonagh, 1975). In addition, ozone disrupts enzymatic activity of bacteria by acting on the sulfhydryl groups of certain enzymes. Beyond the cell membrane and cell wall, ozone may act on the nuclear material within the cell. Ozone has been found to affect both purines and pyrimidines in nucleic acids (Giese and Christensen, 1954; Scott and Lesher, 1963).

The first site of action for virus inactivation is the virion capsid, particularly its proteins (Cronholm et al., 1976 and Riesser et al., 1976). Ozone appears to modify the viral capsid sites that the virion uses to fix on the cell surfaces. High concentrations of ozone dissociate the capsid completely. One researcher found that the mechanism of ozone inactivation of bacteriophage f2 ribonucleic acid (RNA) included releasing RNA from the phage particles after the phage coat was broken into many pieces (Kim et al., 1980). This finding suggests that ozone breaks the protein capsid, thereby liberating RNA and disrupting adsorption to the host pili. Further, the naked RNA may be secondarily inactivated by ozone at a rate less than that for RNA within the intact phage. The mechanism for inactivation of deoxyribonucleic acid (DNA) bacteriophage T4 has been found to be quite similar to RNA inactivation: ozone attacks the protein capsid, liberates the nucleic acid, and inactivates the DNA (Sproul et al., 1982). In contrast, more recent work on the tobacco mosaic virus (TMV) shows that ozone has a specific effect on RNA. Ozone was found to attack both the protein coat and RNA. The damaged RNA cross-links with amino acids of the coat protein subunits. The authors concluded that TMV loses its infectivity because of its loss of protein coating.

Microscopic observation of inactivation of trophozoites of *Naegleria* and *Acanthamoeba* showed that they were rapidly destroyed and the cell membrane was ruptured (Perrine et al., 1984). Perrine and Langlais showed that ozone affect the plugs in *Naegleria gruberi* cysts (Langlais and Perrine, 1984). Depending on the ozonation conditions, these plugs were completely removed or were partially destroyed. It has been speculated that ozone initially affects the *Giardia muris* cysts wall and makes it more permeable (Wickramanayake, 1984c). Subsequently, aqueous ozone penetrates into the cyst and damages the plasma membranes, additional penetration of ozone eventually affects the nucleus, ribosomes, and other ultrastructural components.

3.3.7 Disinfection Parameters

Hoigné and Bader demonstrated that the rate of decomposition of ozone is a complex function of temperature, pH, and concentration of organic solutes and inorganic constituents (Hoigné and Bader, 1975, and 1976). The following sections describe the effects that pH, temperature, and suspended matter have on the reaction rate of ozone and pathogen inactivation.

The ability to maintain a high aqueous ozone concentration is critical from a regulatory disinfection compliance standpoint. This means that factors that accelerate ozone decomposition are undesirable for inactivation because the ozone residual dissipates faster and therefore reduces the CT credit, requiring a corresponding increase in the ozone applied, thus increasing cost.

3.3.7.1 pH

Studies have indicated that pH has little effect on the ability of dissolved ozone residuals to inactivate acid-fast bacteria, such as *Mycobacteria* and *Actinomycetes* (Farooq, 1976). A slight decrease has been found in the virucidal efficacy of ozone residuals as pH decreased (Roy, 1979). However, the opposite effect was observed by Vaughn et al. (1987) (cited in Hoff, 1986). Changes in disinfection efficacy with variations in pH appear to be caused by the ozone decomposition rate. Ozone decomposition occurs faster in higher-pH aqueous solutions and forms various types of oxidants with differing reactivities (Langlais et al., 1991). Tests carried out at constant ozone residual concentration and different pH values showed that the degree of microorganism inactivation remained virtually unchanged (Farooq et al., 1977). More recent studies have indicated decreased virus inactivation by ozone at alkaline pH (pH 8 to 9) for poliovirus 1 (Harakeh and Butler, 1984) and rotaviruses SA-11 and Wa (Vaughn et al., 1987).

Inactivation of *Giardia muris* cysts was found to improve when the pH increased from 7 to 9 (Wickramanayake, 1984a). This phenomenon was attributed to the possible changes in cyst chemistry making it easier for ozone to react with the cyst constituents at the higher pH levels. However, the same study found that inactivation of *Naegleria gruberi* cysts was slower at a pH 9 than at lower pH levels, thereby indicating that pH effects are organism-specific.

3.3.7.2 Temperature

As temperature increases, ozone becomes less soluble and less stable in water (Katzenelson et al., 1974); however, the disinfection and chemical oxidation rates remain relatively stable. Studies have shown that although increasing the temperature from 0 to 30°C can significantly reduce the solubility of ozone and increases its decomposition rate, temperature has virtually no effect on the disinfection rate of bacteria (Kinman, 1975). In other words, the disinfection rate was found to be relatively independent of temperature at typical water treatment plant operating temperatures despite the reduction in solubility and stability at higher temperatures.

3.3.7.3 Suspended Matter

Ozone inactivation of viruses and bacteria contained in aluminum floc (in the size range comparable to those that could typically escape filtration) was not reduced at floc turbidity levels of 1 and 5 NTU (Walsh et al., 1980). This study demonstrated that the microorganisms received no protection from the aluminum floc. Similar results have been obtained for poliovirus 1, coxsackie virus A9, and *E. coli* associated with bentonite clay (Boyce et al., 1981). However, adsorption of the f2 bacteriophage at 1 and 5 NTU of bentonite clay was found to retard the rate of inactivation of ozone (Boyce et al., 1981).

In some instances, river waters heavily polluted with organic matter were ozonated, and the results indicated a degradation of large organic molecules into fragments more easily metabolized by microorganisms. This fragmentation coupled with the inability of ozone to maintain an active concentration in the distribution system, has led to increased slime growth and, consequently, water quality deterioration during distribution (Troyan and Hanson, 1989).

3.3.8 Inactivation of Microorganisms

The following sections contain a description of the disinfection efficiency of ozone in terms of bacteria, virus, and protozoa inactivation.

3.3.8.1 Bacterial Inactivation

Ozone is very effective against bacteria. Studies have shown the effect of small concentrations of dissolved ozone (i.e., 0.6 g/L) on *E. coli*. (Wuhrmann and Meyrath, 1955) and *Legionella pneumophila* (Domingue, et al., 1988). *E. coli*. levels were reduced by 4 logs (99.99 percent removal) in less than 1 minute with a ozone residual of 9 g/L at a temperature of 12°C. *Legionella pneumophila* levels were reduced by greater than 2 logs (99 percent removal) within a minimum contact time of 5 minutes at a ozone concentration of 0.21 mg/L. Results similar to those obtained for *E. coli*. have been found for *Staphyloccus* sp. and *Pseudomonas fluorescens* inactivation. *Streptococcus faecalis* required a contact time twice as long with the same dissolved ozone concentration, and *Mycobacterium tuberculosis* required a contact time six times as long for the same reduction level as *E. coli*.

In regard to vegetative bacteria, *E. coli* is one of the most sensitive types of bacteria. Furthermore, significant difference has been found among all the Gram-negative bacillae, including *E. coli* and other pathogens such as *Salmonella*, which are all sensitive to ozone inactivation. whereas the Gram-positive cocci (*Staphyloccus* and *Streptococcus*), the Gram-positive bacillae (*Bacillus*), and the *Mycobacteria* are the most resistant forms of bacteria. Sporular bacteria forms are always far more resistant to ozone disinfection than vegetative forms (Bablon, et al., 1991), but all are easily destroyed by relatively low levels of ozone.

3.3.8.2 Protozoa Inactivation

Protozoan cysts are much more resistant to ozone and other disinfectants than vegetative forms of bacteria and viruses. *Giardia lamblia* has a sensitivity to ozone that is similar to the sporular forms of *Mycobacteria*. Both *Naegleria* and *Acanthamoeba* cysts are much more resistant to ozone (and all other disinfectants) than *Giardia* cysts. (Bablon et al., 1991). CT products for 99 percent inactivation of *Giardia lamblia* and *N. gruberi* at 5°C were 0.53 and 4.23 mg min/L, respectively (Wickramanayake et al., 1984a and 1984b). Data available for inactivation of *Cryptosporidium* oocysts, suggest that among protozoans, this microorganism is more resistant to ozone (Peeters et al., 1989; Langlais et al., 1990). One study found that *Cryptosporidium* oocysts are approximately 10 times more resistant to ozone than *Giardia* (Owens et al., 1994).

3.3.8.3 Virus Inactivation

Typically, viruses are more resistant to ozone than vegetative bacteria but less resistant than sporular forms of *Mycobacteria* (Bablon, et al., 1991). The most sensitive forms of viruses are phages, and there seems to be little difference between the polio- and coxsackie viruses. The sensitivity of human rotavirus to ozone was determined to be comparable to that of *Mycobacteria* and polio- and coxsackie viruses (Vaughn et al., 1987).

Keller et al. (1974) studied ozone inactivation of viruses by using both batch tests and pilot plant data. Inactivation of poliovirus 2 and coxsackie virus B3 was more than 3 logs (99.9 percent) in the batch tests with an ozone residual of 0.8 mg/L and 1.7 mg/L and a contact time of 5 minutes. Greater than 5 log (99.999 percent) removal of coxsackie virus was achieved in the pilot plant with an ozone dosage of 1.45 mg/L, which provided an ozone residual of 0.28 mg/L in lake water.

3.3.8.4 CT Curves for Giardia Lamblia

CT values shown in Figure 3-9 are based on disinfection studies using in vitro excystation of *Giardia lamblia*. CT values obtained at 5°C and pH 7 were used as the basis for deriving the CT values at other temperatures. A safety factor of 2 has been applied to the values shown in Figure 3-9.

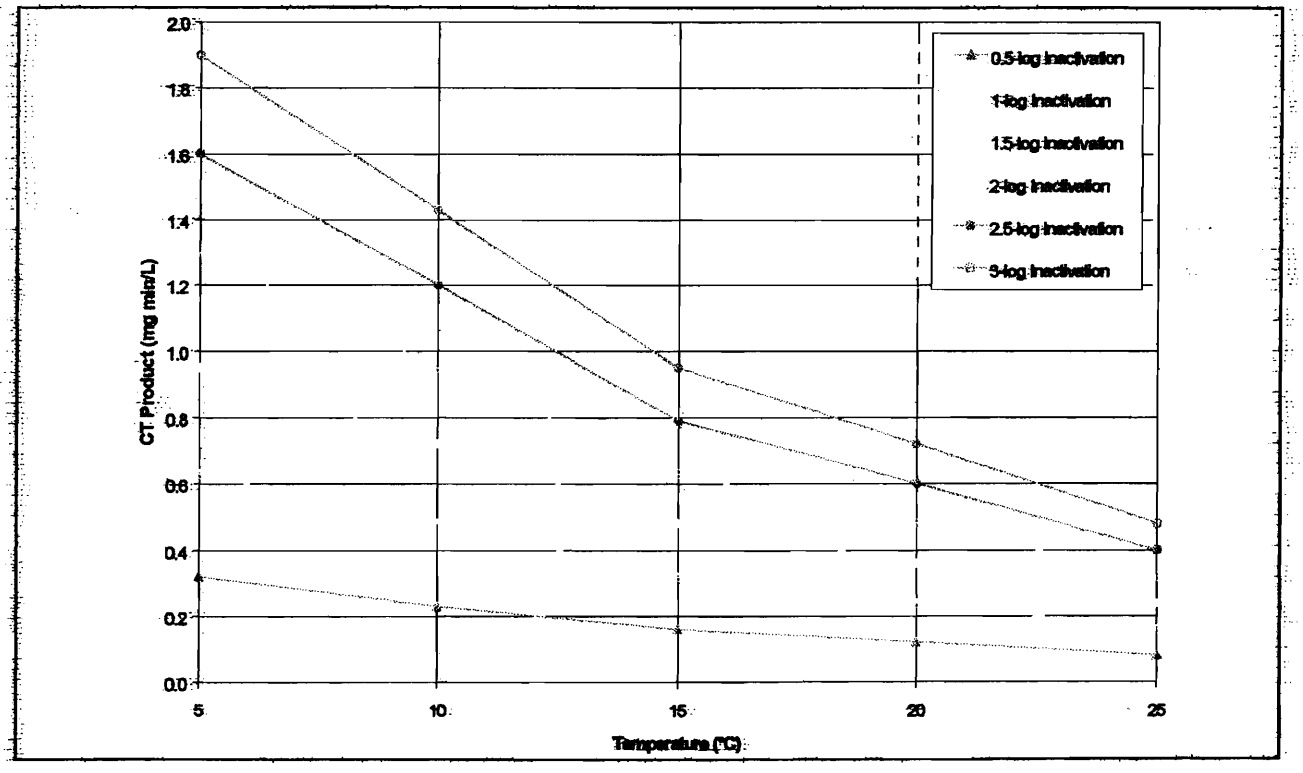


Figure 3-9. CT Values for Inactivation of Giardia Cysts by Ozone (pH 6 to 9)

CT values shown in Figure 3-10 for achieving 2-log inactivation of viruses were determined by applying a safety factor of 3 to data obtained from a previous study on poliovirus 1 (Roy et al., 1982). CT values for 3 and 4-log removal were derived by applying first order kinetics and assuming the same safety factor of 3. Data obtained at a pH of 7.2 was assumed to apply for the pH range of 6 to 9.

Several research groups have investigated the efficiency of ozone for *Cryptosporidium* oocyst inactivation. Table 3-8 summarizes CT values obtained for 99 percent inactivation of *Cryptosporidium* oocysts. Results indicate that ozone is one of the most effective disinfectants for controlling *Cryptosporidium* (Finch, et al., 1994) and that *Cryptosporidium muris* may be slightly more resistant to ozonation than *Cryptosporidium parvum* (Owens et al., 1994). A wide range of CT values has been reported for the same inactivation level, primarily because of the different methods of *Cryptosporidium* measurements employed and pH, temperature, and above all, ozonation conditions.

