United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/national-list/petitioned

Document Type:

☒ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☐ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

**PETITION FOR LISTING ON NATIONAL LIST OF APPROVED AND PROHIBITED SUBSTANCES**
**SEC. 2118. [7 U.S.C. 6517] NATIONAL LIST**


| | |
|---|---|
| Petitioner name: | Waldo Moraga, President - CEO<br>ECO2MIX, Inc. |
| Address: | 2023 N Gateway Blvd, Suite 106<br>Fresno, CA 93727 |
| Telephone number: | 559-666-0558 |
| Email address: | wmoraga@eco2mix.com |
| Date of petition: | November 30, 2020 |
| Send to: | USDA/AMS/NOP, Standards Division<br>Attention: National List Manager<br>1400 Independence Ave. SW<br>Room 2646-So., Ag Stop 0268,<br>Washington, DC 20250-0268 |


Summary of request:

Previous actions by NOSB and NOP allow synthetic carbon dioxide to be used as an ingredient in organic labeled processed food products:

**§205.605 Nonagricultural (nonorganic) substances allowed as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))."**

**(b) Synthetic allowed:**

**- Carbon dioxide**

This petition is a request for NOBS and NOP to allow synthetic carbon dioxide in organic crop production:

**§205.601 Synthetic substances allowed for use in organic crop production.**
**(a) As algicide, disinfectants, and sanitizer, including irrigation system cleaning systems.**
**(j) As plant or soil amendments.**

In 2007, NOBS determined that synthetic carbon dioxide is a substance that is allowed without restrictions as an ingredient in or on processed products labeled as "organic" or "made with organic" under §205.605, confirming that synthetic carbon dioxide was compatible with organic production practices.

ITEM A

ECO2MIX, Inc. is submitting this petition as a request to allow synthetic carbon dioxide to be used without restrictions to adjust water pH ($H^+$ concentration) to be used in irrigation and for spray over plant leaves.

1. **The substance chemicals and common name.**

   Carbon dioxide ($CO_2$) – gas, liquid and solid (dry ice). Currently allowed under §205.605 Synthetic allowed for processed foods.

   More information is included in the petition database at:

   https://www.ams.usda.gov/sites/default/files/media/Carbon%20Dioxide%201%20Petition.pdf

   https://www.ams.usda.gov/sites/default/files/media/Carbon%20Dioxide%202%20Petition.pdf

   *Please also refer to Appendix A

2. **The manufacturer's or producer's name, address and telephone number.**

   There are many, please see Appendix B*, that includes a list of known carbon dioxide source facilities in the United States included in the original petition, we may add to that list these two new sources since 2005.

   Tulare, CA – Ethanol – Air Liquida Industrial U.S. LP
   Keyes, CA – Ethanol – Messer Americas

3. **The intended or current use of the substance.**

   Carbon dioxide is used in a water pH adjustment process. Dissolved carbon dioxide in water makes carbonic acid, which reduces water pH, therefore increasing $H^+$ concentration and neutralizing bicarbonates.

   Water pH adjustment is common practice in agriculture. Irrigation water sources are usually alkaline and with bicarbonates above the maximum desired levels for proper irrigation water quality. This requires some form of pH control to be used to irrigate the crops.

   Water pH cannot drop below pH 5.0 when carbonic acid (dissolved $CO_2$) is used in the acidification process. This characteristic makes the use of carbonic acid the safer and most secure process for water pH adjustment when compared to alternatives.

4. **A list of the crop, livestock or handling activities for which the substance will be used.**

   Carbonic acid will be used on almost every crop. Especially those that are under drip, micro-sprinkler, sprinkler, or pivot irrigation that requires water pH acidification and bicarbonate neutralization (to prevent scale build-up).

   The process is simple. The action to dissolve carbon dioxide ($CO_2$) in water ($H_2O$) makes carbonic acid ($H_2CO_3$), as the following formula describes:

   $H_20 + CO_2 \rightarrow H_2CO_3$

   The water pH adjustment process can be manually controlled, as well as automatically controlled, by adding a pH probe and controller that adjusts the carbon dioxide ($CO_2$) injection to maintain target pH values in the water.

**5. The source of the substance and a detailed description of its manufacturing procedures from the basic component to the final product.**

*See original petition in Appendix A

**6. Ancillary Substances**

Petitioner is unaware of any ancillary substances present in carbon dioxide.

**7. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.**

**OMRI reviews:**

**Carbon Dioxide**
Ruling body: LPO
Status: Allowed
Class: Processing ingredients and Aids
Origin: Non-agricultural
Description: INS 290
Rule reference: LPO Guidance Annex 1, Table 3.1; 3.6;4
Date active: June 30, 2020

**Carbon Dioxide**
Ruling body: LPO
Status: Allowed
Class: Crop Pest, Weed, and Disease Control
Rule reference: LPO Guidance Annex 1, Table 2
Date active: June 30, 2020

**Carbon Dioxide**
Ruling body: LPO
Status: Allowed
Class: Processing Pest Control
Rule reference: LPO Guidance Annex 1, Table 2, LPO Guidelines Article 172
Date active: June 30, 2020

**Carbon Dioxide**
Ruling body: NOP
Status: Allowed with Restrictions
Class: Processing Pest Control
Origin: Synthetic
Description: For use as a pesticide only in conjunction with the facility pest management practices provided for in paragraphs 205.271(a) and (b) and only if those practices are not effective to prevent or control pests alone
Rule reference: NOP reference 205.605(b); 205.271(c); Guidance 5023
Date active: April 4, 2019

**Carbon Dioxide**
Ruling body: NOP
Status: Allowed
Class: Crop Management Tools and Production Aids
Origin: Non-synthetic
Description: Non-synthetic forms are allowed. May also be used in post-harvest handling of raw agricultural commodities.
Rule reference: NOP Reference 205.105; Guidance 5023
Date active: April 4, 2019

**Carbon Dioxide**
Ruling body: COR
Status: Allowed with restrictions
Class: Crop Management Tools and Production Aids
Description: For soil and greenhouse use. For controlled atmosphere storage.
Rule reference: CGSB Reference 32.311 Table 4.3
Date active: June 20, 2019

**Carbon Dioxide**
Ruling body: NOP
Status: Allowed
Class: Processing Non-agricultural ingredients and Processing Aids
Origin: Non-agricultural Synthetic
Description: May be used as an ingredient or processing aid. May also be used in post-harvest handling of raw agricultural commodities.
Rule reference: NOP reference 205.605(b); 205.270(b); Guidance 5023
Date active: April 4, 2019

**Carbon Dioxide**
Ruling body: COR
Status: Allowed with restrictions
Class: Processing Non-agricultural ingredients and Processing Aids, Processing Pest Controls
Origin: Non-Agricultural Synthetic/Non-synthetic
Description: Prohibited as a food additive for the carbonation of wine or mead. For use as a processing aid. For use in facility pest management. For use in controlled atmosphere storage in post-harvest handling.
Rule reference: CGSB Reference Table 6.5; 32.311 Table 6.3; Table 8.3; Table 8.2
Date active: April 4, 2019

**Canadian General Standard Board reviews:**

**Carbon Dioxide**
Status: Allowed
Class: 4.3 Crop production aids and materials. For soil and greenhouse use and for controlled atmosphere storage
6.3 Ingredients classified as food additives. Carbonation of wine or mead is prohibited
6.5 Processing aids
8.2 Facility pest management substances
8.3 Post-harvest substances. For controlled atmosphere storage
Origin: From non-synthetic and synthetic sources

**Washington State Department of Agriculture**

**Carbon Dioxide**

| | |
|---|---|
| Ruling body: | WSDA |
| Status: | Allowed with restrictions |
| Class: | CPA – Crop Production Aid, DPC – Disease and Pest Control, FSA – Fertilizer and Soil Amendment, LPA – Livestock Production Aid, PH – Processing and Handling |
| Origin: | IGI Carbon Dioxide |
| Description: | Preventative practices must be implemented prior to use (NOP 205.271) |
| Date active: | December 21, 2017 |

*See also Appendix C

**7 Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.**

***e-CFR (updated November 9, 2020)***
***Title 21 - Food and Drugs***
***Chapter I – Food and Drug Administration, Department of Health and Human Services***
***Subchapter B – Food for human consumption***
***Part 184 – Direct Food Substances Affirmed as Generally Recognized as Safe (GRAS)***
***Subpart B - Listing of specific substances Affirmed as GRAS***

***§184.1240 Carbon dioxide.***

*(a) Carbon dioxide (empirical formula $CO_2$, CAS Reg. No. 124-38-9) occurs as a colorless, odorless, noncombustible gas at normal temperatures and pressures. The solid form, dry ice, sublimes under atmospheric pressure at a temperature of −78.5 °C. Carbon dioxide is prepared as a byproduct of the manufacture of lime during the "burning" of limestone, from the combustion of carbonaceous material, from fermentation processes, and from gases found in certain natural springs and wells.*

*(b) The ingredient must be of a purity suitable for its intended use.*

*(c) In accordance with §184.1(b)(1), the ingredient is used in food with no limitations other than current good manufacturing practice. The affirmation of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following current good manufacturing practice conditions of use:*

*(1) The ingredient is used as a leavening agent as defined in §170.3(o)(17) of this chapter; a processing aid as defined in §170.3(o)(24) of this chapter; and a propellant, aerating agent, and gas as defined in §170.3(o)(25) of this chapter.*

*(2) The ingredient is used in food at levels not to exceed current good manufacturing practice.*

*(d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.*

*[48 FR 57270, Dec. 29, 1983, as amended at 73 FR 8607, Feb. 14, 2008]*

**Washington State Department of Agriculture**

- Material Registration Certificate # 2962
- IGI Carbon Dioxide

Petitioner is unaware of carbon dioxide for use in water pH control being restricted in any other organic standard board.