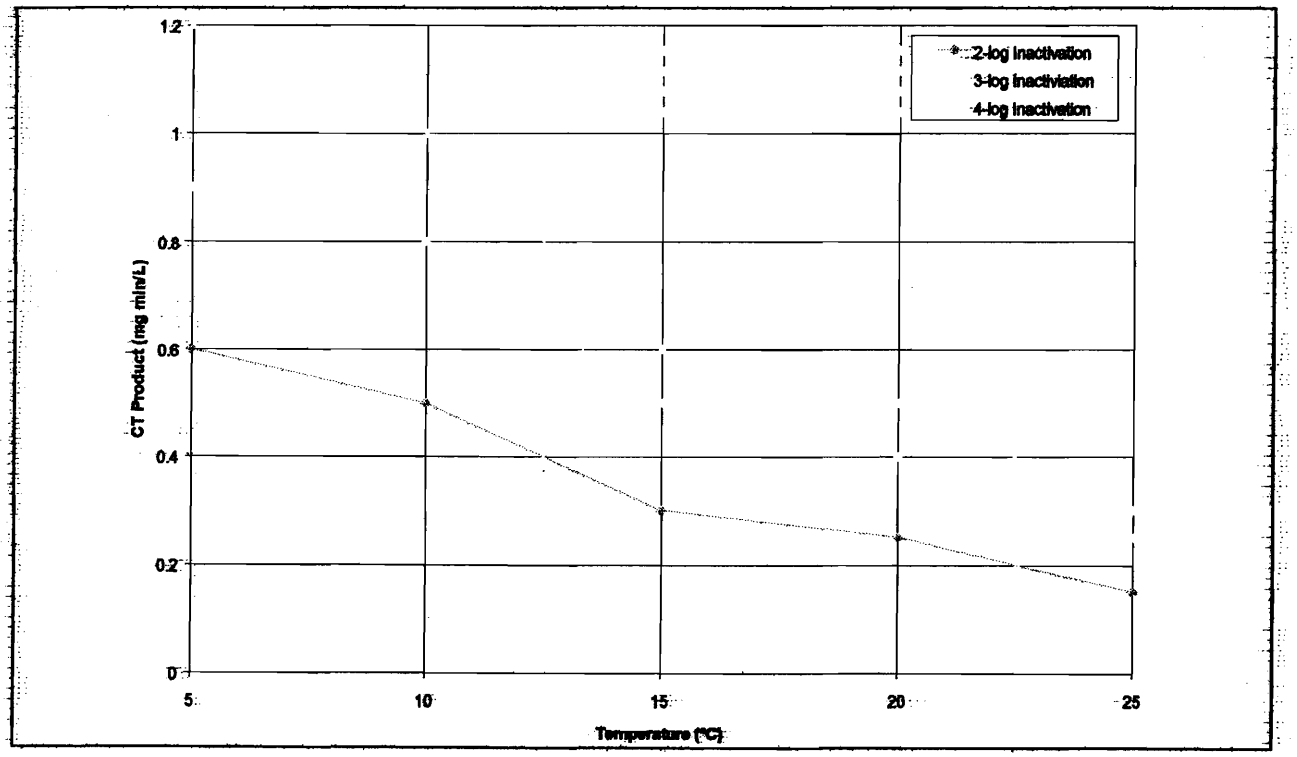


Figure 3-10. CT Values for Inactivation of Viruses by Ozone (pH 6 to 9)

Table 3-8. Summary of Reported Ozonation Requirements for 99 Percent Inactivation of *Cryptosporidium* Oocycts

Species	Ozone protocol	Ozone residual (mg/L)	Contact time (min)	Temperature (°C)	CT (mg.min/L)	Reference
C. baileyi	Batch liquid, modified batch ozone	0.6 & 0.8	4	25	2.4 - 3.2	Langlais et al., 1990
C. muris	Flow through contactor, continuous gas			22 - 25	7.8	Owens et al., 1994
C. parvum	Batch liquid, batch ozone	0.50 0.50	18 7.8	7 22	9.0 3.9	Finch et al., 1993
C. parvum	Batch liquid, batch ozone	0.77 0.51	6 8	Room	4.6 4	Peeters et al., 1989
C. parvum	Batch liquid, continuous gas	1.0	5 & 10	25	5 - 10	Korich et al., 1990
C. parvum	Flow through contactor, continuous gas			22 - 25	5.5	Owens et al., 1994

Ozone dose and contact time (CT) requirements for the inactivation of *Cryptosporidium* oocysts in drinking water when using ozone has not been established similar to the CT values for viruses and *Giardia* cyst inactivation. Inactivation requirements (log removals) for *Cryptosporidium* oocysts have not been established. In addition, as shown in Table 3-8, the CT requirements reported in the literature vary from study to study which adds uncertainty to design CT requirements for specific applications or regulatory needs.

3.4 Ozonation Disinfection Byproducts

Ozone does not form halogenated DBPs (TTHMs and HAA5s) when participating in oxidation/reduction reactions with NOM but it does form a variety of organic and inorganic byproducts. Table 3-9 and Figure 3-11 show the principal known byproducts associated with ozonation. However, if bromide ion is present in the raw water halogenated DBPs may be formed. These brominated DBPs appear to pose a greater health risk than non-brominated DBPs.

The DBPR provides reduced monitoring opportunities (i.e., quarterly rather than monthly samples) if the system demonstrates that the average source water bromide concentration is less than 0.05 mg/L based upon representative monthly bromide measurements for one year. Systems can remain on the reduced monitoring schedule until the running annual average source water bromide concentration, computed quarterly, is equal to or greater than 0.05 mg/L based upon representative monthly measurements.

For compliance monitoring for bromate, systems must use the ion chromatography analytical method as specified in *USEPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Revision 1.0* (USEPA, 1997).

If the average of samples covering any consecutive four-quarter period exceeds the MCL, the system is in violation of the MCL and must notify the public pursuant to 40 CFR §141.32. The system must also report to the State pursuant to 40 CFR §141.134. If the system fails to complete 12 consecutive months' monitoring, compliance with the MCL for the last four-quarter compliance period must be based on an average of the available data.

3.6 Operational Considerations

3.6.1 Process Considerations

Because ozone is such a strong oxidant, it will react with many organic and inorganic compounds present in the water. Ozone is used to remove tastes and odors by breaking down organic compounds, and to aid in the removal of iron and manganese by oxidizing these compounds to less soluble forms. These demands should be satisfied before any ozone is available to satisfy primary disinfection requirements. The presence and concentration of these compounds can dictate the location of ozone addition, depending on the process goals.

Stolarik and Christie (1997) present the results of 10 years of operation at the 600 mgd Los Angeles Aqueduct Filtration and Ozone Facility. Operational experiences at this facility showed lower particle counts (greater than one micron) with ozone use. The optimum ozone concentration in the gas phase applied was found to be 6 percent when using the cryogenic oxygen production facilities, and 4 to 5 percent when using liquid oxygen (LOX).

3.6.2 Space Requirements

Storage of LOX is subject to regulations in building and fire codes. These regulations will impact the space requirements and may dictate the construction materials of adjacent structures if the certain setback requirements cannot be met. In general, the footprint for ozone generated from air is smaller than that required for chloramination and chloride dioxide applications. However, the footprint area for ozone generated from pure oxygen is comparable to that of chlorine dioxide because of the additional area needed for storage.

3.6.3 Material Selection

Ozone-resistant materials should be used from the ozone generators through the off-gas destruct unit. If oxygen is used for the feed gas, oxygen resistant materials should be used up to the generators. Pure oxygen piping should be specially cleaned after installation for oxygen service, which increases construction cost. Materials for air preparation systems can be those normally used for compressed air systems. Langlais et al. (1991) recommended that piping beyond the desiccant dryers be ozone-resistant, as some backflow and ozone diffusion can occur. If a receiver is provided following the desiccant dryer, the piping should be ozone-resistant, downstream of the pressure regulator. Ozone-resistant (oxygen resistant as well if high purity oxygen is the feed gas) check valves should be placed in the piping ahead of the generator.

Ozone-resistant materials include the austenitic (300 series) stainless steels, glass and other ceramics, Teflon and Hypalon, and concrete. The 304 series stainless steels can be used for "dry" ozone gas (also for oxygen), 316 series should be used for "wet" service. Wet service includes piping in the contactors and all off-gas piping and the off-gas destruct unit. Teflon or Hypalon should be used for gasket materials. Concrete should be manufactured from Type II or Type IV cement. Typical practice in the United States is to provide 3 inches of cover for reinforcing to prevent corrosion by either ozone gas or ozone in solution, although Fonlupt (1979) reports that 4 cm (1.13 inches) is adequate for protection. Hatches for access into contactors should be fabricated from 316 series stainless steels and provided with ozone-resistant seals.

3.6.4 Ozone System Maintenance

Stolarik and Christie (1997) provide a good overview of the operational and maintenance requirements during the 10 years of operating the 600 mgd Los Angeles Aqueduct Filtration and Ozone Plant. The ozone system has been available 97.1 percent of the time over the 10 year period.

Fuse failure and generator cleaning comprised the major maintenance chores on the ozone generators during the first years. Fuse failure was caused by a malfunction when its glass dielectric tube failed. Vessels are cleaned every three years or when exit gas temperatures rise due to Fe_3O_4 deposits on the ground electrode/heat exchanger surfaces.

Rod shaped ceramic diffusers worked well as ozone diffusers for the initial two years. These were replaced by sintered stainless steel and ultimately a modified ceramic diffuser.

3.6.5 Ozone Safety

Concern for safety even at the risk of being overcautious, would be to follow practices that have been successfully applied to other oxidants over the years. This would be to generally isolate the ozonation system from the remainder of the plant. This should not be interpreted to mean a separate building, but rather separate rooms, separate exterior entrances, separate heating and ventilation systems, noise control, etc. This method already is manifested in some of the European ozonation plants, but on a lesser scale.

Ozone generators should be housed indoors for protection from the environment and to protect personnel from leaking ozone in the case of a malfunction. Ventilation should be provided to prevent excess temperature rise in the generator room, and to exhaust the room in the case of a leak. Adequate space should be provided to remove the tubes from the generator shell and to service the generator power supplies. Air prep systems tend to be noisy; therefore, it is desirable to separate them from the ozone generators. Off-gas destruct units can be located outside if the climate is not too extreme. If placed inside, an ambient ozone detector should be provided in the enclosure. All rooms should be properly ventilated, heated, and cooled to match the equipment-operating environment.

Continuous monitoring instruments should be maintained to monitor levels of ozone in the rooms. Self-contained breathing apparatuses should be located in hallways outside the rooms liable to ozone hazards. Ambient ozone exposure levels, which have been proposed by appropriate U.S. organizations, are summarized below. The maximum recommended ozone levels are as follows:

- **Occupational Safety and Health Administration.** The maximum permissible exposure to airborne concentrations of ozone not in excess of 0.1 mg/L (by volume) averaged over an eight-hour work shift.

- **American National Standards Institute/American Society for testing Materials (ANSI/ASTM).** Control occupational exposure such that the worker will not be exposed to ozone concentrations in excess of a time weighted average of 0.1 mg/L (by volume) for eight hours or more per workday, and that no worker be exposed to a ceiling concentration of ozone in excess of 0.3 mg/L 9by volume) for more than ten minutes.

- **American Conference of Government Industrial Hygienists (ACGIH).** Maximum ozone level of 0.1 mg/L (by volume) for a normal eight hour work day or 40 hour work week, and a maximum concentration of 0.3 mg/L (by volume) for exposure of up to 15 minutes.

- **American Industrial Hygiene Association.** Maximum, concentration for eight hour exposure of 0.1 mg/L (by volume).

There is a question of whether prolonged exposure to ozone may impair a worker's ability to smell or be aware of ozone levels at less than critical levels. Awareness of an odor of ozone should not be relied upon. Instrumentation and equipment should be provided to measure ambient ozone levels and perform the following safety functions:

- Initiate an alarm signal at an ambient ozone level of 0.1 mg/L (by volume). Alarms should include warning lights in the main control panel and at entrances to the ozonation facilities as well as audible alarms.

- Initiate a second alarm signal at ambient ozone levels of 0.3 mg/L (by volume). This signal would immediately shut down ozone generation equipment and would initiate a second set of visual and audible alarms at the control panel and at the ozone generation facility entrances. An

emergency ventilation system capable of exhausting the room within a period of 2 to 3 minutes also would be interconnected to the 0.3 mg/L ozone level alarm.

Ozone gas is a hazardous gas and should be handled accordingly. Ambient ozone levels should be monitored and equipment shut- down and alarmed when levels exceed 0.1 ppm. Emergency ventilation is typically provided for enclosed areas. Building and fire codes will provide additional guidance. The OSHA exposure limit for an 8-hour shift is 0.1 ppm by volume. The pungent odor of ozone will provide warning to operators of any possible ozone leak.

3.7 Summary

3.7.1 Advantages and Disadvantages of Ozone Use

The following list highlights selected advantages and disadvantages of using ozone as a disinfection method for drinking water (Masschelein, 1992). Because of the wide variation of system size, water quality, and dosages applied, some of these advantages and disadvantages may not apply to a particular system.

Advantages

- Ozone is more effective than chlorine, chloramines, and chlorine dioxide for inactivation of viruses, *Cryptosporidium*, and *Giardia*.
- Ozone oxidizes iron, manganese, and sulfides.
- Ozone can sometimes enhance the clarification process and turbidity removal.
- Ozone controls color, taste, and odors.
- One of the most efficient chemical disinfectants, ozone requires a very short contact time.
- In the absence of bromide, halogen-substitutes DBPs are not formed.
- Upon decomposition, the only residual is dissolved oxygen.
- Biocidal activity is not influenced by pH.

Disadvantages

- DBPs are formed, particularly by bromate and bromine-substituted DBPs, in the presence of bromide, aldehydes, ketones, etc.
- The initial cost of ozonation equipment is high.
- The generation of ozone requires high energy and should be generated on-site.
- Ozone is highly corrosive and toxic.
- Biologically activated filters are needed for removing assimilable organic carbon and biodegradable DBPs.
- Ozone decays rapidly at high pH and warm temperatures.
- Ozone provides no residual.

- Ozone requires higher level of maintenance and operator skill.

3.7.2 Summary Table

Table 3-12 presents a summary of the considerations for the use of ozone as a disinfectant.

Table 3-12. Summary of Ozone Disinfection Considerations

Consideration	Description
Generation	Because of its instability, ozone should be generated at the point of use. Ozone can be generated from oxygen present in air or high purity oxygen. The feed gas source should be clean and dry, with a maximum dewpoint of -60°C. Ozone generation consumes power at a rate of 8 to 17 kWhr/kg O_3. Onsite generation saves a lot of storage space.
Primary uses	Primary uses include primary disinfection and chemical oxidation. As an oxidizing agent, ozone can be used to increase the biodegradability of organic compounds destroys taste and odor control, and reduce levels of chlorination DBP precursors. Ozone should not be used for secondary disinfection because it is highly reactive and does not maintain an appreciable residual level for the length of time desired in the distribution system.
Inactivation efficiency	Ozone is one of the most potent and effective germicide used in water treatment. It is effective against bacteria, viruses, and protozoan cysts. Inactivation efficiency for bacteria and viruses is not affected by pH; at pH levels between 6 and 9. As water temperature increases, ozone disinfection efficiency increases.
Byproduct formation	Ozone itself does not form halogenated DBPs; however, if bromide ion is present in the raw water or if chlorine is added as a secondary disinfectant, halogenated DBPs, including bromate ion may be formed. Other ozonation byproducts include organic acids and aldehydes.
Limitations	Ozone generation is a relatively complex process. Storage of LOX (if oxygen is to be the feed gas) is subject to building and fire codes.
Points of application	For primary disinfection, ozone addition should be prior to biofiltration/filtration and after sedimentation. For oxidation, ozone addition can be prior to coagulation/sedimentation or filtration depending on the constituents to be oxidized.
Safety considerations	Ozone is a toxic gas and the ozone production and application facilities should be designed to generate, apply, and control this gas, so as to protect plant personnel. Ambient ozone levels in plant facilities should be monitored continuously.

3.8 References

1. Alceon Corp. 1993. Overview of Available Information on the Toxicity of Drinking Water Disinfectants and Their By-products. Cambridge, MA.

2. Amy, G.L., M.S. Siddiqui. 1991. "Ozone-Bromide Interactions in Water Treatment." Conference proceedings, AWWA Annual Conference, Philadelphia, PA.

3. AWWA (American Water Works Association). 1990. *Water Quality and Treatment.* F.W. Pontius (editor), McGraw-Hill, New York, NY.

4. Bablon, G.P., C. Ventresque, R.B. Aim. 1988. "Developing a Sand-GAC Filter to Achieve High Rate Biological Filtration." *J. AWWA.* 80(12):47.

5. Bablon, G., et al. 1991. "Practical Application of Ozone: Principles and Case Studies." *Ozone in Water Treatment Application and Engineering.* AWWARF.

6. Billen, G., et al. 1985. Action des Populations Bactériennes Vis-á-Vis des Matiéres Organiques dans les Filtres Biologiques. Report to Compagnie Générale des Eaux, Paris.

7. Boyce, D.S., et al. 1981. "The Effect of Bentonite Clay on Ozone Disinfection of Bacteria and Viruses in Water." *Water Res.* 15:759-767.

8. Bringmann, G. 1954. "Determination of the Lethal Activity of Chlorine and Ozone on *E. coli.*" *Z. f., Hygiene.* 139:130-139.

9. Chang, S.L. 1971. "Modern Concept of Disinfection." *J. Sanit. Engin. Division.* 97:689-707.

10. Cronholm, L.S., et al. 1976. "Enteric Virus Survival in Package Plants and the Upgrading of the Small Treatment Plants Using Ozone." Research Report No. 98, Water Resources Research Institute, University of Kentucky, Lexington, KY.

11. DeMers, L.D. and R.C. Renner. 1992. *Alternative Disinfection Technologies for Small Drinking Water Systems.* AWWARF and AWWA, Denver, CO.

12. Dimitriou, M.A. (editor). 1990. *Design Guidance Manual for Ozone Systems.* International Ozone Association, Norwalk, CN.

13. Domingue, E. L., et al. 1988. "Effects of Three Oxidizing Biocides on *Legionella pneumophila*, Serogroup 1." *Appl. Environ. Microbiol.* 40:11-30.

14. Eighmy, T.T., S.K. Spanos, J. Royce, M.R. Collins, J.P. Malley. 1991. "Microbial Activity in Slow Sand Filters." Conference proceedings, Slow Sand Filtration Workshop, Timeless Technology for Modern Applications, Durham, NH.

15. Farooq, S. et al.1977. "The Effect of Ozone Bubbles on Disinfection." *Progr. Water Ozone Sci. Eng.* 9(2):233.

16. Farooq, S. 1976. *Kinetics of Inactivation of Yeasts and Acid-Fast Organisms with Ozone.* Ph.D. Thesis, University of Illinois at Urbana-Champaign, IL.

17. Farvardin, M.R. and A.G. Collins. 1990. "Mechanism(s) of Ozone Induced Coagulation of Organic Colloids." Conference proceedings, AWWA Annual Conference, Cincinnati, OH.

18. Finch, G. R., E.K. Black, and L.L. Gyürék. 1994. "Ozone and Chlorine Inactivation of *Cryptosporidium.*" Conference proceedings, Water Quality Technology Conference, Part II, San Francisco, CA.

19. Franson, M.H., Eaton, A.D., Clesceri, L.S., and Greenberg, A.E., (editors). 1995. *Standard Methods for the Examination of Water and Wastewater, Nineteenth Edition.* American Public Health Association, AWWA, and Water Environment Federation, Washington D.C.

20. Georgeson, D.L. and A.A. Karimi. 1988. "Water Quality Improvements with the Use of Ozone at the Los Angeles Water Treatment Plant." *Ozone Sci. Engrg.* 10(3):255-276.

21. Giese, A.C. and E. Christensen. 1954. "Effects of Ozone on Organisms." *Physiol. Zool.* 27:101.

22. Glaze W.H., M. Koga, D. Cancilla. 1989a. "Ozonation Byproducts. 2. Improvement of an Aqueous-Phase Derivatizition Method for the Detection of Formaldehyde and Other Carbonyl Compounds Formed by the Ozonation of Drinking Water." *Environ. Sci. Technol.* 23(7):838.

23. Glaze, W.H., M. Koga M., D. Cancilla, et al. 1989b. "Evaluation of Ozonation Byproducts from Two California Surface Waters." *J. AWWA.* 1(8):66.

24. Glaze, W.H., et al.1987. "The Chemistry of Water Treatment Processes Involving Ozone, Hydrogen Peroxide, and Ultraviolet Radiation." *Ozone Sci. Engrg.* 9(4):335.

25. Glaze, W.H., and J.W. Kang. 1988. "Advanced Oxidation Processes for Treating Groundwater Contaminated with TCE and PCE: Laboratory Studies." *J. AWWA.* 88(5):57-63.

26. Glaze, W.H., H.S. Weinberg, S.W. Krasner, M.J. Sclimenti. 1991. "Trends in Aldehyde Formation and Removal Through Plants Using Ozonation and Biological Active Filters." Conference proceedings, AWWA, Philadelphia, PA.

27. Goldstein, B.D., and E.M. McDonagh. 1975. "Effect of Ozone on Cell Membrane Protein Fluorescence I. *in vitro* Studies Utilizing the Red Cell Membrane." *Environ. Res.* 9:179-186.

28. Gordon, G. K. Rankness, D. Vornehm, and D. Wood. 1989. "Limitations of the Iodometric Determination of Ozone." *J. AWWA.* 81(6):72-76.

29. Gordon, G., W.J. Cooper, R.G. Rice, and G.E. Pacey. 1992. *Disinfectant Residual Measurement Methods,* second edition. AWWARF and AWWA, Denver, CO.

30. Gurol, M.D. and M. Pidatella. 1983. "A Study of Ozone-Induced Coagulation." Conference proceedings, ASCE Environmental Engineering Division Specialty Conference. Allen Medine and Michael Anderson (editors), Boulder, CO.

31. Haag, W.R. and J. Hoigné. 1984. "Kinetics and Products of the Reactions of Ozone with Various Forms of Chlorine and Bromine in Water." *Ozone Sci. Engrg.* 6(2):103-14.

32. Hann, V.A. 1956. "Disinfection of Drinking Water with Ozone." *J. AWWA.* 48(10):1316.

33. Harakeh, M.S. and M. Butler. 1984. "Factors Influencing the Ozone Inactivation of Enteric Viruses in Effluent." *Ozone Sci. Engrg.* 6:235-243.

34. Hiltebrand, D.J., A.F. Hess, P.B. Galant, and C.R. O'Melia. 1986. "Impact of Chlorine Dioxide and Ozone Preoxidation on Conventional Treatment and Direct Filtration Treatment Processes." Conference proceedings, AWWA Seminar on Ozonation: Recent Advances and Research Needs, Denver, CO.

35. Hoff, J.C. 1986. *Inactivation of Microbial Agents by Chemical Disinfectants*, U. S. Environmental Protection Agency, EPA/600/2-86/067.

36. Hoigné J. and H. Bader. 1976. Role of Hydroxyl Radical Reactions in Ozonation Processes in Aqueous Solutions, *Water Res.* 10: 377.

37. Hoigné J., and H. Bader. 1988. "The Formation of Trichloronitromethane (chloropicrin) and Chloroform in a Combined Ozonation/Chlorination Treatment of Drinking Water." *Water Res.* 22(3):313.

38. Hoigné J., and H. Bader. 1983b. "Rate Constants of Reaction of Ozone with Organic and Inorganic Compounds in Water - II. Dissociating Organic Compounds." *Water Res.* 17:185-194.

39. Hoigné J., and H. Bader. 1977. "The Role of Hydroxyl Radical Reactions in Ozonation Processes in Aqueous Solutions." *Water Res.* 10:377-386.

40. Hoigné J., and H. Bader. 1983a. "Rate Constants of Reaction of Ozone with Organic and Inorganic Compounds in Water - I. Non-dissociating Organic Compounds." *Water Res.* 17:173-183.

41. Hoigné, J. and Bader, H. 1975. Ozonation of Water: Role of Hydroxyl Radicals as Oxidizing Intermediates. *Science*, Vol. 190, pp. 782.

42. Huck, P.M., P.M. Fedorak, and W.B. Anderson. 1991. "Formation and Removal of Assimilable Organic Carbon During Biological Treatment." *J. AWWA.* 83(12):69-80.

43. IOA (International Ozone Association). 1989. *Photometric Measurement of Low Ozone Concentrations in the Gas Phase.* Standardisation Committee--Europe.

44. Katz, J. 1980. *Ozone and Chlorine Dioxide Technology for Disinfection of Drinking Water.* Noyes Data Corporation, Park Ridge, New Jersey.

45. Katzenelson, E., et al. 1974. "Inactivation Kinetics of Viruses and Bacteria in Water by Use of Ozone." *J. AWWA.* 66:725-729.

46. Keller, J.W., R.A. Morin, and T.J. Schaffernoth. 1974. "Ozone Disinfection Pilot Plants Studies at Laconia, New Hampshire." *J. AWWA.* 66:730.

47. Kim, C.K., et al. 1980. "Mechanism of Ozone Inactivation of Bacteriophage f2." *Appl. Environ. Microbiol.* 39:210-218.

48. Kinman, R.N. 1975. "Water and Wastewater Disinfection with Ozone: A Critical Review." *Crit. Rev. Environ. Contr.* 5:141-152.

49. Krasner, S.W., W.H. Glaze, H.S. Weinberg, et al. 1993. "Formation of Control of Bromate During Ozonation of Water Containing Bromide." *J. AWWA.* 85(5):62..

50. Krasner, S.W., et al. 1989. "The Occurrence of Disinfection By-products in US Drinking Water." *J. AWWA.*81(8):41.

51. Langlais, B., et al. 1990. "New Developments: Ozone in Water and Wastewater Treatment. The CT Value Concept for Evaluation of Disinfection Process Efficiency; Particular Case of Ozonation for Inactivation of Some Protozoa, Free-Living Amoeba and *Cryptosporidium*." Presented at the Int. Ozone Assn. Pan-American Conference, Shreveport, Louisiana, March 27-29.

52. Langlais, B., D.A. Reckhow, and D.R. Brink (editors). 1991. *Ozone in Drinking Water Treatment: Application and Engineering.* AWWARF and Lewis Publishers, Boca Raton, FL.

53. Langlais B. and D. Perrine. 1986. "Action of Ozone on Trophozoites and Free Amoeba Cysts, Whether Pathogenic of Not." *Ozone Sci. Engrg.* 8(3):187-198.

54. LeChevallier, M.W., W.C. Becker, P. Schorr, and R.G. Lee. 1992. "Evaluating the Performance of Biologically Active Rapid Filters." *J. AWWA.* 84(4):136-146.

55. LeLacheur, R.M., P.C. Singer, and M.J. Charles. 1991. "Disinfection By-products in New Jersey Drinking Waters." Conference proceedings, AWWA Annual Conference, Philadelphia, PA.

56. Logsdon, G.S., S. Foellmi, and B. Long. 1992. "Filtration Pilot Plant Studies for Greater Vancouver's Water Supply." Conference proceedings, AWWA Annual Conference, Vancouver, British Columbia.

57. Malley, J.P., T.T. Eighmy, M.R. Collins, J.A. Royce, and D.F. Morgan. 1993. "The True Performance and Microbiology of Ozone - Enhanced Biological Filtration." *J. AWWA.* 85(12):47-57.

58. Masschelein, W.J. 1992. "Unit Processes in Drinking Water Treatment." Marcel Decker D,C., New York , Brussels, Hong Kong.

59. Masschelein, W.J. 1977. "Spectrophotometric Determination of Residual Ozone in Water with ACVK." *J. AWWA.* 69:461-462.

60. McGuire, M.J., S.W. Krasner, and J. Gramith. 1990. Comments on Bromide Levels in State Project Water and Impacts on Control of Disinfection Byproducts Metropolitan Water District of Southern California.

61. McKee, H.C., R.E. Childers, and V.B. Parr 1975. *Collaborative Study of Reference Method for Measurement of Photochemical Oxidants in the Atmosphere*, EPA EPA-650/4-75-016, Washington, D.C. February.

62. McKnight A., and D.A. Reckhow. 1992. "Reactions of Ozonation Byproducts with Chlorine and Chloramines." Conference proceedings, AWWA Annual Conference, Vancouver, British Columbia.

63. MWDSC and JMM (Metropolitan Water District of Southern California and James M. Montgomery Consulting Engineers). 1992. "Pilot Scale Evaluation of Ozone and peroxone." AWWARF and AWWA, Denver, CO.