**8 The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.**

CAS number: 124-38-9
RTECS number: FF6400000
EEC number: 204-696-9

**9 The substance physical and chemical properties**

Physical properties: colorless, odorless, non-flammable gas or white opaque solid ("dry ice"); can be liquid under pressure ("supercritical $CO_2$"). Boiling point: not available. Freezing Point: -56.6 $^oC$. Vapor Pressure at 70 $^oF$: 830 psi.

a. Carbon dioxide is usually the end product of other processes and therefore has relatively little chemical interactions with other substances used in organic production.
b. As a basic component of the atmosphere, carbon dioxide has a high environmental persistence. This is not a negative, except to the overarching concern of global warming. At the rates occurring in the atmosphere, it is completely non-toxic and is exempt from having an LD50.
c. All oxygen breathing organisms will suffocate in pure $CO_2$, not from a toxic effect of the gas itself, but because of the lack of oxygen. There are no other direct effects on human health from the substance.
d. Most of the sources of carbon dioxide are reclaiming the substance from other primary processes. That is to say, it is recycling substances that would otherwise be given off into the atmosphere. Ethanol production also has some positive environmental impact in reducing the amount of non-renewable energy needed.

**10 Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environment Health Studies.**

*See MSDS attached as Appendix D

**11 Research information about the substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from The National List.**

a. Matthias C. Rillig, Sara F. Wright & Valerie T. Eviner - "The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation comparing effects on five plant species" Nov 2001. – See Appendix E*
b. D.E. Akin, B.A. Kimball, J.R. Mauney, R.L. LaMorte, G.R. Hendrey, K. Lewin, J. Nagy, R.N. Gates – "Influence of enhanced CO2 concentration and irrigation on sudangrass digestibility" Nov 1993. – See Appendix E*
c. Mike Amaranthus, Ph.D., Mar 2008, ACRES, The Voice of Eco Agriculture, Vol. 38 No. 3 – See Appendix E*

d. Shawn Ashkan and David Zoldoske – "CO2 Enrichment Can Boost Yields and Help Mitigate Climate Change", RESOURCE, May/June 2018. – See Appendix E*
e. H.Z. Enoch and J. M. Olesen – "Plant response to irrigation with water enriched with carbon dioxide", New Phytol, Tansley Review No. 54, 1993 – See Appendix E*

*Also see original petition in Appendix A

**12 A "Petition Justification Statement" which provides justification for any of the following actions requested in the petition:**

Natural irrigation water pH and bicarbonate concentration varies from site to site. Generally, water pH is high and needs to be treated in order to fit crop requirements and increase yields.

In crop production, there is an ideal water pH where almost all nutrients are more available for the plants, and the plants benefit from this nutrient availability. In general, ideal pH value is between pH 6.0 to 6.5 for most crops. The use of dissolved carbon dioxide, in the form of carbonic acid, is the only pH control method that mimics nature. Plant roots naturally release $CO_2$ to make carbonic acid and absorb the minerals present near the radicular zone.

*See Appendix F

## *A. Inclusion of a Synthetic on the National List (7 C.F.R. §§ 205.601)*

- *Explain why the synthetic substance is necessary for the production or handling of an organic product.*

Carbon dioxide is an essential component of plant growth. The element participates in the process of photosynthesis, is part of the most natural acidification process in nature and provides a source of carbon to the plant and the soil subsequent to its use in irrigation water. Water acidification is a necessary practice for most of the crops cultivated and irrigated using drip, micro-sprinklers, sprinklers or pivots. Water acidification aids plants in absorbing more nutrients from the water/fertilizer/soil solution. Due to the use of carbon dioxide, the reduction of bicarbonates helps in keeping emitters clean from scale build-up, maintaining water distribution uniformity with the highest efficiency during the year, preventing scale in pipes, and saving energy (per each mm of scale inside of a pipe, 11% more energy is needed to deliver water to the field).

According to the FDA, carbon dioxide is Generally Recognized As Safe (GRAS), independent from the source (non-synthetic or synthetic). For these reasons, we do not recognize any drawback in the use of $CO_2$ to produce carbonic acid to adjust water pH, neither for irrigation or for use in spray pesticides and/or foliar fertilizers over the leaves as an inert ingredient (included as inert in EPA List 4A).

- *Describe any non-synthetic substances, synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substance.*

According to petitioner understanding, the only non-synthetic substance allowed for use in crop production is carbon dioxide, which is sourced from fermentation, without restrictions.

The current synthetic substance allowed for use in water pH adjustment in organic agriculture is sulfur. The methods for making sulfurous acid include burning sulfur and mixing the sulfur dioxide gas resulting from that combustion with water using a sulfur burner machine.

- *Describe the beneficial effects to the environment, human health, or farm eco- system from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural methods.*

  The use of dissolved carbon dioxide (carbonic acid) to reduce water pH is the only method that mimics nature. It is the only natural acidification process that, when the treated water reaches the soil, stores the gas-off $CO_2$ underground. This is especially true in low or non-tilling farming. Carbonic acid (dissolved $CO_2$ in water) is a stable compound that stays in the solution for many hours before the $CO_2$ is released from the water, and in that case, is going to provide carbon to the soil microbiology present in the wetted bulb.

**Conclusions**

Carbon dioxide is a common and abundant substance in nature, which is available from processing air and from natural fermentation processes. Under the Organic Food Production Act and the Final Rule, carbon dioxide from such natural sources is considered non-synthetic and does not require listing.

However, carbon dioxide is produced from many sources in different ways. Long distance transport is not feasible in any form- gas, liquid or solid. Other than from the air and fermentation processes, this substance is obtained from mines and from oil and natural gas refining. These sources are considered synthetic. Because of substantial long-distance transport obstacles, this substance is not commercially available at many locations as a non-synthetic. Therefore, this petition is for synthetic carbon dioxide gas to be allowed in the acidification of water for the use in irrigation or to spray over the plant leaves.

Carbon dioxide is the most “natural” substance available that can be used to reduce water pH values to optimum levels for crop cultivation. $CO_2$ is safe and its use is established in organic production under §205.605(b) - Synthetics allowed.

Carbon dioxide produced by fermentation (as in Ethanol Steam Reformers) is a non-synthetic substance that is allowed without restriction in crop production. Pursuant to a petition received in 2005, synthetic carbon dioxide was extensively reviewed by a Technical Advisory Panel and was determined by NOSB to be compatible with organic production practices in 2007 and is allowed in processed products under §205.605(b).

The purpose of this petition is to request that synthetic carbon dioxide be allowed for use in organic crop production, without restriction under National List §205.601- Synthetic substances allowed for use in organic crop production.

Waldo Moraga

President – CEO

Eco2Mix, Inc.

# Appendix A

# Original petition November 14, 2005

# CCOF

CCOF
*Organic Certification* *Trade Association* *Education & Outreach* *Political Advocacy*



**Petition for Amending the National List of the USDA's National Organic Program**

ITEM A

CCOF, Inc. is submitting this petition to change the listing of Carbon Dioxide from synthetic in §205.605(b)(8) to non-synthetic §205.605(a) in the National List of Nonagricultural (non-organic) substances allowed in or on processed products labeled as "organic" or "made with organic...."

ITEM B

1. The substance's common name.
Carbon Dioxide

2. The manufacturer's name, address and telephone number.
There are many. See attached list of Known $CO_2$ Source Facilities in the United States.

3. The intended or current use of the substance such as use as a pesticide, animal feed additive, processing aid, nonagricultural ingredient, sanitizer or disinfectant.

4. A list of the crop, livestock or handling activities for which the substance will be used. If used for handling (including processing), the substance's mode of action must be described.
Carbon dioxide has many uses in Handling. Included in this petition:

| Handling Activity | Type of Use | Mode of Action |
|---|---|---|
| Grains Storage | Pest control/fumigant | Modifying atmosphere of storage bins by replacing oxygen with $CO_2$ kills pests by suffocation and prevents new ones. |
| Herbs and Spices | Pest control/fumigant | Same as above. |
| Beverages: Soda, fruit juice, and beer | Ingredient for carbonation | Carbonation results from $CO_2$ being pumped into beverages. Also retards microbial breakdown. |
| Production of natural flavors and extracts | Processing aid: extracting agent | Oleoresins can be separated from other plant components in a supercritical liquid $CO_2$ environment. Temperature and pressure vary to wash soluble compound from plant bulk. |
| Oil Production | Processing aid: extracting agent | $CO_2$ can help break up plant parts to enable oil to be extracted without hexane. It also improves the antioxidant content of oil, allowing it to keep better. |
| Chicken processing | Slaughtering agent | Chickens placed in pure $CO_2$ cannot breathe and suffocate. |
| Milk Handling | Processing aid: microbial control | $CO_2$ is dissolved into milk (post pasteurization) to inactivate microbial decomposition. Keeps microbes from obtaining oxygen. |



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| | | |
|---|---|---|
| Seed treatment for sprout production | Processing aid: disinfectant | Alfalfa seed can be soaked in concentrated $CO_2$ solution to kill seed borne pathogens as alternative to high chlorine. |
| Whipped cream | Propellant | Aids ejection of food from aerosol can. |
| Fruit storage | Pest control | Pre-conditioning stone fruits in $CO_2$ helps them withstand controlled atmosphere storage. |
| Coffee decaffeination | Processing aid: extracting agent | Separates caffeine from coffee without harmful chemicals. |

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.