64. Morris, J.C. 1975. "Aspects of the Quantitative Assessment of Germicidal Efficiency." *Disinfection: Water and Wastewater.* J.D. Johnson (editor). Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

65. Owens, J. H., et al. 1994. "Pilot-Scale Ozone Inactivation of *Cryptosporidium* and *Giardia*." Conference proceedings, Water Quality Technology Conference, Part II, San Francisco, CA.

66. Peeters, J. E. et al. 1989. "Effect of Disinfection of Drinking Water with Ozone or Chlorine Dioxide on Survival of *Cryptosporidium parvum* Oocysts." *Appl. Environ. Microbiol.* 55(6):1519-1522.

67. Perrine, D., et al. 1984. "Action d l'Ozone sur les Trophozoites d'Amibes Libres Pathogens ou Non." *Bull Soc.Frnac.* Parasitol. 3:81.

68. Prendiville, D.A. 1986. "Ozonation at the 900 cfs Los Angeles Water Purification Plant." *Ozone Sci. Engrg.* 8:77.

69. Price, M.L. 1994. *Ozone and Biological Treatment for DBP Control and Biological Stability.* AWWARF and AWWA, Denver, CO, pp. 252.

70. Rachwal, A.J., et al. 1988. "Advanced Techniques for Upgrading Large Scale Slow Sand Filters." *Slow Sand Filtration- Recent Developments in Water Treatment Technology*, Ellis Horwood Ltd, Chichester, U.K.

71. Reckhow, D.A., J.K. Edzwald, and J.E. Tobiason. 1993. "Ozone as an Aid to Coagulation and Filtration." AWWARF and AWWA, Denver, CO.

72. Reckhow, D.A., P.C.Singer, and R.R. Trussell. 1986. Ozone as a coagulant aid. Seminar proceedings, Ozonation, Recent Advances and Research Needs, AWWA Annual Conference, Denver, CO.

73. Reckhow, D.A., J.E. Tobiason, M.S. Switzenbaum, R. McEnroe, Y. Xie, X. Zhou, P. McLaughlin, and H.J. Dunn. 1992. "Control of Disinfection Byproducts and AOC by Pre-Ozonation and Biologically Active In-Line Direct Filtration." Conference proceedings, AWWA Annual Conference, Vancouver, British Columbia.

74. Regli, S., J.E. Comwell, X. Zhang., et al. 1992. *Framework for Decision Making: An EPA Perspective.* EPA 811-R-92-005, EPA, Washington, D.C.

75. Rehme, K.A., J.C. Puzak, M.E. Beard, C.F. Smith, and R.J. Paur. 1980. *Evaluation of Ozone Calibration Procedures*, EPA-600/S4-80-050, EPA, Washington, D.C, February.

76. Renner, R.C., M.C. Robson, G.W. Miller, and A.G. Hill. 1988. "Ozone in Water Treatment - The Designer's Role." *Ozone Sci. Engrg.* 10(1):55-87.

77. Rice, R.G. 1996. Ozone Reference Guide. Electric Power Research Institute, St. Louis, MO.

78. Rice, R.G., P.K. Overbeck, K. Larson. 1998. Ozone Treatment for Small Water Systems. Presented at the First International Symposium on Safe Drinking water in Small Systems. NSF International/PAHP/WHO, Arlington, VA, May 10-13, 1998.

79. Riesser, V.W., et al. 1976. "Possible Mechanisms of Poliovirus Inactivation by Ozone." *Forum on Ozone Disinfection*, E. G. Fochtman, R.G. Rice, and M.E. Browning (editors), pp. 186-192, International Ozone Institute, Syracuse, NY.

80. Rittman, B.E. 1990. "Analyzing Biofilm Processes Used in Biological Filtration." *J. AWWA.* 82(12):62.

81. Roy, D. 1979. *Inactivation of Enteroviruses by Ozone.* Ph.D. Thesis, University of Illinois at Urbana-Champaign.

82. Roy, D., R.S. Engelbrecht, and E.S.K. Chian. 1982. "Comparative Inactivation of Six Enteroviruses by Ozone." *J. AWWA.* 74(12):660.

83. Scott, D.B.M. and E.C. Lesher. 1963. "Effect of Ozone on Survival and Permeability of Escherichia coli." *J. Bacteriol.* 85:567-576.

84. Singer P.C. 1992. "Formation and Characterization of Disinfection Byproducts." Presented at the First International Conference on the Safety of Water Disinfection: Balancing Chemical and Microbial Risks.

85. Singer, P.C., et al. 1989. "Ozonation at Belle Glade, Florida: A Case History." Conference proceeding, IOA Ninth Ozone World Conference.

86. Song, R., et al. 1997. "Bromate Minimization During Ozonation." *J. AWWA.* 89(6):69.

87. Sproul, O. J., et al. 1982. "The Mechanism of Ozone Inactivation of Waterborne Viruses." *Water Sci. Technol.* 14:303-314.

88. Staehelin, J., R.E. Bühler, and J. Hoigné. 1984. "Ozone Decomposition in Water Studies by Pulse Radiolysis. 2 OH and HO_4 as Chain Intermediates." *J. Phys. Chem.* 88:5999-6004.

89. Stolarik, G. F., and J.D. Christie. 1997. "A Decade of Ozonation in Los Angeles." Conference proceedings, IOA Pan American Group Conference, Lake Tahoe, NV.

90. Suffet, I.H., C. Anselme, and J. Mallevialle. 1986. "Removal of Tastes and Odors by Ozonation." Conference proceedings, AWWA Seminar on Ozonation: Recent Advances and Research Needs, Denver, CO.

91. Tobiason, J.E., J.K. Edzwald, O.D. Schneider, M.B. Fox, and H.J. Dunn. 1992. "Pilot Study of the Effects of Ozone and Peroxone on In-Line Direct Filtration." *J. AWWA.* 84(12):72-84.

92. Tomiyasu, H., and G. Gordon. 1984. "Colorimetric Determination of Ozone in Water Based on Reaction with Bis-(terpyridine)iron(II)." *Analytical Chem.* 56:752-754.

93. Troyan, J.J. and S.P. Hansen. 1989. *Treatment of Microbial Contaminants in Potable Water Supplies Technologies and Costs.* Noyes Data Corporation, Park Ridge, New Jersey.

94. Umphries, M.D., et al. 1979. "The Effects of Pre-ozonation on the Formation of Trihalomethanes." *Ozonews.* 6(3).

95. USEPA. 1997. USEPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Revision 1.0. EPA A/600/r-98/118.

96. Van Dijk, J.F.M., and R.A. Falkenberg. 1977. "The Determination of Ozone Using the Reaction with Rhodamine B/Gallic Acid." Presented at Third Ozone World Congress sponsored by the IOA, Paris, France, May.

97. Van Gunten, U. and J. Hoigné. 1996. "Ozonation of Bromide-Containing Waters: Bromate Formation through Ozone and Hydroxyl Radicals." *Disinfection By-Products in Water Treatment*, Minear, R.A. and G.L. Amy (editors). CRC Press, Inc., Boca Raton, FL.

98. Van Hoof, F., J.G. Janssens, H. van Dijck. 1985. "Formation of Mutagenic Activity During Surface Water Pre-ozonation and Its Removal in Drinking Water Treatment." *Chemosphere,* 14(5):501.

99. Vaughn, J.M., et al. 1987. "Inactivation of Human and Simian Rotaviruses by Ozone." *Appl. Env. Microbiol.* 53:2218-2221.

100. Walsh, D.S., et al. 1980. "Ozone Inactivation of Floc Associated Viruses and Bacteria." *J. Environ. Eng. Div.* ASCE. 106:711-726.

101. Ward, S.B. and D.W. Larder. 1973. "The Determination of Ozone in the Presence of Chlorine." *Water Treatment Examination.* 22:222-229.

102. Wickramanayake, G.B., et al. 1984b. "Inactivation of Giardia lamblia Cysts with Ozone." *Appl. Env. Microbiol.* 48(3):671-672.

103. Wickramanayake, G.B., et al. 1984a. "Inactivation of Naegleria and Giardia cysts in Water by Ozonation." *J. Water Pollution Control Fed.* 56(8):983-988.

104. Wickramanayake, G.B. 1984c. *Kinetics and Mechanism of Ozone Inactivation of Protozoan Cysts.* Ph.D. dissertation, Ohio State University, Columbus, OH.

105. Wuhrmann, K., and J. Meyrath. 1955. "The Bactericidal Action of Ozone Solution. *Schweitz." J. Allgen. Pathol. Bakteriol.*, 18:1060.

106. Yamada, H. and I. Somiya. 1989. "The Determination of Carbonyl Compounds in Ozonated Water By the PFBOA Method." *Ozone Sci. Engrg.* 11(2):127.

107. Zabel, T.F. 1985. "The Application of Ozone for Water Treatment in the United Kingdom - Current Practice and Recent Research." *Ozone Sci. Engrg.* 7(1):11.

Author: **Utriainen, J. and T. Holopainen. 2001.**

Nitrogen Availability modifies the ozone responses of Scots pine seedlings exposed inan open-field system.

Tree Physiology. 21:1205-13.

Abstract:
Three-year old Scots pine (Pinus sylvestrisL.) seedlings were exposed to either ambient or elevated (1.5-1.6x ambient) ozone concentrations [O3] for three growing seasons in an open field fumigation facility where they were irrigated during the growing season with a nutrient solution providing nitrogen (N) at 70 (LN treatment), 100 (control) or 150% (HN treatment of the optimum supply rate. Treatment effects were most evident during the third year of exposure when the ambient [O] + HN treatment enhanced whole-plant biomass, root/shoot dry weight ration, needle pigment concentrations and the number of chloroplast plastoglobuli in the mesophyll cells in current-year (C) needles, whereas it reduced starcjh accumulation in C needles and abscission pf 2-year-old (C + 2) needles. In the control fertilization, 3 years of exposure to elevated [O3] decreased stem-base diameter and increased K concentration and electron density of chloropolast stroma in C needles. Plants in the HN treatment exposed for 3 years to elevated [O3] had significantly lower heights, current-year main shoot length and root/shoot dry mass ratio than control plants, and increased abscission of C+2 needles. In contrast, O3-induced changes in the ultrastructure of mesophyll cells were most evident in seedlings grown for 3 years in the LN treatment. We conclude that, in Scots pine, a relatively O3-tolerant species, chronic O3 exposure leads to cumulative growth reduction, increased needle abscission and changes in carbon allocation that are strongly influenced by plant N availability.

E-951, Water Quality Handbook for Nurseries
Oklahoma Cooperative Extension Service

7. Capturing and Recycling Irrigation Water to Protect Water Supplies

Sharon L. von Broembsen, Extension Plant Pathologist

The preceding chapters describe irrigation, nutrient, and pest management practices for reducing nutrients and pesticides in runoff water leaving a nursery site. This chapter presents a different approach in which water quality is protected by retaining irrigation runoff on the nursery site and then reusing it within the nursery. This capture and recycle strategy may have significant advantages for some nurseries.

The runoff standards that nurseries must meet vary with geographic location. Those located near outstanding resource waters or large population centers are likely to bear the most scrutiny. Since 1990, the Oklahoma Department of Agriculture has been monitoring nutrients and pesticides in runoff from ornamental nurseries in the Illinois River Basin (an Oklahoma designated Scenic River) to establish baselines for nursery effluents. More importantly, it also has been working with these nurseries to reduce effluent contamination to acceptable levels through a voluntary compliance program. This program has been very successful--the cooperating nurseries have made the management changes necessary to achieve the target levels and have shown the public that they are doing their part toward protecting water quality. Although no statewide standards have been set for nursery effluents so far, the Illinois River Basin studies would allow realistic levels to be set for Oklahoma nurseries.

Most nurseries have found it difficult not to exceed the discharge limits occasionally--mistakes, miscalculations, and accidents do happen. Some nurseries have found that certain pollution prevention practices do not fit their production methods. In such cases, the most reliable pollution control may be achieved by capturing and recycling runoff. In this way, potential contaminants are totally contained on site. With the capture and recycle approach, runoff is captured in retention basins, mixed with fresh water as appropriate, and recycled onto crops.

The design of a system to capture and recycle irrigation water must be site specific. The number of retention basins needed to capture runoff depends on the topography of the nursery. Sites with only one major gradient might only need one retention basin, but most nursery sites have more than one gradient and require more retention basins. In the basic system, runoff is captured at

low points in the nursery, then pumped to a storage pond at a high point for redistribution. Captured runoff water can be treated before or during transfer to storage to eliminate plant pathogens or improve water quality. The quality of captured runoff can also be improved by mixing it with fresh water before reuse.

During storm events, retention basins may not be able to retain all the runoff from a nursery site, particularly if it receives off-site drainage. Provisions should be made to hold a minimal amount of storm water before discharge occurs. This is important because the pollutant levels in the first flush of storm water through a system can be high. After this initial phase, however, the concentration of pollutants in discharged water is usually much lower than in normal irrigation runoff because dilution occurs. Although no retention limits have been set for Oklahoma, other states require that 1/2 to 1 inch of storm water be retained before discharging. Most rain events do not produce enough storm water to exceed these retention limits and so do not result in discharge. Discharge of storm water is governed by a different set of permitting regulations than normal day-to-day runoff from irrigation.

Capturing and recycling runoff is not a substitute for good pollution prevention practices. These management practices should be well established before implementing the capture and recycle strategy. Most existing nurseries adopting the capture and recycle strategy choose to phase in the installation process over a period of years. Different parts of a nursery may be placed under the capture and recycle approach, starting with those that lend themselves to this most easily or those with the most serious water quality problem. By phasing in capture and recycle technology, the capital outlay can be spread over a number of years and adjustments in management practices made gradually.

Other Advantages of the Capture and Recycle Strategy

In addition to protecting the environment, the capture and recycle strategy has many other important advantages. In fact, many nurseries had already adopted capture and recycling systems to deal with their specific needs even before the current emphasis on environmental protection. For some nurseries, the most important reason to adopt capture and recycle methods has been that using recycled water can result in major savings on the cost of water. For others, the most compelling reason has been to assure that an adequate supply of sufficiently high quality water would be available when needed during production.