Carbon dioxide comes from many sources and regional conditions determine which source is available in which area. (See attached list of known $CO_2$ sources in the United States.) However, as a natural occurring material a non-synthetic designation is appropriate. Major sources are described here:

a. By-product of oil refinery operations - In order to generate hydrogen for oil refining, methane gas is exposed to steam to create hydrogen and carbon dioxide. The reaction is as follows:

$$CH_4 + 2\ H_2O \rightarrow CO_2 + 4\ H_2$$

The resulting mixture still has some moisture and uncracked methane in it. To purify the carbon dioxide, the water is removed by drying. Then the mixture is distilled in a column under pressure which removes hydrocarbons and hydrogen. Once the $CO_2$ is purified, the amount of impurities is in the parts per billion (ppb) range. It could be argued that the methane gas is non-synthetic, but there is a chemical change that happens under steam and pressure to crack the methane into carbon dioxide and hydrogen. This is the primary type of $CO_2$ available in urban areas near either coast, or areas where there are oil refineries. Capturing the $CO_2$ prevents it from rising into the air as pollution from the refinery.

b. By-product of ethanol production - Ethanol for fuel is produced by fermentation of the natural sugars in corn or other grains. Carbon dioxide is given off in this reaction and is captured for many uses. The reaction is as follows:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

There are impurities associated with the carbon dioxide produced this way, such as aldehydes, glycerol, higher alcohols, and acids. These impurities may be removed either by use of activated carbon absorbers (the Backus process) or by the Reich process of purification.

c. By-product of ammonia production - The reaction and process here is the same as for oil refineries except that air is involved in the process so that the ratio of hydrogen to nitrogen is sufficient to synthesize ammonia. For each ton of ammonia produced, more than a ton of carbon dioxide is generated.

d. Underground wells - Deposits of carbon dioxide occur underground, frequently in association with natural gas deposits. The primary deposits are located in Mississippi, New Mexico and Colorado. These deposits frequently have natural gas as an impurity and this is separated from the $CO_2$ by absorption.

e, f, g, h. By-products of chemical synthesis - Carbon dioxide is given off in reactions to produce sulfuric acid, phosphoric acid, ethylene oxide and from co-generation plants. Some of these plants purify and sell the $CO_2$ produced from these reactions.

6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.

None available except the NOSB TAP review from 1995 (attached). In the 1995 TAP review, one reviewer stated the substance should be considered non-synthetic, one reviewer said either synthetic or non-synthetic, and one said synthetic. All three reviewers recommended carbon dioxide for the National List. The NOSB noted that it could be from several sources and it was not always easy to determine which source was available. Therefore they added it to the National List as synthetic so that both natural and synthetic sources could be used.

7. Information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers.
Not Applicable.

8. The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contains the petitioned substance.
CAS number: 124-38-9
RTECS number: FF6400000
EEC number: 2046969

9. The substance's physical properties and chemical mode of action including (a) chemical interactions with other substances, especially substances used in organic production; (b) toxicity and environmental persistence; (c) environmental impacts from its use or manufacture; (d) effects on human health; and, (e) effects on soil organisms, crops, or livestock.
Physical properties: colorless, odorless, non-flammable gas or white opaque solid ("dry ice"); can be liquid under pressure ("supercritical $CO_2$"). Boiling Point: -78.5 °C. Freezing Point: -56.6 °C. Vapor pressure at 70 °F: 856 psi.
For modes of action in the various uses in organic handling, see the chart above in section #3.

a. Carbon dioxide is usually the end product of other processes and so has relatively little chemical interaction with other substances used in organic production.
b. As a basic component of the atmosphere, it has a high environmental persistence but this is not bad except to the overarching concern about global warming. At the rates occurring in air it is completely non-toxic and is exempt from having an LD50.
c. Most of the sources of carbon dioxide are reclaiming the substance from other primary processes. As such it is recycling something that would otherwise be given off into the atmosphere. However, it is likely to be released into the atmosphere anyway after its use in organic handling and so no evaluation can be made as to the overall impact on global warming and $CO_2$ enrichment of the atmosphere from organic uses. The manufacture of refined petroleum, ethanol, ammonia, or energy are the primary manufacturing processes that carbon dioxide is purified from and each of them have significant negative environmental impacts. Ethanol production also has some positive environmental impact in reducing the amount of non-renewable energy needed. However, the purification of the $CO_2$ from these sources does not substantially change the impact that the primary process has on the environment.
d. All oxygen breathing organisms will suffocate in pure $CO_2$, not from a toxic effect of the gas itself, but because of the lack of oxygen. There are no other direct effects on human health from the substance.
e. Although not relevant for organic handling uses, plants benefit from carbon dioxide enrichment of their environment, such as can be done in greenhouses.

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.
MSDS attached.

11. Research information about the substance which includes comprehensive substance research reviews and research bibliographies, including reviews and bibliographies which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List.

Ahmadi, H., W.V Biasi, E.J Mitcham. 1999. "Control of Brown Rot Decay of Nectarines with 15% Carbon Dioxide Atmospheres." Journal of the American Society for Horticultural Science. Nov 1999. v. 124 (6) p. 708-712. Alexandria, Va. DNAL, 81 SO12
Abstract: Effects of short-term exposure to a 15% $CO_2$ atmosphere on nectarines [Prunes persica (L.) Batsch (Nectarine Group) 'Summer Red'] inoculated with Monilinia fructicola (Wint.) (Causal agent of brown rot) were investigated. Nectarines were inoculated with spores of M. fructicola and incubated at 20 degrees C for 24, 48 or 72 hours and then transferred to storage in either air or air enriched with 15% $CO_2$ at 5 degrees C. Fruit were removed from storage after 5 and 16 days and were examined for brown rot decay immediately and after ripening in air for 3 days at 20 degrees C. Non-inoculated nectarines were stored and treated likewise for evaluation of post-harvest fruit attributes to determine their tolerance to 15% $CO_2$. Incubation period after

inoculation, storage duration, and storage atmosphere had highly significant effects on fruit decay. After 3 days ripening in air at 20 degrees C, the progression of brown rot disease was rapid in all inoculated nectarines, demonstrating the fungistatic effect of 15% $CO_2$.

American Society for Horticultural Science. In the special section: "Modified Atmosphere Packaging--Toward 2000 and Beyond." Paper presented at a symposium held July 28-31, 1999, Minneapolis, Minnesota. DNAL, SB317.5.H68

Abstract: The tolerances of horticultural commodities to CO2 are outlined, as are also the associated biochemical and physiological aspects of differences in tolerance between and within commodity types. These tolerances are related to responses to the use of modified atmosphere packaging (MAP) during storage. Commodities vary widely in their responses to elevated CO2, and low tolerance to the gas limits its use to maintain quality in some cases. Factors such as cultivar and post-harvest treatment before imposing high CO2 can influence responses of commodities to CO2, but are rarely considered in cultivar selection or in commercial application. A better understanding of the physiology and biochemistry of commodity responses to CO2 is required for increased use of MAP.

Cossentine, J.E., et al. "Fumigation of Empty Fruit Bins with Carbon Dioxide to Control Diapausing Codling Moth Larvae and Penicillium Expansum Link. ex Thom Spores." HortScience. American Society for Horticultural Science. 2004 Apr. v. 39, no. 2 1022656803 p. 429-432. DNAL, SB1.H6

Daniels, James A.; Rajagopalan Krishnamurthi, Syed Rizvi. A Review of Effects of Carbon Dioxide on Microbial Growth and Food Quality. J. Food Protection. Ames, IA 1985. v. 48 (6) p. 532-537. DNAL 44.8 - J824.

Abstract: Carbon dioxide is effective for extending the shelf-life of perishable foods by retarding bacterial growth. The overall effect of carbon dioxide is to increase both the lag phase and the generation time of spoilage microorganisms; however, the specific mechanism for the bacteriostatic effect is not known. Displacement of oxygen and intracellular acidification were possible mechanisms that were first proposed then discounted by early researchers. Rapid cellular penetration and alteration of cell permeability characteristics have also been reported, but their relation to the overall mechanism is not clear. Several researchers have proposed that carbon dioxide may first be solubilized into the liquid phase of the treated tissue to form carbonic acid ($H_2CO_3$), and investigations by the authors tend to confirm this step, as well as to indicate the possible direct use of carbonic acid for retarding bacterial spoilage. Most recently, a metabolic mechanism has been studied by a number of researchers whereby carbon dioxide in the cell has negative effects on various enzymatic and biochemical pathways. The combined effects of these metabolic interferences are though to constitute a stress on the system, and result in a slowing of the growth rate. The degree to which carbon dioxide is effective generally increases with concentration, but high levels raise the possibility of establishing conditions where pathogenic organisms such as Clostridium Botulinum may survive. It is thought that such risks can be minimized with proper sanitation and temperature control, and that the commercial development of food packaging systems employing carbon dioxide will increase in the coming years.