Water costs vary greatly depending on the source. Water may have to be purchased from an expensive community water supply. It may need to be pumped a significant distance from underground or surface supplies, entailing high electrical costs for pumping. Source water may require treatment--e.g., by flocculation, filtration, acidification, or decontamination--before it can be

used for crop production. All these factors contribute to the final cost of irrigation water. For many production systems, a significant portion of that cost is lost when runoff leaves the nursery. Some nurseries cannot be sure they will be able to acquire enough good quality water for their needs at any cost. Water supplies may be unavailable, restricted, or poor quality during drought periods when production need is great. Capturing storm water and irrigation runoff and storing it for later use is advantageous in these situations.

Some nurseries faced with tightly managing nutrients and pesticides to keep these constantly below effluent limits have switched to the capture and recycle strategy. This allows more flexibility in the use of different forms of fertilizers for different stages of plant growth, in scheduling applications, and in meeting emergencies such as disease outbreaks. For example, soluble fertilizers can be used for propagation and for pushing the growth of certain crops, and slow-release fertilizers can be used at higher levels without concern about spikes of nutrients in effluents. The capture and recycle method also acts as a safety net in case of an accident, mistake, or miscalculation, particularly with regard to pesticides. Finally, the capture and recycle strategy demonstrates to the public a clear effort to protect the environment.

Disadvantages of the Capture and Recycle Strategy

There are several disadvantages to the capture and recycle strategy. The most obvious is the cost of retention basins, storage ponds, and additional pumping capacity. These costs can, in many cases, be recovered through savings in water costs. New types of management skills will be needed to manage a recycling system, with the inevitable learning curve of any new technology. There has also been some fear that recycled herbicides could damage sensitive crops, but this can be avoided with proper management. Likewise, buildup of salts in recycled water can be effectively managed by dilution with fresh water if this becomes a problem.

However, the main disadvantage of the capture and recycle strategy may be the possibility that waterborne pathogens recycled back onto crops will increase disease problems and force nurseries to decontaminate recycled water. Studies have shown that plant pathogenic fungi such as Phytophthora and Pythium spp. are present in nursery runoff at relatively high concentrations and can sometimes be detected in recycled irrigation water at the point of delivery to crops. Since there are no scientifically derived thresholds for levels of pathogens in irrigation water, it is easy to see why growers may feel compelled to decontaminate recycled irrigation water before reuse. On the other hand, many nurseries have been recycling irrigation water for years without decontaminating and have not experienced increased disease problems.

Managing Plant Pathogens in Recycled Irrigation Water

When it comes to managing plant pathogens in recycled irrigation water, every nursery situation is unique. But an important first step in any situation is to determine if pathogens are present in irrigation water and to what extent. Once this is done, various management practices can be considered to reduce contamination.

Samples should be taken at the irrigation water source, at points of runoff, and at points where recycled water is delivered back to plants. These samples can be analyzed by a diagnostic laboratory for pathogens of importance, such as *Pythium* and *Phytophthora* spp. Another practical way to sample irrigation water is to use plant parts--e.g. lemon or rhododendron leaves-- to "bait" these pathogens out of the water. For more information on sampling and testing irrigation water for plant pathogens, contact the Plant Disease Diagnostic Laboratory, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.

The risk of using recycled irrigation water can best be evaluated by looking for pathogens in recycled water at the points of reuse. Even where pathogens are present in runoff water at relatively high concentrations, there may be few or no pathogens detectable at the points of reuse. This results because there are natural processes acting in the system to reduce pathogens. Plant pathogens tend to settle to the bottom of retention basins and storage ponds during even limited storage. Natural biological and physical processes, such as microbial degradation or unfavorable water conditions, destroy many plant pathogens or render them unable to infect. Finally, if captured water is mixed with fresh water before reuse, any remaining pathogens will be diluted even further.

Crops that are highly susceptible to waterborne pathogens such as *Phytophthora* spp. (e.g., rhododendron, citrus, Lawson cypress, dogwood) should be grouped together in the same part of the nursery. That way, pathogen-free fresh water can be reserved for these areas and for propagation. Recycled water that is used with no treatment other than settling, holding, and dilution should be used only for hardier or more mature plants that are relatively resistant to waterborne pathogens. By following these strategies, nurseries may find that decontamination of recycled water is not necessary. However, if large parts of the nursery contain crops highly susceptible to waterborne pathogens, decontamination of recycled water may be warranted.

Another consideration in devising any overall disease management strategy that is often overlooked is the need to test the irrigation water source for plant pathogens. Ground water drawn from properly constructed wells and water for human consumption should be pathogen free. However, water drawn from surface sources such as lakes and rivers may contain waterborne pathogens and may require decontamination before use in propagation or highly susceptible crops.

If decontamination of source water or recycled water is warranted, there are a

number of options. First, filtration may be used to eliminate plant pathogens. Modern sand filters remove most plant pathogens, except bacteria and viruses, to a practical level, but do not sterilize the water. This leaves many of the natural biological control organisms in place, which is an important advantage. Microfiltration to smaller pore sizes removes almost all plant pathogens, but it is only useful for low flow rates and low volumes such as those required in propagation areas. Several more stringent methods of decontamination can be used, provided water is filtered to a reasonably clean level before treatment. These decontamination methods have been adapted from purification methods for drinking water or swimming pool water and include the use of ultraviolet light, ozone, or chlorine. These are all effective in eliminating plant pathogens and other microorganisms, but they require careful management to achieve the desired effect.

Summary

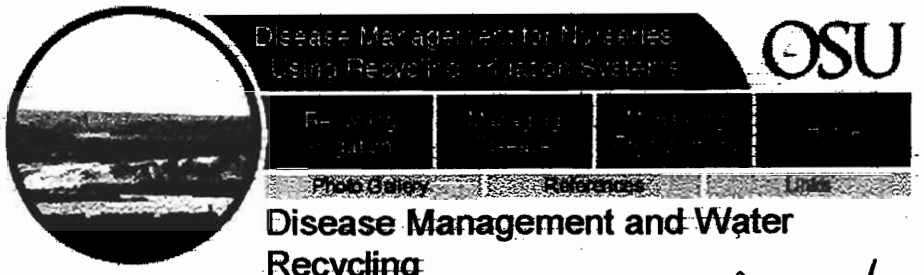
The capture and recycle strategy used in conjunction with other pollution prevention practices is an effective way to protect the quality of water supplies. It also has many other advantages for nurseries, such as reduced water costs, an assured supply of good quality water, and more flexibility in crop production. The major drawbacks of this strategy are the cost of building retention basins and storage ponds and the potential impact of using recycled water on disease management. It may not be the answer for every nursery; however, nurseries currently using the capture and recycle strategy are strong proponents of this technology as advancement for the nursery industry.

Shown here is a retention basin for capturing irrigation runoff and equipment for pumping captured water to a storage pond at a higher point in the nursery. There, the water can be mixed with fresh water and held for later use.

E-951 Home Page

P*E*A*R*L

Oklahoma Cooperative Extension Service
Division of Agricultural Sciences and Natural Resources



Disease Management and Water Recycling

Von Broembsen, S. L. 2002. Disease Management for Nurseries Using Recycling Irrigation Systems. Stillwater: Oklahoma State University

~~http://~~ http://zoospore.okstate.edu/nursery/index.html

Water recycling effects all aspects of a nursery's management. Disease management becomes a critical aspect of the overall management program. Most successful nurseries will already have an effective disease management program in place. However as recycling is implemented, certain practices within that program should receive higher priority.

"Routine scouting, especially of plant materials susceptible to water borne pathogens such as Phytophthora spp., should be a high priority with recycling irrigation."

Routine scouting for disease is very important. Scouting should occur weekly for all plant materials. Crops highly susceptible to *Phytophthora* spp. or other waterborne pathogens should receive careful scrutiny. Routine scouting will have the added benefit of increasing confidence in the recycling system by demonstrating that the overall disease management program is working. It should not be necessary to apply either additional preventative treatments or remedial treatments.

Nursery sanitation is also a very important factor for recycling systems. Many plant pathogens produce large numbers of infective structures, called propagules, during the disease process. This is especially true of waterborne root pathogens, such as *Phytophthora* spp. Diseased plants will release pathogen propagules into drainage water and will increase the levels of these pathogens in captured runoff. Removing diseased plants quickly from the system

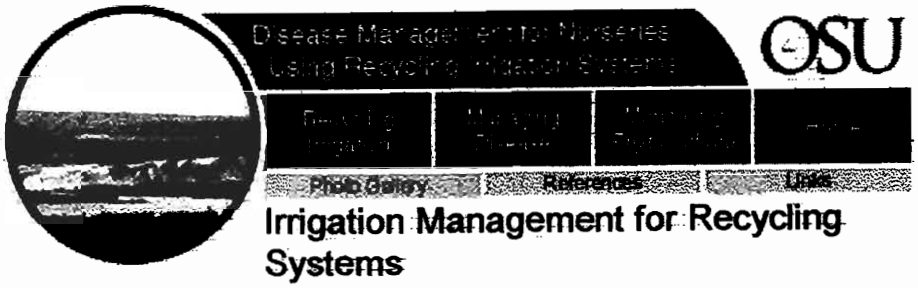
"Diseased plants should be removed quickly to prevent pathogens from draining into runoff."

priority.

Research has shown that irrigation runoff often contains significant numbers of plant pathogens such as *Phytophthora* spp. and that when this water is reused to irrigate healthy susceptible plants, root infections can result. But many factors (e.g., plant susceptibility and age, pathogen concentrations, cultural practices, environmental conditions) will interact to determine if disease results. It is important that all these factors be taken into consideration when making decisions about implementing disease management practices with recycling systems.

And finally, irrigation water in different parts of a recycling irrigation system will be of different quality with respect to plant pathogens. Careful irrigation management will be needed to make sure that the pathogen status of each type of water matches the susceptibility of the plant materials receiving that water.

Back to Managing Disease in Recycling Irrigation Systems



Irrigation Management for Recycling Systems

Assessing the Pathogen Status of Irrigation Water

Before specific irrigation management practices to prevent, reduce or eliminate pathogens in irrigation water can be considered, the extent to which pathogens are present in irrigation water must be determined. Water should be sampled at various points in the irrigation system, since pathogens may be problematic only in certain parts of the system. Samples should be taken at the irrigation water source, at points of runoff, and at points where recycled water is delivered back to plants. Water samples also should be taken at different times during the production cycle as pathogens may be seasonal and affected by changes in production practices.

"Source water drawn from lakes, ponds, canals and streams should be sampled and tested for pathogens."

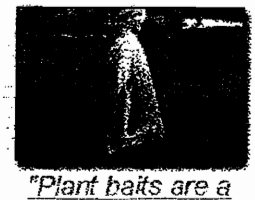
An important but often overlooked consideration for all nurseries is the need to test the source of irrigation water for plant pathogens. Ground water drawn from properly constructed wells and water suitable for human consumption should be pathogen free. However, water drawn from surface water sources such as lakes and rivers may contain waterborne pathogens and may require decontamination before use in propagation operations and for susceptible crops.

"Samples of runoff should be taken at fast flowing points in channels and ditches."

Sampling runoff from different parts of the nursery will determine if captured runoff is

"Recycled water should be sampled at the points where it is irrigated back onto crops."

extent. The pathogen status of recycled irrigation water can best be evaluated by assaying for pathogens in water collected at the points of reuse.



"Plant baits are a practical method for detecting pathogens in irrigation water."

A diagnostic laboratory can analyze water samples for waterborne pathogens and should be consulted before samples are taken for specific instructions on how to take and submit samples. A practical way to sample irrigation water is to place certain plant parts, e.g. lemon leaves or green pears, in irrigation water where they can become infected by plant pathogens that may be in the water. These plant "baits" are very sensitive to low levels of pathogens. Plant bait samples can also be analyzed by a diagnostic laboratory or can be tested on-site with commercially available detection kits.

For more information about water sampling and analysis for *Phytophthora* spp. see Methods for Monitoring *Phytophthora* spp.

Matching Pathogen Status With Irrigation Use

Once the pathogen status of water in different parts of the recycling system are know, different qualities of water can be used accordingly. Crops that are highly susceptible to water-borne pathogens such as *Phytophthora* spp. (e.g. rhododendron, citrus, Lawson cypress, and dogwood) should be grouped together in the same part of the nursery. That way pathogen-free fresh water or water that has been decontaminated can be reserved for these areas and for propagation areas. Where recycled water is used with no treatment other than retention and dilution, it should only be used for hardier or more mature plants that tend to be more resistant to waterborne pathogens. There is little known about acceptable thresholds for different pathogens on different crops.

By following these strategies, nurseries may find that stringent decontamination of all recycled water is not necessary. However, if large parts of the nursery contain crops susceptible to waterborne pathogens, treating water to remove plant pathogens from recycled water is advised.

Treating Irrigation Water to Remove Plant Pathogens

If decontamination of source water or recycled water is warranted, there are a number of options. Retention and dilution of contaminated runoff can greatly reduce the levels of plant pathogens present. Pathogens tend to settle out of contaminated water while it is stored and pathogen concentrations can be reduced by dilution with water that is free of pathogens. Filtration may be used to remove plant pathogens. Modern sand filters which use graded sand reduce the numbers of fungal spores and nematodes in water, but have little effect on bacteria. Micro-filtration to even smaller pore sizes can remove most fungal and bacterial plant pathogens, but it is only useful for low flow rates and low volumes such as those required in propagation areas and greenhouses.


Retention and dilution


Filtration


Chlorination

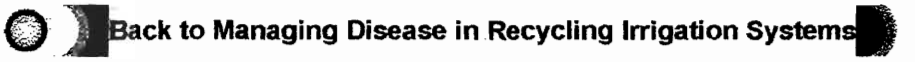

Ozonation

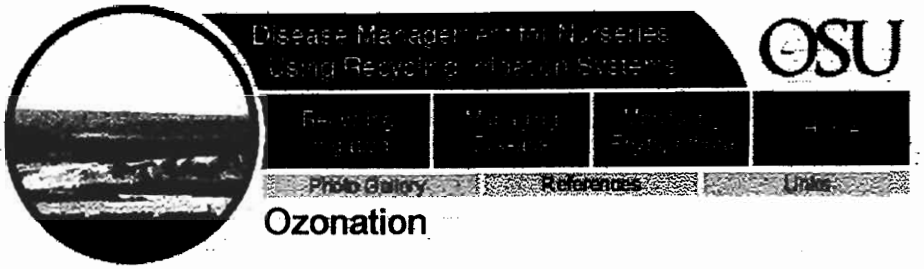

UV light

Several more stringent methods can also be used provided water is filtered to a reasonably clean level before decontamination. These decontamination methods have been adapted from purification methods for drinking water or swimming pool water and include the use of chlorine, ozone or ultraviolet (UV) light. All three methods very effective in eliminating plant pathogens and other microorganisms from water but they require careful management to achieve the desired effect.

Obviously no single water treatment process will be work for all recycling systems. Different nurseries have different water quality problems, different irrigation demands, and different abilities to invest space and capital in treatment equipment. For some systems, complete decontamination may not be economically or technologically feasible. Each system will have to accommodate the unique requirements and conditions of the nursery for which it is designed.

ACKNOWLEDGEMENT: The author thanks J. D. MacDonald, University of California--Davis and J. W. Pscheidt, Oregon State University for reviewing this section on water treatment


Back to Managing Disease in Recycling Irrigation Systems



Ozonation

Ozone is a powerful oxidizing agent is now commonly used as a component of systems for purification of drinking water, swimming pool water, and municipal and industrial waste water. More recently it has been used for treating recycled irrigation water. Treatment with ozone involves bubbling the gas through water, using fine bubbles to ensure a good contact with the solution. Excess ozone must be deactivated (usually by venting through an activated charcoal filter) before release to the atmosphere, as it is a severe nasal and throat irritant, and poses health risks to workers.

"This unit produces sufficient ozone for a small to medium sized nursery."

Production of ozone. Ozone is commonly produced by passing a high voltage electrical discharge across a dry, oxygen rich gas. About 10% of the energy supplied is used to make ozone and the remainder is lost, primarily as heat. Use of a 100% oxygen stream rather than air results in production of 21 times as much ozone.

Factors affecting activity. The disinfective capacity of ozone is affected by organic matter, pH, conductivity, and the amount and type of Fe-chelate. If ozone reacts with organic matter, the amount of ozone remaining in solution and available for killing microorganisms is reduced. The rate of breakdown of ozone to oxygen and hydroxyl ions is increased at high pH. Ozonation increases pH so it may be necessary to add acid to the treatment chamber to maintain optimum pH. Solution conductivity influences the effectiveness of

ozone treatment. Effectiveness is reduced at higher conductivities due to more ozone reacting with the increased concentrations of ions in solution. Iron chelates react with ozone and reduce the amount of ozone in solution available for killing microorganisms. The effectiveness of ozone treatment varies markedly with the amount and type of iron chelate. Pesticides may be destroyed in water by ozone treatment. While this may be viewed as a disadvantage, if water is being discharged into the environment then pretreatment with ozone to break down pesticides may in fact be an advantage.

"Effective ozonation requires prefiltration to remove interfering substances and a collection tank to increase exposure time."

Activity against pathogens. Ozone kills micro-organisms by oxidation of cell structures and processes. It is also very reactive with any inanimate organic matter. In the process of oxidation, oxygen and hydroxyl ions are produced and pH increases. Ozone has been shown to be effective against fungi, bacteria and viruses. It is a more powerful oxidizing agent than chlorine and work with human pathogens has shown a more rapid kill of bacteria and viruses than with chlorine. The killing effect depends on concentration of ozone in solution, contact time and the type of microorganism. For disinfection of drinking water the dose of ozone is about 0.4 mg/L and the contact time about 4 minutes. The concentration of ozone required to kill fungal spores is considerably higher than the levels used to inactivate bacteria and viruses. For example, a concentration of 3.8 mg/L for 2 minutes and 1.5 mg/L for 20 minutes inactivated spores of *Botrytis cinerea, Mucor piriformis* and *Phytophthora parasitica* suspended in water.

Vanachter *et al.* (1988) showed that in pure water, and in nutrient solution containing Fe-DPTA or Fe-EDTA, spores of *Fusarium oxysporum* f.sp. *lycopersici* were killed after 10 minutes. The maximum ozone concentrations measured in the nutrient solutions were 1.11 and 0.60 mg/L. When *F.s. lycopersici* spores and *Clavibacter michiganense* cells were introduced 35 minutes after starting ozone generation, most spores and cells were killed after 1 minute and all were killed after 5 minutes. Runia (1988) demonstrated that ozone treatment of spore suspensions of *Fusarium oxysporum* f.sp. *melonogea* and *Verticillium dahliae* in nutrient solution for 20 minutes resulted in complete elimination of infectivity.

When spores of *Fusarium* were suspended in distilled water, bubbling ozone into the suspension caused 100% mortality in less than 5 minutes–the

shortest practical treatment interval (MacDonald and Kabashima, 1998). However, achieving 100% mortality of spores suspended in nursery effluent water required almost 20 minutes of ozonation. This time interval varied between effluent samples, and appeared to be related to the time required to achieve an ozone concentration of 0.4 ppm. In distilled water, that concentration was achieved in 1-2 minutes, but in effluent samples, ozone accumulated much more slowly. This is because ozone reacts with, and is rapidly depleted by, organic materials in the water. A pre-filter to remove particulate organic matter before water is treated should improve effectiveness.

If the water to be treated does not contain significant quantities of organic matter, or large numbers of resistant propagules (e.g. chlamydospores of *Thielaviopsis basicola*, microsclerotia of *Verticillium* spp), ozone treatment should prove adequate to disinfect water. However, treatment efficacy is a function of ozone concentration and exposure duration. To achieve sufficient exposures at times of peak irrigation demand, nurseries will need to install collection tanks. Ozonated water is transferred to the tanks prior to pumping back into the irrigation system. Tank volume must be matched to pumping rates to assure an adequate residence time for efficacy. This can add considerably to the infrastructure, and tends to limit ozone to small to medium sized nurseries

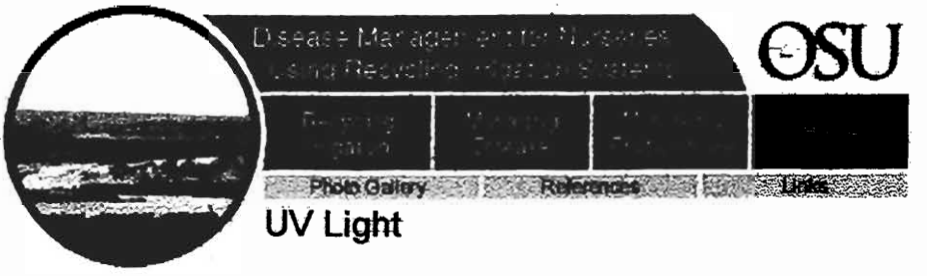
Adverse effects and health hazards. Potential disadvantages of ozone treatment include loss of ozone from the system resulting in harm to human health and crop phytotoxicity, and the breakdown of iron chelates resulting in iron deficiency. The first objection is overcome by use of a well designed and constructed treatment system, with automatic alarms should there be a leak. The second objection is overcome by use of appropriate iron chelates. In addition, ozone attacks most metals.

Although the smell of ozone was thought by some to be refreshing in low concentrations, it is in fact toxic both to humans and plants. The background atmospheric concentration of ozone in surface air at sea level is approximately 0.01-0.03 ppm. The characteristic metallic smell of ozone is usually detected by humans at 0.02 to 0.05 ppm, below the level at which it is harmful. Levels above 0.1 ppm result in dryness of the respiratory tract, nose and throat. Levels of 1 ppm for 30 minutes or more produce headaches. Automatic monitoring of ozone levels in the air of the treatment facility will probably be necessary.

Advantages and disadvantages of ozone for water disinfection

Advantages	Disadvantages
Powerful disinfectant; more powerful than chlorine	High capital cost
Wide spectrum of biocidal activity	Relatively high running cost
Can monitor disinfection process easily by rise in redox value	Relatively long treatment time (20-30 minutes); thus holding tanks for batch treatment may be necessary
No noxious products formed in treated water	A high concentration of ozone is required to kill fungal spores
Ozone formed on site so no transport or storage needed	Effectiveness declines markedly in water with high organic matter
Adds oxygen to water	May need to reduce pH for best results and re-adjust pH after treatment
	Some Fe-chelates, and possibly Mn and pesticides destroyed
	Risk to human and crop health if ozone leaks from treatment chamber so need to monitor atmospheric ozone level

Back to Treating Irrigation Water to Remove Plant Pathogens



UV Light

Some water companies use ultraviolet (UV) light in preference to chlorination as a method for treating drinking water supplies, and UV is widely used to disinfect some recirculation systems (e.g., fish tanks, water cooling towers). UV light also has been used to disinfect recirculated nutrient solutions in hydroponic growing systems, but application of this technology to nursery recirculation has been more limited.

UV light sources and activities. A number of UV light sources are available, with differing capabilities and costs. The most common source is low pressure mercury vapor lamps, which emit photons in a band around 254 nm. High pressure mercury vapor lamps also emit photons around 254 nm, but have the added effect of emissions 190 nm, which forms ozone *in situ* from dissolved water. Xenon flashlamps also are used as UV sources. These have the advantage of pulsed power emissions, making them a higher power source of photon emissions. The disadvantage is that xenon lamps emit a broad spectrum of electromagnetic energy (UV, visible and infrared), so they do not convert all electrical energy into UV. The most efficient conversion of electrical energy into UV is the krypton/fluorine excimer laser. This source is virtually monochromatic, emitting virtually all photons at 248 nm, with the added advantage of pulsed power kinetics. While capable of peak power emissions in the megawatt range (as compared to milliwatts for mercury lamps), these units are still in developmental stages for the purposes of water disinfection.

Electromagnetic radiation with a wavelength of 100-400 nm is termed ultra-violet light. UV rays in the range 200-280 nm (UV-C rays) have a killing effect on microorganisms, the optimum effect being 240-280 nm with a peak around 265 nm. UV light is selectively absorbed by nucleic acids (DNA, RNA) in organisms, resulting in genetic and physiological damage. Absorption is maximal at approximately 254 nm, and UV damage to cells is a well-known process, leading to skin cancers in humans. All cells possess chemical repair mechanisms that can correct the damage to nucleic acids caused by UV. However, very high exposure doses will exceed the repair capacity of cells, leading to physiological disruption and death. Exposing zoospores of *Phytophthora* to emissions from a low pressure mercury vapor lamp for as little as 5 seconds

can cause nearly 100% mortality. The dose of radiation required to kill a particular microorganism is generally expressed as mJ/cm^2 and is the product of radiation intensity and exposure time. These factors must be considered in system design.

Factors affecting activity. Water quality is the predominant factor influencing the efficacy of UV. While UV light can pass through 25 cm of pure water with relatively little attenuation, entry into fouled water might be limited to only a few millimeters. The presence of suspended solids such as colloidal clays are one factor in this phenomenon, but a major factor is the presence of dissolved organics, which may give the water an amber color and which are highly absorptive in the UV band. Spores suspended in fouled water may not receive a lethal dose as they pass through a UV treatment chamber. Since UV radiation is a constant from the source lamps, the only way to increase exposure dose is to slow down flow rate (i.e., increase potential exposure time). However, at low rates, flow within treatment chambers may become laminar, allowing some spores to pass through the chamber without ever coming close to the UV source. Turbulence must be maintained. If turbulence is maintained, it is possible that pathogen propagules suspended in fouled water will only pass close to the UV source for very brief intervals. Hence the interest in high-power UV sources that can deliver lethal doses in the millisecond range.

"For UV light to be effective, all interfering substances must be removed and exposure increased using turbulence."

Activity against pathogens. The lethal dose for many fungi, bacteria and viruses in clear water is less than 200 mJ/cm^2 (Steffan, 1990). Chlamydospores of *Verticillium dahliae* and *Thielaviopsis basicola* are notable exceptions requiring doses of 500 and 3000 mJ/cm^2 or greater respectively. Runia (1988) reported that in demineralized water with no organic matter a 5-10 minute exposure time to UV light at 254 nm resulted in a 30-50% reduction in infectivity of *Phytophthora nicotianiae*. Runia and Klomp (1990) reported that a low capacity (9-18 L/h) flat-film lamp with a high radiation intensity (430-800 mJ/cm^2) eliminated *Fusarium* completely and achieved 48-74% kill of

Verticillium spores. A different lamp design with water flowing around the lamp at 200-400 L/h and receiving 100-200 mJ/cm^2 radiation resulted in only 2-18% kill of *Fusarium* and *Verticillium*. With both lamp designs increasing the flow rate resulted in a higher percentage kill. This was attributed to greater water turbulence at the higher flow rate and a greater 'hit' of fungal structures.

More recently, Runia and Nienhuis (1992) noted that a high pressure lamp was 90% effective against *Fusarium* at a dose of 25-30 mJ/cm^2 and, assuming a linear dose/response relationship, estimated a dose of 100 mJ/cm^2 was required for 99.9% elimination. Ewart and Chrimes (1980) found significant reductions in total bacterial numbers, fluorescent pseudomonads and pectolytic bacteria when treating the nutrient solution from a tomato crop. Stanghellini *et al.* (1984) obtained complete control of root rot of spinach caused by *Pythium aphanidermatum* by treating the circulating nutrient solutions with a 30 mW/cm^2/s UV light at 253.7 nm for 3 sec (90 mJ/cm^2). The flow rate was 7.44 m^3/h and water was passed through a sand filter before treatment.

UV light is active against fungal and bacterial pathogens if the water to be treated is sufficiently clear to avoid UV quenching. Thus, UV is best suited to situations where the water supply is either clean at the outset, or cleaned prior to treatment. Cleaning water to remove particulates or dissolved organics can be accomplished, but also creates a waste disposal problem which must be accounted for. *Thielaviopsis basicola* is one pathogen that appears to be particularly difficult to kill by UV, suggesting that some other treatment should be considered for disinfecting water collected from crops which are very susceptible to this fungus.

There also appear to be synergistic effects in UV / ozone treatments for microbial disinfection. Ozone absorbs UV light very effectively and the resultant ozone breakdown product, the hydroxyl radical, is an even more powerful oxidant than ozone. The use of combined ozone/ UV systems are also very effective in breaking down chemical pollutants in water. UV light in conjunction with metal catalysts degrade pesticides in water.