Dziezak, J.D. "Innovative separation process finding its way into the food industry." Food Technology. Chicago, IL 1986. v. 40 (6) p. 66 - 69. DNAL 389.8 - F7398.

Abstract: A technological process is described, using carbon dioxide in its supercritical state to selectively extract and fractionate desirable components from a food mixture in 1 step. The basic supercritical process is illustrated schematically and discussed. Various supercritical processing applications (e.g., the production of supercritically-decaffeinated tea) are described. The energy savings of supercritical fluid extraction for the food industry are cited.

Hotchkiss, J.H., Chen, J.H., Lawless, H.T. 1999. "Combined Effects of Carbon Dioxide Addition and Barrier Films on Microbial and Sensory Changes in Pasteurized Milk." Journal of Dairy Science. Apr 1999. v. 82 (4) American Dairy Science Association, Savoy, Ill. p. 690-695. DNAL, 44.8 J822

Abstract: The growth of psychrotrophic microorganisms is an important factor in the deterioration of refrigerated pasteurized milk. Dissolved $CO_2$ inhibits certain spoilage microorganisms in foods provided that the packaging offers a sufficient barrier to $CO_2$ evolution. The objectives of this work were, first, to estimate the sensory threshold for dissolved $CO_2$ in 2% milk and, second, to determine the relationship between microbial growth and package barrier properties for pasteurized milk to which $CO_2$ had been added at

concentrations near the flavor threshold. Pasteurized milk was inoculated with a cocktail of spoilage microorganisms, packaged in different barrier film pouches, and stored at 6.1 degrees C for up to 28 d. The addition of $CO_2$ at concentrations of 8.7 and 21.5 mM increased the time needed to reach 10(6) cfu/ml from 6.4 d (no $CO_2$) to 8.0 and 10.9 d, respectively, in low barrier pouches. In high barrier pouches, the time needed to reach 10(6) cfu/ml was increased to 9.7 and 13.4 d, respectively, at $CO_2$ concentrations of 8.7 and 21.5 mM. This increase represents an increase in shelf-life of approximately 25 to 200%. Microbial counts had longer lag times and lower growth rates and took longer to reach stationary growth as the concentration of $CO_2$ increased in all films than did the control milk. The control milk curdled in less than 17 d, but the test milk in the high barrier packaging had not curdled at 28 d. These data suggest that the shelf-life of pasteurized refrigerated milk could be extended by at least 25 to 200% at $CO_2$ concentrations near the sensory threshold.

Mann, D.D., et al. "Efficient Carbon Dioxide Fumigation of Wheat in Welded-steel Hopper Bins." Applied Engineering in Agriculture. Jan 1999. v. 15 (1) p. 57-63. DNAL, S671.A66
Abstract: Two welded-steel hopper bins were modified for fumigation with carbon dioxide ($CO_2$) and a method for efficiently purging the air from the bins was developed. Concentrations of $CO_2$ during experimental fumigations were less than the concentrations predicted theoretically, but were high enough to kill more than 99% of caged adult rusty grain beetles in three separate experiments. Between 58 and 75% of the $CO_2$ initially added remained in the bin at the time when the $CO_2$ concentrations peaked. The positive results from this research mean that stored-product insects in stored grain can be controlled using $CO_2$ rather than continuing to rely on synthetic insecticides and fumigants that present health and environmental concerns.

Mazzoni, A.M., et al. "Supercritical Carbon Dioxide Treatment to Inactivate Aerobic Microorganisms on Alfalfa Seeds." Journal of Food Safety. Dec 2001. v. 21 (4). p. 215-223. Food & Nutrition Press, Inc., Trumbull, Conn. DNAL, TP373.5.J62
Abstract: The supercritical carbon dioxide (SC- $CO_2$) process involves pressurizing $CO_2$ in a chamber which generates liquid phase of carbon dioxide. Pressurized liquid $CO_2$ has a strong extraction capability of organic and inorganic compounds. The recent studies have also demonstrated that antimicrobial effect of SC- $CO_2$ due extraction some cellular components of microorganisms. The efficacy of a supercritical carbon dioxide treatment on alfalfa seeds contaminated with Escherichia coli K12 was tested at 2000, 3000, and 4000 psi at 50 degrees C. Samples were treated for 15, 30, and 60 min at each pressure. Treated seeds were evaluated in terms of germination characteristics. For aerobic plate count, the effect of pressure in the range of 2000-4000 psi was not statistically significant ($p > 0.05$) even though 85.6% inactivation was achieved at 4000 psi for 60 min. For E. coli, the reductions for 2000, 3000, and 4000 psi treatments for 15 min were 26.6, 68.1, and 81.3%, respectively. As the time was increased from 15 to 60 min at 4000 psi, the percent E. coli reduction increased from 81.3% to 92.8%. The percent germination for all treatments was over 90%. There was no significant difference ($p > 0.05$) in the germination rate of treated and untreated seeds. Supercritical carbon dioxide treatments demonstrated a reduction of E. coli K12 and total aerobic counts without affecting the germination characteristics of alfalfa seeds ($p < 0.05$). This study was a step in the direction of improving safety of alfalfa seeds used to produce fresh sprouts, which have been the cause of several outbreaks.

United States Agricultural Research Service. Carbon Dioxide Extracts Seed Oils Replacement for Hexane Solvent Process. Agricultural research - United States Agricultural Research Service. Washington, D.C. The Service. Mar 1982. v. 30 (9) p. 8-9.

Watkins, C.B. "Responses of Horticultural Commodities to High Carbon Dioxide as Related to Modified Atmosphere Packaging." HortTechnology July/Sept 2000. v. 10 (3). p. 501-506.

12. A ``Petition Justification Statement" which provides justification for one of the following actions requested in the petition:
Carbon dioxide is currently widely used in organic handling. In light of the court decision that supported Mr. Harvey regarding the use of synthetic materials in processing, we request that the National List be changed so that carbon dioxide is moved from synthetic in §205.605(b)(8) to non-synthetic §205.605(a) in the National List of Nonagricultural (non-organic) substances allowed in or on processed products labeled as "organic" or "made with organic."

History and Alternatives

As described above, there are many sources of $CO_2$ throughout the country. Some of these sources, from ethanol fermentation and from underground wells, could be evaluated as non-synthetic. The NOSB recognized this during the first review of $CO_2$, but decided that it was very difficult for buyers to determine the source of $CO_2$ when they purchase it, and therefore it would be appropriate to allow both the synthetic and non-synthetic forms. Therefore it was added to the National List of Synthetics allowed (§205.605(b)) so that untraceable sources remained permissible.

The chart below indicates the main alternatives for the uses of carbon dioxide. In all cases (except possibly the chicken slaughter using physical means) the alternatives are far more toxic and less acceptable or prohibited in an organic handling system. Because of a fairly long history of use in organics, there has not been much investigation into alternatives that might be acceptable for organic food.

| Handling Activity | Type of Use | Alternatives |
|---|---|---|
| Grains Storage/Herbs and Spices | Pest control/fumigant | Methyl bromide, other chemical fumigants. |
| Beverages: Soda, fruit juice, and beer | Ingredient for carbonation | None for soda and juice, beer uses recycled $CO_2$ from its own fermentation. |
| Natural flavors, extracts, oils, decaffeination. | Processing aid: extracting agent | Hexane, other synthetic and non-synthetic alcohols. |
| Chicken processing | Slaughtering agent | Decapitation. |
| Milk Handling | Processing aid: microbial control | Chemical preservatives. |
| Seed treatment for sprout production | Processing aid: disinfectant | High levels of chlorine. |
| Whipped Cream | Propellant | Chlorofluorocarbons. |

Interpretation of the Organic Food Production Act (OFPA)

Here are a few OFPA citations for the discussion below:

§6502 DEFINITIONS.

(21) Synthetic. The term 'synthetic' means a substance that is formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources, except that such term shall not apply to substances created by naturally occurring biological processes.

§6510 HANDLING.

(a) In General. For a handling operation to be certified under this chapter, each person on such handling operation shall not, with respect to any agricultural product covered by this chapter

(1) add any synthetic ingredient during the processing or any post harvest handling of the product

§6512 OTHER PRODUCTION AND HANDLING PRACTICES.

If a production or handling practice is not prohibited or otherwise restricted under this chapter, such practice shall be permitted unless it is determined that such practice would be inconsistent with the applicable organic certification program.

Argument 1: Under the definition of synthetic used in both OFPA and the final rule, the starting substance would have to be derived from a plant, animal or mineral source. Carbon dioxide from air is not from any of these sources and so the synthetic definition does not apply here. Therefore, following §6512, cited above, carbon dioxide should be permitted because the use of gas is not prohibited or restricted under the chapter.

Argument 2: In §6510 of OFPA, only synthetics used as ingredients are not allowed. Noting the carbon dioxide usage chart, only for carbonation and possibly as a propellant would carbon dioxide be considered an ingredient. Its other uses are processing aids, extracting agents, and pest control and as such are not be required to be non-synthetic in origin. $CO_2$ could therefore be required as an ingredient in carbonation from one of the non-synthetic sources discussed above, but any source could be used for the other purposes.

Interpretation of the National Organic Program Final Rule, 7 CFR Part 205

This petitioner is aware of various efforts by the NOSB and others to re-classify groups of substances used as processing aids, cleaning agents, extractants, and packaging materials. Because of the many uses of carbon dioxide, some would fall under each sub group under discussion. Two such interpretations in particular could apply already.

Section 205.271(c) is the Facility Pest Management Practice Standard. This paragraph states, "(c) If the practices provided for in paragraphs (a) and (b) are not effective to prevent or control pests, a non-synthetic or synthetic substance consistent with the National List may be applied." Concerning its use as pest control through atmospheric modification of a grain or herb storage facility, carbon dioxide is part of this practice standard. Regardless of the outcome of the Harvey lawsuit on ingredients used in processed food, $CO_2$ should stay on the National List for this specific use to meet this section of the rule.

The second interpretation concerns the uses of carbon dioxide as an extracting agent for natural flavors, oils, and decaffeination of coffee and tea. There is no definition of extraction in either OFPA or the NOP Final Rule. The NOSB proposed this definition: "Extraction: The concentration, separation and removal of a substance from a plant, animal microbiological or mineral source. Materials used in plant crop and animal production may be extracted in any way that does not result in synthetic reaction as defined by §2103(21). The products of any other methods of extraction shall be considered on a case by case basis and reviewed for compatibility under OFPA §2119(m)(1-7)." (NOSB, 1995; Austin, Texas). There is nothing that requires that an extracting agent be non-synthetic since there is none of it left in the final product and no chemical change has occurred in the extraction. The final rule only prohibits volatile synthetic solvents in §205.270(c)(2), but would allow anything else on the National List.

Availability Issues

Because carbon dioxide is produced in many places in many ways, and because transporting the highly pressurized gas or liquid for long distances is not particularly feasibly or desirable, some regions are at a very distinct disadvantage if it were required to source non-synthetic sources. Ethanol plants, a common source, tend to be in the grain producing areas of the country, while the mined sources are in Mississippi, New Mexico, and Colorado. In industrialized areas such as California and the Northeastern seaboard, the primary availability is from oil refining operations. When California companies requested their suppliers to try to get an ethanol source, they were informed that they would have to get an entire railcar full, about 80 tons of carbon dioxide. The storage capacity of a medium size grain processing operation is about 24 tons, making a railcar commitment not feasible. Organic handling uses are a miniscule percentage of all the $CO_2$ used in the country and so this situation is not likely to change in the near future.

Conclusion

Carbon dioxide is an important part of many Organic Handling Systems for a wide variety of uses in a wide variety of foods. It is safe, better than the alternatives, and compatible with organic production. It is essential that it remain available to Organic systems in the face of changing regulatory issues. Therefore this petition is a request to re-classify carbon dioxide in whatever way is necessary to enable its continued use.

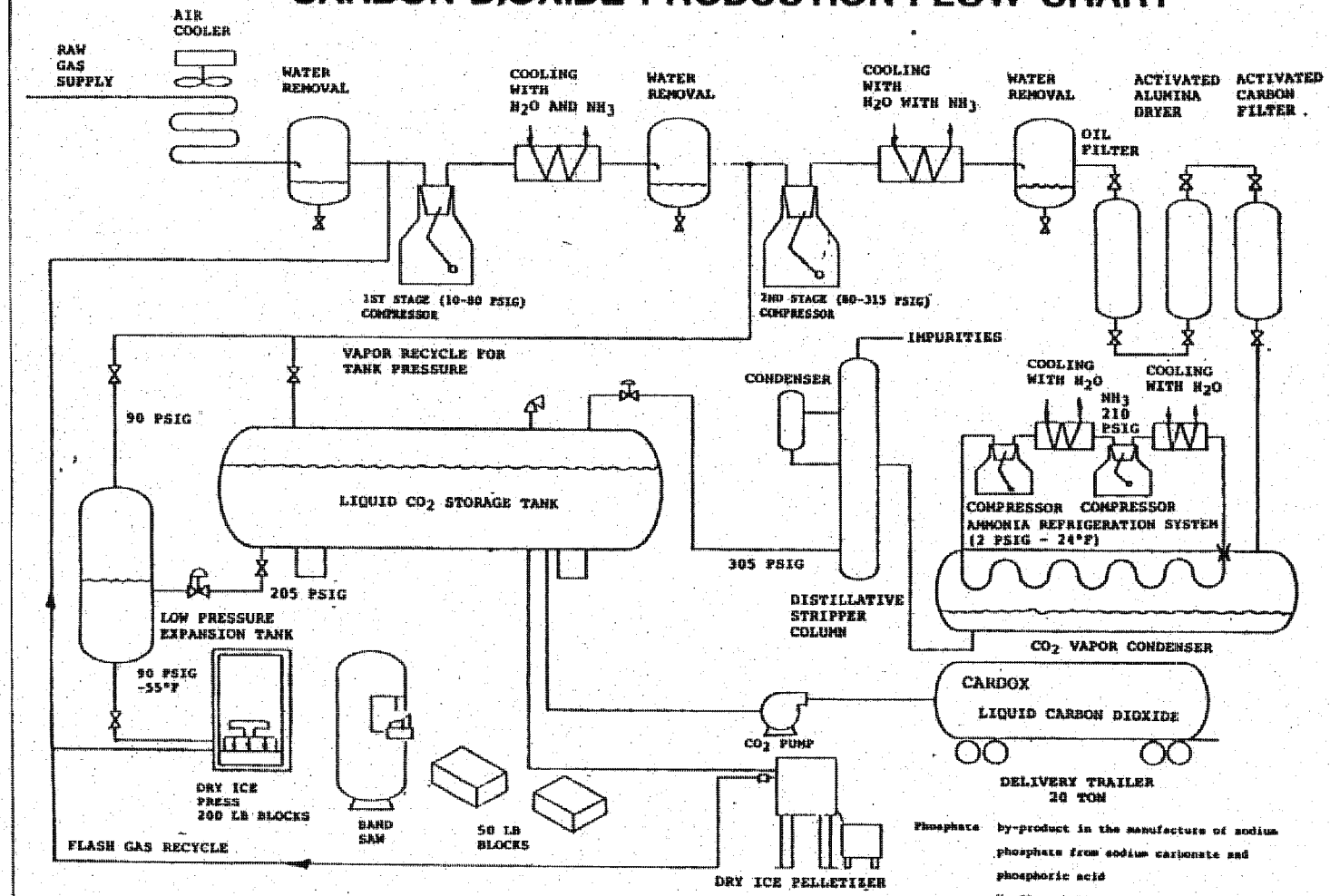
Other References

Air Liquide Group personal communication with: David Cheng, Bay area sales agent; Christine Boisrobert, Chicago office; and Tom Kuruc, manager of $CO_2$ and $N_2O$ manufacturing unit.
Kirk Othmer Encyclopedia of Chemical Technology, 3rd edition.
National Organic Standards Board, 1995. TAP review of Carbon Dioxide.

Attachments:

1. MSDS for Carbon Dioxide
2. Sources of $CO_2$ in the United States (courtesy of Air Liquide Group)
3. Carbon Dioxide Production Flow Chart
4. Original TAP Review for Carbon Dioxide.

# CARBON DIOXIDE PRODUCTION FLOW CHART




## SOURCES OF RAW $CO_2$

**Natural Gas Wells** — in purity up to 99.7% or more

**Fermentation** — by-product in the fermentation of ethanol, whiskey, beer, and other distilled products

$C_6H_{12}O_6$ ——— $2C_2$ H$_5$OH + $2CO_2$
carbohydrate — ethyl alcohol

**Ammonia** — by-product in the manufacture of hydrogen from natural gas for ammonia production

$CH_4 + O_2$ ——— $CO_2 + 2H_2$

**Hydrogen** — by-product in the manufacture of hydrogen for catalytic crackers in oil refineries and other petrochemical processes

$CH_4 + 2H_2O$ ——— $4H_2 + CO_2$

**Phosphate** — by-product in the manufacture of sodium phosphate from sodium carbonate and phosphoric acid

$Na_2CO_3 + H_3PO_4$ ——— $Na_2HPO_4 + CO_2 + H_2O$
sodium carbonate — phosphoric acid — sodium phosphate

**SNG** — by-product in the manufacture of substitute natural gas from residual fuel oil and steam

residual fuel oil + steam ——— methanol + $CO_2$

**Ethylene Oxide** — by-product in the manufacture of ethylene oxide

$C_2H_4$ + Air + Heat + Catalyst ——— $C_2H_4O + CO_2$

### COMPRESSION

Commercial carbon dioxide is manufactured from by-product stock obtained from a number of different sources. The product typically enters the system as a warm, wet gas. The $CO_2$ gas is then pressurized in turbine or piston driven compressors in at least two stages to approximately 300 psig. Carbon dioxide cooling is accomplished with water and ammonia heat exchangers after each compression step, with moisture removal after each stage of compression.