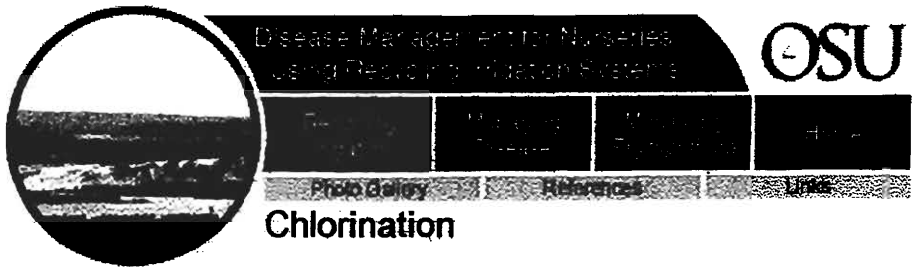
Disadvantages. One disadvantage of treating water with UV light is that it breaks down iron chelate. This can be an important concern in hydroponic or nutrient film technique systems, where loss of iron chelate can induce iron deficiency chlorosis. Stanghellini *et al.* (1984) recorded a fall in the iron content in nutrient solution from 4.5 to 0.1 mg/L after 24 h of UV treatment. Daughtrey and Schippers (1980) found that UV treatment of nutrient film technique solution from a

tomato crop led to the development of pinkish roots and foliar iron deficiency.

Advantages and disadvantages of UV light for water disinfection

Advantages	Disadvantages
Low capital cost for low capacity system used with high quality water	High capital costs for high capacity system and poor quality water
Wide spectrum of biocidal activity	Relatively high running cost
UV generated on site	Effectiveness declines rapidly in water with suspended solids or dissolved organics, necessitating pre-filter, turbulent flow, treatment in series
No noxious products in treated water	
No effect on pH	
Short treatment time allows continous flow systems	Fe-chelates may be destroyed
Easily maintained	No residual (downstream) effects as with chlorine

Back to Treating Irrigation Water to Remove Plant Pathogens

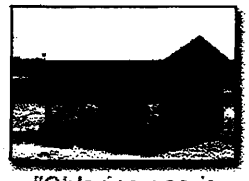


Chlorination

Chlorine treatment has been widely used to eliminate bacteria and viruses from drinking water supplies. Chlorine is one of the most effective chemicals for control of plant pathogenic fungi in water used for irrigation of greenhouse crops. However, its widespread adoption for treatment of recycled water in other crops has been somewhat deterred by several problems. Chlorine can be phytotoxicity to certain crops, is rapidly inactivated by dissolved and suspended organic matter in recirculated water, is difficult measure chlorine readily in nutrient solution, and poses important human health hazards.

Chlorination of water is generally achieved by adding metered amounts of sodium hypochlorite solution, calcium hypochlorite solution, or chlorine gas. Chlorine that is present in solution as chlorine, hypochlorite or hypochlorous acid is known as free or available chlorine. These molecules are very reactive and will readily combine with organic matter, ammonia or nitrogen in oxidation reactions. Chlorine tied up in this manner is termed combined or unavailable. The amount of chlorine inactivated by chemical reaction (the chlorine demand) depends on the impurities, particularly organic matter, in a water supply. Hence the killing effect of chlorine is quickly reduced in a peat leachate solution as the chlorine combines with organic matter. In order to be certain that spores of target fungi are exposed to available chlorine at the required minimum concentration it is necessary to maintain that level of residual free chlorine in the water. Thus, continuous monitoring of the residual chlorine concentration is required.

A chlorine residual is still present in drinking water after treatment in order to maintain a potable supply should there be contamination along the distribution system. Although a distributed residual chlorine dose should in theory offer similar benefits if applied to a crop grown in a recycled watering system, this is not the case in practice. For crops in an inert substrate, or a nutrient film system, residual chlorine



"Chlorine gas is effective but poses major human health risks."

sufficient to kill pathogens may be phytotoxic and for crops grown in organic media the residual dose will soon be lost.

Chlorine products for treating water. Sodium hypochlorite. Commercial sodium hypochlorite solutions sold for use as a disinfectant or a bleaching agent generally contains 10-14% available chlorine (100,000 - 140,000 mg/L). The product is diluted to achieve the target concentration (e.g. 2 mg/L.) in the water supply. During storage chlorine gas is gradually lost and the % available chlorine falls. Sodium hypochlorite exerts its disinfectant and bleaching properties through oxidation reactions. If the material being oxidized is a living microorganism, then cell processes and structures are disrupted and the organism is killed. However, if the hypochlorite reacts with root cells of a plant, some of these may be killed. Sodium hypochlorite is may be used in water treatment systems in preference to gaseous chlorine to avoid the hazard of handling and storing poisonous gas.

Calcium hypochlorite. This product, like sodium hypochlorite, is available as a solution, but the concentration of available chlorine (35%) is greater. Calcium hypochlorite also produces the hypochlorite ion and hypochlorous acid when dissolved in water. It has been used experimentally for treating water in horticultural crops although sodium hypochlorite is the more usual source. If there is a risk of sodium accumulating to a phytotoxic level by continually dosing with sodium hypochlorite in an enclosed system, calcium hypochlorite may be a preferable source of chlorine.

"Calcium hypochloride injection is an effective method of decontaminating nursery runoff."

Chlorine gas. Chlorine gas is the cheapest form of chlorine, and has long been used to treat municipal drinking water. However, chlorine gas is phytotoxic and deadly poisonous to humans, and must be used with great caution. While some nurseries have successfully employed gas-based chlorination systems, many have been discouraged from this approach due to stringent environmental and public health regulations governing gas storage and use. As with sodium and calcium hypochlorites, gaseous chlorine produces the hypochlorite ion and hypochlorous acid when dissolved in water.

Factors affecting activity. The killing effect of chlorine depends on concentration, time, water quality (especially organic matter content), temperature and pH. The residual concentration in drinking water is

generally around 0.5 mg/L, but this level is not sufficient for killing most plant pathogenic fungi. There is no single exposure dose that assures mortality of all plant pathogens under all conditions. Thus, a key requirement for chlorination (or any other disinfection process) is an effective assay to detect target pathogens.

Concentration and Time. Zoospores of *P. cinnamomi* were shown to be killed by exposure to 2 mg/L residual chlorine at 18 C for one minute (Smith, 1979). Exposures to 15 mg/L for 30 seconds and to 10 mg/L for 10 seconds were also effective. Mycelium containing chlamydospores was killed when immersed for 24 hours in a solution of 100 mg/L chlorine, or for 4 hours in a solution of 200 mg/L chlorine. Treatment in a solution of 50 mg/L chlorine for 24 hours was ineffective. At pH 4.0, concentrations of free chlorine less than 100 mg/L were effective at killing mycelium of pythiaceous fungi, and zoospores were killed without the addition of chlorine (Pittis, 1981). Price and Fox (1984) found that *Fusarium oxysporum* f. sp. *dianthi* conidia in nutrient solution were killed by exposure to 5 mg/L chlorine for 15 minutes. Runia (1988) reported that spores of *F. oxysporum* f. sp. *lycopersici* in nutrient solution were generally killed by exposure to 1 mg/L chlorine for 2 hours, although in some tests some spores survived a concentration of 5 mg/L for 2 hours. Datnoff and Kroll (1987) demonstrated that chlorine at 2 mg/L for 24 hours at 25 C apparently killed resting spores of *Plasmodiophora brassicae* and prevented club root in cabbage plants in laboratory tests. A higher concentration for a shorter time (20 mg/L for 5 minutes) was also effective but this treatment was phytotoxic to the cabbage plants.

Chlorine will react with phenols and with unsaturated bonds in organic matter, and also with reducing agents such as Fe 2+ and Mn 2+. All these reactions increase the chlorine demand of water and thus reduce the disinfection capacity of a given chlorine concentration and exposure time. Chlorine also reacts with ammonia in solution to form chloramines, often termed combined chlorine residuals. Chloramines, like chlorine, have significant disinfecting power. Generally however, a greater concentration of combined chlorine residual than of free chlorine residual is required to accomplish a given kill in a specified time

pH. A lower pH, which favors the formation of hypochlorous acid over hypochlorite ion, is more effective for disinfection. The activity of hypochlorous acid to that of hypochlorite ion is of the order of 100:1. However, although the acid is more toxic than the ion, it is also unstable. Hypochlorous acid reacts with hypochlorite to produce chlorate and hydrochloric acid and this decomposition is self-accelerating as the pH falls. To achieve the best activity over a period of time, therefore, it is often necessary to maintain an alkaline

pH. Decomposition is kept to a minimum in commercial hypochlorite solutions by the presence of sodium hydroxide. Spores of *Alternaria tenuis* were killed more readily by calcium hypochlorite at pH 6-7.5 than at pH 8 (Segall, 1968). A hypochlorite concentration of 100 mg/L at pH 7.6 has the same effect on *Bacillus subtilis* spores as a concentration of 1000 mg/L at pH 9.

Temperature. The killing effect of chlorine on *P. cinnamomi* zoospores was shown to be slightly lower at 23 C than at 18 C (Smith, 1979). Other workers have found that the effectiveness of chlorine declined rapidly below 10 C.

Activity against bacteria. Lacey *et al.* (1972) demonstrated that chlorination of contaminated irrigation water gave control of bacterial rot of iris caused by *Erwinia chrysanthemi* and *E. carotovora* spp. *carotovora*. Water was treated with 20 mg/L chlorine for 1 hour. They found that the effectiveness of chlorination was reduced in dirty water and when the number of bacteria was increased. In the presence of 10% sterile peat and with a contact time of 15 seconds, sodium hypochlorite was effective against *Erwinia carotovora* and *Pseudomonas marginalis* at 1% and against *Xanthomonas campestris* at 10%, but was ineffective against *Clavibacter michiganense*, *Pseudomonas corrugate* and *Xanthomonas graminis* (Thompson and Williams, 1986). Ewart and Chrimes (1980) reported that chlorination of nutrient solution to 3 mg/L in a nutrient film technique tomato crop reduced the numbers of potentially pathogenic types of bacteria. But treatment at this rate markedly reduced root development and there was still evidence of root damage even at 0.5 mg/L chlorine. Sodium hypochlorite at 25 mg/L or greater completely inhibited growth on agar plates of *Pseudomonas* sp. that cause of bacterial wilt of sweet pepper (Teoh and Chuo, 1978). When plants grown in granite chips were irrigated with water chlorinated at 15 mg/L or greater there was no plant death from bacterial wilt; treatment at 10 mg/L was ineffective. The growth and yield of plants was reduced as chlorine concentration increased above 10 mg/L. Affected plants showed leaf chlorosis and stunted growth.

Phytotoxicity. There are reports of chlorine treatment of irrigation water resulting in phytotoxic symptoms in a growing crop, particularly when the crop is grown in a hydroponic system or in an inert substrate. Phytotoxicity may result from oxidation of root cells or cell contents, from the presence of toxic chlorate in the chlorine supply or from an accumulation of sodium ions in a recirculating system. Although chlorine treatment will be more effective against phytopathogenic microorganisms in crops grown in nutrient film technique systems and in inert substrates, equally the chlorine will be in very intimate contact with roots and will react with organic matter including root cells.

Sodium chlorate may occur as a contaminant in sodium hypochlorite solutions and the amount may slowly increase with storage. It is highly toxic to plant growth. Accumulation of sodium ions in a recirculating solution will occur with continual application of sodium hypochlorite and this may prove toxic to some crops, e.g. carnation (Price and Fox, 1984). A high sodium concentration will alter the sodium/potassium ratio in solution and plants may develop symptoms of induced potassium deficiency.

Experiments by Frink and Bugbee (1987) indicated that irrigation water with a residual chlorine concentration < 1 mg/L should not adversely affect growth or appearance of most potted plants and vegetable seedlings grown in a peat/perlite/vermiculite medium. Plants were irrigated from above twice-weekly with much of the water contacting the foliage. Growth of geranium and begonia declined at 2 mg/L, pepper and tomatoes at 8 mg/L, lettuce at 18 mg/L and broccoli, marigold and petunia at 37 mg/L. Germination of vegetable seedlings was unaffected. Affected plants showed reduced weight and leaf chlorosis. Irrigation water containing 10 mg/L chlorine has been applied to a wide range of nursery stock subjects grown in peat-based media without adverse effect on plant growth. Treated subjects include *Azalea, Berberis, Calluna, Chamaecyparis lawsoniana, Cotoneaster, Deutzia scabra, Erica cinerea, Hydrangea, Ilex acruifolium, Thuja occidentalis, Viburnum* spp. and *Weigela florida* (Scott *et al.* 1984). Smith (1979) reported that the high concentration of chlorine (200 mg/L) required to kill mycelium of *P. cinnamomi* was phytotoxic to newly rooted cuttings of *Abelia*, *Caryopteris*, *Fuchsia* and *Rosmarinus* when used as routine watering from June to October. As this discussion demonstrates, chlorine treatments that successfully achieve pathogen mortality pose a risk of phytotoxicity. Hence, chlorine-based treatments require careful biological and chemical monitoring for successful use.

Other adverse effects and health hazards. Sodium hypochlorite is corrosive and may damage metal parts of irrigation systems. Decomposition of sodium hypochlorite in incorrectly designed, sealed containers may lead to explosion. As previously mentioned chlorine gas is highly toxic to humans. Sodium hypochlorite causes burns on eyes and skin and if ingested causes internal irritation and damage. Formaldehyde reacts with hypochlorite to produce a carcinogen (bis-chloromethyl ether). Treatment of drinking water and municipal waste water with chlorine is being discontinued in some countries because of reaction of chlorine with humic substances to form trihalomethanes which may be harmful to human health.

Advantages and disadvantages of chlorine for water disinfection

Advantages	Disadvantages
Low capital cost	Effectiveness declines rapidly in water with particulates or organic substances
Low running cost	Less powerful disinfectant than ozone
Wide spectrum of biocidal activity	Risk of crop phytotoxicity
Simple operating system	Effectiveness affected by pH
Rapid action	Risk to human and crop health if chorine leaks from treatment system
No effect on Fe-chelates or pesticides	Risk of corrosion of metal equipment
Can continuously monitor and control chlorine level in water	Potential for production of trihalomethanes and carcinogens which are harmful to human health

Back to Treating Irrigation Water to Remove Plant Pathogens

DISINFECTION OF RECIRCULATING NUTRIENT SOLUTIONS BY SLOW SAND FILTRATION

W. Wohanka
Research Station Geisenheim, Department of Phytomedicine
Von-Lade-Str. 1, D-65366 Geisenheim, Germany

Abstract

In two experiments the efficacy of slow sand filtration against *Fusarium oxysporum* f.sp. *cyclaminis* was proved on small pilot filter devices. The filter containers consisted of plastic pipes with a length of 217 cm and an effective filter surface of 279 cm^2. The filtration rate was 200 l/m^2h. The filter bed was biologically activated by recirculating the nutrient solutions from NFT cultures of geraniums.