### PURIFICATION

The high pressure carbon dioxide vapor first passes through an oil filter where residual oil from compressor lubrication is removed. The $CO_2$ then enters a dryer where moisture is removed to a dewpoint below -50°F. The $CO_2$ then passes through activated carbon filters which remove any traces of alcohols, oils, or organic contaminants that might otherwise have objectionable taste or odor. The carbon dioxide then enters an ammonia-cooled condenser where the $CO_2$ is liquified at about 305 psig. Finally the $CO_2$ feeds into a distillative stripper column where methane, carbon monoxide, hydrogen, and other non-condensable gases are boiled off. The carbon dioxide liquid at 300 psig, 3°F leaving the distillative stripper is completely purified and of food-grade quality.

### LIQUEFACTION

The ammonia refrigeration system is operated with two stages of compression and two heat exchangers; one following each stage of compression. The ammonia removes heat from the $CO_2$ vapor condenser at 2 psig, -24°F. The carbon dioxide is liquified, leaving at approximately 300 psig. Carbon dioxide liquid then flows through an expansion valve entering a 450 ton capacity storage tank where temperature and pressure are maintained at about 300 psig, -20°F. All tanks are equipped with a safety relief valve and a vapor recycle loop where tank vapor and flash gas are piped back to the second stage of compression.

### DRY ICE PRODUCTION

For production of dry ice blocks, $CO_2$ liquid is drawn to a low pressure expansion tank where it is expanded to 90 psig, -55°F and then piped to a dry ice press. The $CO_2$ pressure is then dropped to atmospheric through an orifice producing $CO_2$ snow. The $CO_2$ snow is compressed into 200 lb. blocks with hydraulic pressure and then cut into 50 lb. blocks for shipping. The carbon dioxide liquid is also used to produce dry ice pellets in a Cardox dry ice pelletizer which are then shipped in insulated boxes.

### QUALITY CONTROL

Continuous quality control testing is performed at all Cardox $CO_2$ processing plants. Reports of all testing are reviewed by Cardox Quality Control Personnel. Test samples are sent at periodic intervals to the corporate lab for detailed, independent analysis to insure maintenance of pure, high-quality carbon dioxide production.

### DISTRIBUTION

Liquid carbon dioxide is pumped into tank trailers and rail cars at approximately 200 psig. During transport, this pressure will often rise, depending on distance traveled, generally to a pressure no higher than 300 psig. The customers storage tank would normally be maintained at a pressure between 275 and 305 psig. Cardox has a number of production plants located throughout the country resulting in a reliable and efficient distribution network.

# NOSB Materials Database

## Identification

**Common Name** **Carbon dioxide** **Chemical Name**

**Other Names**

**Code #: CAS** **Code #: Other**

**N. L. Category** Non-agricultural **MSDS** ◉ yes ○ no

## Chemistry

**Family**

**Composition** $CO_2$.

**Properties** colorless, odorless gas or white opaque solid.

**How Made** Can be recovered from flue gases from coal burning, from synthetic ammonia and hydrogen plants, from fermentation of sugars, by a lime-kiln operation, from sodium phosphate manufacture, or from natural carbon dioxide gas wells. All of these processes are in commercial use and which is used is determined by local individual conditions. (details of each process are in the Kirk-Othmer Encyclopedia of Chemical Technology).

## Use/Action

**Type of Use** Processing

**Specific Use(s)** carbonation of beverages. propellent, extraction method, fumigant

**Action** acts as a preservative to inhibit growth of mold and bacteria, as well as being a flavor enhancer.

**Combinations**

## Status

**OFPA**

**N. L. Restriction**

**EPA, FDA, etc**

**Directions**

**Safety Guidelines**

**State Differences**

**Historical status**

**International status**

## OFPA Criteria

2119(m)1: chemical interactions Not Applicable

2119(m)2: toxicity & persistence Not Applicable

2119(m)3: manufacture & disposal consequences

2119(m)4: effect on human health

2119(m)5: agroecosystem biology Not Applicable

2119(m)6: alternatives to substance

2119(m)7: Is it compatible?

## References

Kirk-Othmer Encyclopedia of Chemical Technology, 3rd. Ed.

see also attached.

# USDA/TAP Reviewer
# Comment Form

Material: Carbon Dioxide

Reviewer: Bob Durst

---

Is this substance Natural or Synthetic? Explain (if appropriate)

This substance is natural.

This material should be added to the National List as:

_____ Synthetic Allowed _____ Prohibited Natural

_____ Non–synthetic (allowed ingredient) _____ Non–synthetic (allowed processing aid)

_____ This material does not belong on the National List because:

Are there any restriction or limitations that should be placed on this material by use or application on the National List?

Must be listed on the label if it is an ingredient (carbonated beverage for instance).

Please comment on the accuracy of the information in the file:

The file is accurate.

Any additional comments or references?

It should be used only in an oil–free grade.

Do you have a commercial interest in this material? __X__ No; ____ Yes

Signature_____ Date _____

---

Comments on the 7 criteria in the Organic Foods Production Act:

1) Detrimental interactions: None

2) Toxicity, breakdown products, persistence: No problems.

3) Contamination during manufacturing and disposal:

4) Human health effects: Suffocation hazard in extreme concentrations.

5) Interactions with ecosystem: No detrimental ones.

6) Alternatives: In some applications nitrogen.

7) Compatible with sustainable agriculture: Yes.

# TAP REVIEWER COMMENT FORM for USDA/NOSB

**Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.**

**This file is due back to us by:** August 29, 1995

**Name of Material:** Carbon Dioxide

**Reviewer Name:** Mary C. Mulry

**Is this substance Synthetic or non-synthetic? Explain (if appropriate)**

Non-Synthetic

**If synthetic, how is the material made? (please answer here if our database form is blank)**

**This material should be added to the National List as:**

_✓_ **Synthetic Allowed** ____ **Prohibited Natural**

or, _X_ **Non-synthetic (Allowed as an ingredient in organic food)**

_X_ **Non-synthetic (Allowed as a processing aid for organic food)**

**or,** ____ **this material should not be on the National List**

**Are there any use restrictions or limitations that should be placed on this material on the National List?**

**Please comment on the accuracy of the information in the file:**

Good accurate infmtion

**Any additional comments? (attachments welcomed)** Supercrit. $CO_2$

May also be used as an extraction method — decaffeination of coffee + tea, essential oil extraction, etc (see references). Also used as a fumigant for herbs + spices.

**Do you have a commercial interest in this material?** ____ **Yes;** _X_ **No**

**Signature** _____ **Date** 9/20/95

# TAP REVIEWER COMMENT FORM for USDA/NOSB

**Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.**

**This file is due back to us by:** August 8

**Name of Material:** Carbon Dioxide

**Reviewer Name:** DR Joseph Montecalvo JR.

---

**Is this substance Synthetic or non-synthetic? Explain (if appropriate)**

Synthetic

**If synthetic, how is the material made? (please answer here if our database form is blank)**

**This material should be added to the National List as:**

✓ **Synthetic Allowed** ____ **Prohibited Natural**

or, ____ **Non-synthetic (Allowed as an ingredient in organic food)**

____ **Non-synthetic (Allowed as a processing aid for organic food)**

**or,** ____ **this material should not be on the National List**

**Are there any use restrictions or limitations that should be placed on this material on the National List?** See USES

**Please comment on the accuracy of the information in the file:** good

**Any additional comments? (attachments welcomed)**

Also used as a propellant for aerosols foods (ie. whipped cream, cheese fruit) Also used as a cryogenic freezing operation for foods.