The results of this study show that only a very small amount of the microconidia of *F. oxysporum* f.sp. *cyclaminis* could pass through the filter bed. Immediately after inoculation the mean population density of the pathogen in the supernatant water layer was 79100 cfu/ml in experiment 1 and 86290 cfu/ml in experiment 2. The highest average concentration in the filtrate was 75 cfu/ml 4 hours after inoculation in experiment 1 and 88 cfu/ml 3 hours after inoculation in experiment 2. Concentrations of nutrients, pH- and EC-values of the nutrient solutions were not significantly affected by slow sand filtration. However, the oxygen content decreased to zero in experiment 1 and to 1.6 %saturation in experiment 2.

1. Introduction

Slow sand filtration is one of the oldest water treatment processes and was developed more than 100 years ago. It greatly reduced the dangers to human health associated with the use of polluted surface water sources (Ellis, 1991). It is a simple, highly effective and the least expensive method; therefore it is currently used for community water supplies in developing countries.

Raw water passes very slowly through a bed of fine sand at a flow rate of 100 to 300 l/m^2h. Soon after the filter process begins, a filter skin forms on the top surface of the filter bed. It consists of organic and inorganic material and a wide variety of micro-organisms breaking down the organic matter. This biological activity extends through the upper layer of the sand bed, perhaps to a depth of about 40 cm (Visscher et al., 1987).

Earlier investiga
filtration is a very
pythiaceaeous fungi
water (Kemp et al.,
Furthermore, high ef
Thielaviopsis basicc
Verticillium dahliae
prevented the spreac
caused by *Xanthomona*
culture with recircu
and has a considerat
flower break virus (

Investigations wi
shown that the micrc
eliminated only witl
(Wohanka, 1990). Exp
aimed at clarifying
slow sand filtratio
increased by an impr

2. Material and met

2.1 Construction

Four slow sand f
to the scheme in fi
plastic pipes with
of 19.5 cm. The eff
filter bed (sand qu
35 cm thick drainag
(16-32, 8-16, 2-8 m
in a 50 mm wide pip
outlets at the leve

The supernatant
switches at levels
The maximum volume

The filtration r
reduction valve (D
Germany). To get a
approximately 200 l
water head 140 cm;
the outlet structu
effective filter s

The filter beds
recirculating the
geraniums (1 m^2 cul
solutions were prep
Molitor (1988).

Acta Horticulturae 382, 1995
Soil and Substrate Infestation and Disinfestation

Earlier investigations have shown that slow sand filtration is a very reliable method eliminating pythiaceaeous fungi from nutrient solutions or drainage water (Kemp et al., 1992; Friedel et al., 1991). Furthermore, high efficacy could be observed against *Thielaviopsis basicola*, *Cylindrocladium* spp. and *Verticillium dahliae*. Moreover, slow sand filtration prevented the spreading of bacterial wilt in geraniums caused by *Xanthomonas campestris* pv. *pelargonii* in a NFT culture with recirculating nutrient solution (Wohanka, 1993) and has a considerably high efficacy against pelargonium flower break virus (Berkelmann et al., 1993).

Investigations with *Fusarium oxysporum* f.sp. *pisi* have shown that the microconidia of this fungus could be eliminated only with an efficacy of approximately 70-80 % (Wohanka, 1990). Experiments presented in this paper are aimed at clarifying the question whether the efficiency of slow sand filtration against *Fusarium oxysporum* could be increased by an improved filter design.

2. Material and methods

2.1 Construction of the filter units

Four slow sand filter devices were constructed according to the scheme in fig. 1. The filter containers consisted of plastic pipes with a length of 217 cm and an inner diameter of 19.5 cm. The effective filter surface was 279 cm^2. The filter bed (sand quality see tab. 1) was supported by a 35 cm thick drainage system of three layers of graded gravel (16-32, 8-16, 2-8 mm). The raw water flowed from a 1/4" tube in a 50 mm wide pipe, integrated in the filter bed with outlets at the level of the upper sand surface.

The supernatant water layer was regulated by two float switches at levels of 56 and 21 cm above the sand surface. The maximum volume of the supernatant water was 16.7 litres.

The filtration rate was controlled by a flow meter with a reduction valve (D10A614N; Fischer & Porter, Göttingen, Germany). To get a continuous filtration rate of approximately 200 l/m^2h a centrifugal pump ('RENA C40'; 10W, water head 140 cm; RENA, Annecy, France) was integrated into the outlet structure. One experimental filter unit with an effective filter surface of 279 cm^2 delivered 5.6 l/h.

The filter beds were biologically activated by recirculating the nutrient solutions from NFT cultures of geraniums (1 m^2 culture area per unit). The nutrient solutions were prepared according the recommendations of Molitor (1988).

Ten days before inoculation the filter skins were scraped off, decreasing the head loss from an average of 87 to 41 hPa in experiment 1 and from 59 to 18 hPa in experiment 2.

2.2 Inoculation

A freshly isolated strain of *F. oxysporum* f.sp. *cyclaminis* was used. After two weeks of cultivation on malt agar by daylight at room temperature the conidia were drained off with sterile distilled water. Approximately 1 litre conidia suspension was mixed with the supernatant nutrient solution of each filter unit to adjust the conidia concentration to approximately 80000 cfu/ml. The ratio between micro- and macroconidia was about 10:1.

2.3 Sampling

Immediately after inoculation and 1, 2, 3, 4, 5, 6, 24, 48 and 72 hours later samples were taken from the supernatant water and the filter effluent. To determine the concentration of *F. oxysporum* f.sp. *cyclaminis* 5 sub samples (38 µl each) were plated by a spiral plater (Meintrup, Lähden, Germany) on a selective medium according to Komada (1975). Furthermore, total counts of bacteria were estimated on 'standard-I'-agar by the same method (3 sub samples per sample).

3. Results

3.1 Influence of slow sand filtration on nutrient contents, EC- and pH-values, temperature and oxygen content of the nutrient solutions

Nutrient contents, pH- and EC-values of the nutrient solutions were not significantly affected by slow sand filtration (tab. 2 and 3). However, the oxygen content decreased to zero in experiment 1 and to 1.6 %saturation in experiment 2. The temperature of the nutrient solutions varied between 24 and 32 °C (experiment 1) and between 21 and 25 °C (experiment 2) and was not significantly influenced by slow sand filtration.

3.2 Total counts of bacteria in the nutrient solutions

In experiment 1 total counts of bacteria in the supernatant nutrient solutions varied between 10000 and 22000 cfu/ml and in the filtrate between 1600 and 4100 cfu/ml. In experiment 2 the population density in the supernatant solutions varied between 7500 and 31200 cfu/ml and in the filtrate between 800 and 4200 cfu/ml.

3.3 Colony forming *cyclaminis* in

Immediately after
density in the superr
79100 cfu/ml in exper
2. During the first s
decreased by sediment
described in fig. 2.

As demonstrated ir
the fungus was detect
after the filter proc
57.9 cfu/ml). The hig
density 75.0 cfu/ml)
inoculation. 72 hours
of only 1.3 cfu/ml wa

In experiment 2 th
was 1 hour after star
case the highest path
88.2 cfu/ml) could be
After 72 hours an ave

4. Discussion

A previous study w
filter have shown tha
pisi could be elimina
approximately 70-80 %
of this fungus was o
glass) a very expens

Experiments prese
clarifying the quest
filtration against *F.*
improved filter desi
previous study a san
characteristics, esp
distribution, was us
was improved to prev

Instead of *F. oxy*
oxysporum f.sp. *cycl*
Komada agar was used
flow rate of approxi
about 99.9 % could b
efficacy is sufficie
F. oxysporum by reci

Various means of
or adapted for horti
Nienhuis, 1992). Esp

3.3 Colony forming units of *Fusarium oxysporum* f.sp. *cyclaminis* in the nutrient solutions

Immediately after inoculation the average propagule density in the supernatant nutrient solutions was 79100 cfu/ml in experiment 1 and 86290 cfu/ml in experiment 2. During the first six hours after inoculation they rapidly decreased by sedimentation on the filter surfaces as described in fig. 2.

As demonstrated in fig. 3 in experiment 1 the first time the fungus was detectable in the filtrate was two hours after the filter process had started (mean density 57.9 cfu/ml). The highest pathogen concentration (mean density 75.0 cfu/ml) could be observed 4 hours after inoculation. 72 hours after inoculation an average density of only 1.3 cfu/ml was found.

In experiment 2 the first time the fungus was detectable was 1 hour after starting (mean density 2.6 cfu/ml). In this case the highest pathogen concentration (mean density 88.2 cfu/ml) could be observed 3 hours after inoculation. After 72 hours an average density of 10.5 cfu/ml was found.

4. Discussion

A previous study with a pilot device of a slow sand filter have shown that microconidia of *F. oxysporum* f.sp. *pisi* could be eliminated only with a poor efficiency of approximately 70-80 % (Wohanka, 1990). Complete elimination of this fungus was only possible by using 'Siran' (sintered glass) a very expensive filter material.

Experiments presented in this paper are aimed at clarifying the question whether the efficacy of slow sand filtration against *F. oxysporum* could be increased by an improved filter design. In contrast to the above mentioned previous study a sand with more appropriate filter characteristics, especially with a better grain size distribution, was used now. Furthermore, the inlet structure was improved to prevent damaging the filter skin.

Instead of *F. oxysporum* f.sp. *pisi* another pathovar *F. oxysporum* f.sp. *cyclaminis* with a better pigmentation on Komada agar was used. With the improved filter design and a flow rate of approximately 200 l/m^2h a reduction rate of about 99.9 % could be achieved. It is assumed that this efficacy is sufficient to prevent serious distribution of *F. oxysporum* by recirculating nutrient solutions.

Various means of water disinfection have been developed or adapted for horticultural use (Runia, 1991; Runia and Nienhuis, 1992). Especially heating and ozonization are

highly efficient and recommended for recirculating systems. However, these methods generally require a high investment and heating involves a high energy consumption. Therefore, these techniques can economically be used only with large production areas of more than 1 ha (Nienhuis, 1988).

In contrast, slow sand filtration is a simple and low-cost technique easily adaptable to every size of cultivation area. The results of this and earlier studies indicate that the efficacy of slow sand filtration against phytopathogens including fungi, bacteria and even viruses is acceptable under practical conditions (Wohanka, 1993; Berkelmann et al., 1993).

Especially UV-irradiation and ozonization cause changes in the constitution of nutrient solutions (Vanachter et al., 1988; Benoit and Ceustermans, 1993). Except the oxygen depletion slow sand filtration has no negative side effects on nutrient solutions. Contents of micro- and macro-nutrients, pH- and EC-values are not significantly affected.

References

Benoit,F., and Ceustermans,N., 1993. Low pressure UV disinfection also effective for NFT-lettuce. Report of the European Vegetable R&D Centre, Sint-Katelijne-Waver, Belgium, March 1993.

Berkelmann,B., Wohanka,W., and Krczal,G., 1993. Transmission of pelargonium flower break virus (PFBV) by recirculating nutrient solutions with and without slow sand filtration. Acta Horticulturae, in press.

Ellis,K.V., 1991. Water disinfection: A review with some consideration of the requirements of the third world Critical Reviews in Environmental Control 20:341-407.

Friedel,S., Wohanka,W., and Molitor,H.-D., 1991. *Erica*: *Phytophthora* in Fließrinnen bekämpfen. Gärtnerbörse und Gartenwelt 91:69-72.

Kemp,J., Behrens,V., and Wohanka,W., 1992. Langsamfilter verhinderten Ausbreitung von *Phytophthora*. Gartenbau-magazin 5/92:58-60.

Komada,H., 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Reviews of Plant Protection Research 8:114-124.

Molitor,H.-D., 1988. Stock plant cultivation in rockwool with and without recycling the nutrient solution. ISOSC Proceedings of the 7th Intern. Congr. on Soilless Culture 1988, 323-333.

Nienhuis,J.K., 1988. voor grote bedrijv Bloemisterij 43:3{

Rattink,H., 1983. Sp: carnations on art: 141:103-108.

Runia,W.T., 1991. Be: Vakblad voor de B:

Runia,W.T., and Nienl systemen werken n: 47:99.

Vanachter,A., Lieve : Van Assche,C., 19{ disinfestation of Horticulturae 221

Visscher,J.T., Parma: 1987. Slow sand f: Planning, design, International Rev: and Sanitation, T:

Wohanka,W., 1990. Wa: Kulturverfahren - Braunschweig:73-8:

Wohanka,W., 1993. Sl cost techniques f nutrient solution the 8th Intern. C

Nienhuis,J.K., 1988. Thermisch ontsmetten drainwater alleen voor grote bedrijven vordelig. Vakblad voor de Bloemisterij 43:38-39.

Rattink,H., 1983. Spread and control of Fusarium wilt in carnations on artificial substrates. Acta Horticulturae 141:103-108.

Runia,W.T., 1991. Betrouwbare ontsmetting (drain)water. Vakblad voor de Bloemisterij 46:20-22.

Runia,W.T., and Nienhuis,J., 1992. Twee ontsmettings-systemen werken niet goed. Vakblad voor de Bloemisterij 47:99.

Vanachter,A., Lieve Thys,E., Van Wambeke,E., and Van Assche,C., 1988. Possible use of ozon for disinfestation of plant nutrient solutions. Acta Horticulturae 221:295-301.

Visscher,J.T., Parmasivan,R., Raman,A., and Heijnen,H.A., 1987. Slow sand filtration for community water supply: Planning, design, construction, operation and maintenance International Reverence Centre for Community Water Supply and Sanitation, The Hague.

Wohanka,W., 1990. Wasserentkeimung. In: Geschlossene Kulturverfahren - Zierpflanzenbau. Thalacker-Verlag, Braunschweig:73-81.

Wohanka,W., 1993. Slow sand filtration and UV radiation; low cost techniques for disinfection of recirculating nutrient solutions or surface water. ISOSC Proceedings of the 8th Intern. Congr. on Soilless Culture 1992:497-511.