**Do you have a commercial interest in this material?** ____ **Yes;** ✓ **No**

**Signature** [signature] **Date** 7/30/95

# TAP REVIEWER COMMENT FORM for USDA/NOSB

**Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.**

**This file is due back to us by:** August 8

**Name of Material:** Carbon Dioxide

**Reviewer Name:** R THEUER

---

**Is this substance Synthetic or non-synthetic? Explain (if appropriate)** EITHER!

**If synthetic, how is the material made? (please answer here if our database form is blank)**

GOOD INFO ATTACHED

**This material should be added to the National List as:**

____ Synthetic Allowed ____ Prohibited Natural

or, ✓ Non-synthetic (Allowed as an ingredient in organic food)

✓ Non-synthetic (Allowed as a processing aid for organic food)

**or, ____ this material should not be on the National List**

**Are there any use restrictions or limitations that should be placed on this material on the National List?**

NON-SYNTHETIC

**Please comment on the accuracy of the information in the file:**

GOOD

**Any additional comments? (attachments welcomed)**

**Do you have a commercial interest in this material?** ____ Yes; ✓ No

**Signature** [signature] **Date** 8/28/95

# Appendix B

# Known carbon dioxide source facilities in the United States.

## Known Carbon Dioxide Sources in the United States

| PRODUCER | CITY | ST | ZIP | Source Type | Start YR | Nameplate (Capacity) |
|---|---|---|---|---|---|---|
| <Confidential> | Richmond | VA | 23241 | Acid | 85 | 90 |
| | Saint Johns | AZ | 85936 | CO2-P | 90 | 100 |
| | Brandon | MS | 39043 | CO2-P | 88 | 900 |
| | Brandon | MS | 39047 | CO2-P | 88 | 1000 |
| | Guymon | OK | 73942 | CO2-P | | 400 |
| | Denver City | TX | 79323 | CO2-P | 85 | 210 |
| | Odessa | TX | 79761 | CO2-P | 90 | 350 |
| | Green River | WY | 82935 | CO2-P | 91 | 300 |
| | Rock Springs | WY | 82901 | CO2-P | 98 | 400 |
| | Cortez | CO | 81321 | CO2-W | 50 | 150 |
| | Walden | CO | 80480 | CO2-W | 85 | 300 |
| | Brandon | MS | 39601 | CO2-W | 2002 | 700 |
| | Brookhaven | MS | 39601 | CO2-W | 2002 | 700 |
| | Goshen Springs | MS | 39208 | CO2-W | 93 | 300 |
| | Madison | MS | 39110 | CO2-W | 88 | 750 |
| | Star | MS | 39167 | CO2-W | 87 | 1400 |
| | Bueyeros | NM | 88412 | CO2-W | 85 | 270 |
| | Clayton | NM | 88415 | CO2-W | 87 | 180 |
| | Valley | NM | 88412 | CO2-W | 70 | 120 |
| | Price | UT | 84501 | CO2-W | 2002 | 220 |
| | Cumberland | MD | 21501 | Cogen | 2000 | 150 |
| | Poteau | OK | 74953 | Cogen | 91 | 200 |
| | Bayport | TX | 77507 | EO | 82 | 350 |
| | Beaumont | TX | 77701 | EO | 95 | 250 |
| | Cedar Rapids | IA | 52405 | Ethnl | 86 | 500 |
| | Clinton | IA | | Ethnl | 91 | 250 |
| | Eddyville | IA | 52553 | Ethnl | 94 | 240 |
| | Galva | IA | 51020 | Ethnl | 2002 | 150 |
| | Muscatine | IA | 52761 | Ethnl | 1994 | 500 |
| | Decatur | IL | 62523 | Ethnl | 80 | 800 |
| | Pekin | IL | 61554 | Ethnl | 82 | 650 |
| | Pekin | IL | 61554 | Ethnl | 1995 | 300 |
| | Pekin | IL | 61554 | Ethnl | 86 | 300 |
| | Lawrenceburg | IN | 47025 | Ethnl | 88 | 120 |
| | South Bend | IN | 46601 | Ethnl | 85 | 480 |
| | Washington | IN | 47501 | Ethnl | 2000 | 400 |
| | Atchison | KS | 66002 | Ethnl | 67 | 140 |
| | Colwich | KS | 67030 | Ethnl | 2003 | 100 |
| | Russell | KS | 67665 | Ethnl | 2002 | 300 |
| | Hopkinsville | KY | 42240 | Ethnl | 2005 | 150 |
| | Albert Lea | MN | | Ethnl | 2001 | 260 |
| | Bingham Lake | MN | 56118 | Ethnl | 2000 | 260 |
| | Claremont | MN | 55924 | Ethnl | 2004 | 250 |
| | Marshall | MN | 56258 | Ethnl | 89 | 200 |
| | Preston | MN | | Ethnl | 2002 | 400 |
| | Winnebago | MN | 56098 | Ethnl | 98 | 225 |
| | Winthrop | MN | 55396 | Ethnl | 1994 | 120 |
| | Macon | MO | 63552 | Ethnl | 2002 | 400 |
| | Aurora | NE | 68818 | Ethnl | 2001 | 120 |
| | York | NE | 68467 | Ethnl | 98 | 200 |
| | Scotland | SD | 57059 | Ethnl | 98 | 80 |

## Known Carbon Dioxide Sources in the United States

| | | | | | |
|---|---|---|---|---|---|
| Loudon | TN | 37774 | Ethnl | 2001 | 275 |
| Loudon | TN | 37774 | Ethnl | 84 | 350 |
| Monroe | WI | 53566 | Ethnl | 2004 | 400 |
| Oshkosh | WI | 54902 | Ethnl | 2003 | 250 |
| Stanley | WI | 54768 | Ethnl | 2003 | 300 |
| Decatur | AL | 35602 | H2 | 84 | 90 |
| Decatur | AL | 35699 | H2 | 87 | 120 |
| Benicia | CA | 94510 | H2 | 80 | 300 |
| Carson | CA | 90744 | H2 | 87 | 250 |
| El Segundo | CA | 90245 | H2 | 2000 | 600 |
| Long Beach | CA | 90747 | H2 | 67 | 550 |
| Martinez | CA | 94553 | H2 | 79 | 360 |
| Richmond | CA | 94850 | H2 | 79 | 425 |
| Torrance | CA | 90503 | H2 | 78 | 350 |
| Wilmington | CA | 90748 | H2 | 77 | 300 |
| Delaware City | DE | 19706 | H2 | 71 | 500 |
| Augusta | GA | 30917 | H2 | 78 | 150 |
| Barbers Point | HI | 96707 | H2 | x | 40 |
| Chicago | IL | 60607 | H2 | 98 | 250 |
| Wood River | IL | 62095 | H2 | 88 | 250 |
| New Orleans | LA | 70126 | H2 | 81 | 480 |
| New Orleans | LA | 70126 | H2 | 2003 | 200 |
| St. Paul | MN | 55124 | H2 | 79 | 250 |
| Toledo | OH | 43659 | H2 | 70 | 230 |
| Memphis | TN | 37501 | H2 | 84 | 70 |
| Baytown | TX | 77521 | H2 | 78 | 400 |
| Texas City | TX | 77591 | H2 | 71 | 160 |
| Ferndale | WA | 98226 | H2 | 79 | 650 |
| Corpus Christi | TX | 78426 | H2 | 80 | 350 |
| Marmet | WV | 25315 | N.Gas | 82 | 1000 |
| Cherokee | AL | 35616 | NH3 | 80 | 300 |
| Courtright ON | CAN | 48060 | NH3 | 86 | 330 |
| Medicine Hat AB | CAN | | NH3 | 85 | 800 |
| Augusta | GA | 30917 | NH3 | 94 | 1250 |
| Creston | IA | 50801 | NH3 | 77 | 215 |
| Fort Dodge | IA | 50501 | NH3 | 71 | 400 |
| Sioux City | IA | 51054 | NH3 | 88 | 250 |
| East Dubuque | IL | 61025 | NH3 | 84 | 550 |
| Dodge City | KS | 67801 | NH3 | 72 | 300 |
| Donaldsonville | LA | 70346 | NH3 | 77 | 250 |
| Pollock | LA | 71467 | NH3 | 93 | 230 |
| Beatrice | NE | 68310 | NH3 | 90 | 250 |
| Beatrice | NE | 68310 | NH3 | 1995 | 225 |
| Lima | OH | 45801 | NH3 | 70 | 800 |
| Enid | OK | 73701 | NH3 | 77 | 350 |
| Verdigris | OK | 74116 | NH3 | x | 450 |
| Woodward | OK | 73802 | NH3 | 83 | 275 |
| St. Helens | OR | 97051 | NH3 | 80 | 50 |
| Borger | TX | 79007 | NH3 | 77 | 160 |
| Dumas | TX | 79029 | NH3 | 65 | 40 |
| Hopewell | VA | 23860 | NH3 | 2003 | 650 |
| Hopewell | VA | 23860 | NH3 | 55 | 435 |
| Hopewell | VA | 23860 | NH3 | 78 | 500 |

## Known Carbon Dioxide Sources in the United States

| | | | | | |
|---|---|---|---|---|---|
| Cheyenne | WY | 82002 | NH3 | 77 | 450 |
| Lawrence | KS | 66045 | PO4 | 84 | 100 |
| LaPorte | TX | 77572 | SNG | x | 120 |

Source Key:

| | |
|---|---|
| Acid | neutralization of sulfuric acid by-product |
| CO2-P | pipeline from a distant well |
| CO2-W | well (underground CO2 source) at location |
| Cogen | byproduct of Co-generation plant |
| EO | Ethylene oxide production by-product |
| Ethnl | Fermentation of ethanol source |
| H2 | hydrogen plant for oil refining |
| N.Gas | Natural gas well mixed with CO2 |
| NH3 | by-product of ammonia production |
| PO4 | phosphoric acid production by-product |
| SNG | synthetic natural gas plant |

# Appendix C

# Previous reviews by State or private certification programs

PO Box 42560, Olympia, WA 98504-2560 Ph (360) 902-1805
In accordance with Chapter 15.86 RCW and Chapter 16-160 WAC

# Washington State Department of Agriculture

# MATERIAL REGISTRATION CERTIFICATE

is issued to:

IGI, LLC
PO Box 193
Acampo, CA 95220
United States

The products listed below have been verified to comply with the USDA National Organic Standards (7 CFR Part 205):

| # | Product Name | Sub-Type | Type | Annotation |
|---|---|---|---|---|
| 2962 | IGI Carbon Dioxide | Carbon Dioxide | PPC | Preventative practices must be implemented prior to use (NOP 205.271). |

**Types: CPA - Crop Production Aid, DPC - Disease and Pest Control, FSA - Fertilizer and Soil Amendment, LPA - Livestock Production Aid, PH - Processing and Handling**

**WSDA Registered Company #**: 861
Issue Date: 12/21/2017
Registration valid through 10/31/2018

Brenda Book

Brenda Book
Organic Program Manager
DEPARTMENT OF AGRICULTURE
AGR 2291 (R/3/16)

REGISTERED MATERIAL
For Use In Organic Agriculture
Washington State Dept. of Agriculture

# Appendix D

# Material Safety Data Sheet (MSDS)

# SAFETY DATA SHEET

Carbon Dioxide

Airgas
an Air Liquide company

## Section 1. Identification

**GHS product identifier** : Carbon Dioxide

**Chemical name** : Carbon dioxide, gas

**Other means of identification** : Carbonic, Carbon Dioxide, Carbonic Anhydride, R744, Carbon Dioxide USP

**Product type** : Gas.

**Product use** : Synthetic/Analytical chemistry and Medical use.

**Synonym** : Carbonic, Carbon Dioxide, Carbonic Anhydride, R744, Carbon Dioxide USP

**SDS #** : 001013

**Supplier's details** : Airgas USA, LLC and its affiliates
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

**24-hour telephone** : 1-866-734-3438

## Section 2. Hazards identification

**OSHA/HCS status** : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

**Classification of the substance or mixture** : GASES UNDER PRESSURE - Liquefied gas
Simple asphyxiant.

**GHS label elements**

**Hazard pictograms** :

**Signal word** : Warning

**Hazard statements** : Contains gas under pressure; may explode if heated.
May displace oxygen and cause rapid suffocation.
May increase respiration and heart rate.

**Precautionary statements**

**General** : Read and follow all Safety Data Sheets (SDS'S) before use. Read label before use. Keep out of reach of children. If medical advice is needed, have product container or label at hand. Close valve after each use and when empty. Use equipment rated for cylinder pressure. Do not open valve until connected to equipment prepared for use. Use a back flow preventative device in the piping. Use only equipment of compatible materials of construction. Always keep container in upright position.

**Prevention** : Use and store only outdoors or in a well ventilated place.

**Response** : Not applicable.

**Storage** : Protect from sunlight. Store in a well-ventilated place.

**Disposal** : Not applicable.

**Hazards not otherwise classified** : In addition to any other important health or physical hazards, this product may displace oxygen and cause rapid suffocation.
May cause frostbite.

# Section 3. Composition/information on ingredients

**Substance/mixture** : Substance

**Chemical name** : Carbon dioxide, gas

**Other means of identification** : Carbonic, Carbon Dioxide, Carbonic Anhydride, R744, Carbon Dioxide USP

**Product code** : 001013

## CAS number/other identifiers

**CAS number** : 124-38-9

| Ingredient name | % | CAS number |
|---|---|---|
| Carbon Dioxide | 100 | 124-38-9 |

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

**There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.**

**Occupational exposure limits, if available, are listed in Section 8.**

# Section 4. First aid measures

## Description of necessary first aid measures

**Eye contact** : Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention if irritation occurs.

**Inhalation** : Remove victim to fresh air and keep at rest in a position comfortable for breathing. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention if adverse health effects persist or are severe. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

**Skin contact** : Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Get medical attention if symptoms occur. Wash clothing before reuse. Clean shoes thoroughly before reuse.

**Ingestion** : As this product is a gas, refer to the inhalation section.

## Most important symptoms/effects, acute and delayed

### Potential acute health effects

**Eye contact** : No known significant effects or critical hazards.

**Inhalation** : No known significant effects or critical hazards.

**Skin contact** : No known significant effects or critical hazards.

**Frostbite** : Try to warm up the frozen tissues and seek medical attention.

**Ingestion** : As this product is a gas, refer to the inhalation section.

### Over-exposure signs/symptoms

**Eye contact** : No specific data.

**Inhalation** : No specific data.

**Skin contact** : No specific data.

**Ingestion** : No specific data.

## Indication of immediate medical attention and special treatment needed, if necessary

**Notes to physician** : Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

**Specific treatments** : No specific treatment.

## Section 4. First aid measures

**Protection of first-aiders** : No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

**See toxicological information (Section 11)**

## Section 5. Fire-fighting measures

### Extinguishing media

**Suitable extinguishing media** : Use an extinguishing agent suitable for the surrounding fire.

**Unsuitable extinguishing media** : None known.

**Specific hazards arising from the chemical** : Contains gas under pressure. In a fire or if heated, a pressure increase will occur and the container may burst or explode.

**Hazardous thermal decomposition products** : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

**Special protective actions for fire-fighters** : Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Contact supplier immediately for specialist advice. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool.

**Special protective equipment for fire-fighters** : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

## Section 6. Accidental release measures

### Personal precautions, protective equipment and emergency procedures

**For non-emergency personnel** : No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Avoid breathing gas. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.

**For emergency responders** : If specialized clothing is required to deal with the spillage, take note of any information in Section 8 on suitable and unsuitable materials. See also the information in "For non-emergency personnel".

**Environmental precautions** : Ensure emergency procedures to deal with accidental gas releases are in place to avoid contamination of the environment. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

### Methods and materials for containment and cleaning up

**Small spill** : Immediately contact emergency personnel. Stop leak if without risk.

**Large spill** : Immediately contact emergency personnel. Stop leak if without risk. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

## Section 7. Handling and storage

### Precautions for safe handling

**Protective measures** : Put on appropriate personal protective equipment (see Section 8). Contains gas under pressure. Avoid breathing gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.
Avoid contact with eyes, skin and clothing. Empty containers retain product residue and can be hazardous.

# Section 7. Handling and storage

**Advice on general occupational hygiene** : Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.

**Conditions for safe storage, including any incompatibilities** : Store in accordance with local regulations. Store in a segregated and approved area. Store away from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see Section 10). Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F). Keep container tightly closed and sealed until ready for use. See Section 10 for incompatible materials before handling or use.

# Section 8. Exposure controls/personal protection

## Control parameters

### Occupational exposure limits

| Ingredient name | Exposure limits |
|---|---|
| Carbon Dioxide | **ACGIH TLV (United States, 3/2017). Oxygen Depletion [Asphyxiant].**<br>STEL: 54000 mg/m³ 15 minutes.<br>STEL: 30000 ppm 15 minutes.<br>TWA: 9000 mg/m³ 8 hours.<br>TWA: 5000 ppm 8 hours.<br>**NIOSH REL (United States, 10/2016).**<br>STEL: 54000 mg/m³ 15 minutes.<br>STEL: 30000 ppm 15 minutes.<br>TWA: 9000 mg/m³ 10 hours.<br>TWA: 5000 ppm 10 hours.<br>**OSHA PEL (United States, 6/2016).**<br>TWA: 9000 mg/m³ 8 hours.<br>TWA: 5000 ppm 8 hours.<br>**OSHA PEL 1989 (United States, 3/1989).**<br>STEL: 54000 mg/m³ 15 minutes.<br>STEL: 30000 ppm 15 minutes.<br>TWA: 18000 mg/m³ 8 hours.<br>TWA: 10000 ppm 8 hours. |

**Appropriate engineering controls** : Good general ventilation should be sufficient to control worker exposure to airborne contaminants.

**Environmental exposure controls** : Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

## Individual protection measures

**Hygiene measures** : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

**Eye/face protection** : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: safety glasses with side-shields.

**Skin protection**

## Section 8. Exposure controls/personal protection

**Hand protection** : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.

**Body protection** : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

**Other skin protection** : Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

**Respiratory protection** : Based on the hazard and potential for exposure, select a respirator that meets the appropriate standard or certification. Respirators must be used according to a respiratory protection program to ensure proper fitting, training, and other important aspects of use. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

## Section 9. Physical and chemical properties

**Appearance**

**Physical state** : Gas. [Compressed gas.]

**Color** : Colorless.

**Odor** : Odorless.

**Odor threshold** : Not available.

**pH** : Not available.

**Melting point** : Sublimation temperature: -79°C (-110.2 to °F)

**Boiling point** : Not available.

**Critical temperature** : 30.85°C (87.5°F)

**Flash point** : [Product does not sustain combustion.]

**Evaporation rate** : Not available.

**Flammability (solid, gas)** : Not available.

**Lower and upper explosive (flammable) limits** : Not available.

**Vapor pressure** : 830 (psig)

**Vapor density** : 1.53 (Air = 1) Liquid Density@BP: Solid density = 97.5 lb/ft3 (1562 kg/m3)

**Specific Volume ($ft^3$/lb)** : 8.7719

**Gas Density (lb/$ft^3$)** : 0.114

**Relative density** : Not applicable.

**Solubility** : Not available.

**Solubility in water** : Not available.

**Partition coefficient: n-octanol/water** : 0.83

**Auto-ignition temperature** : Not available.

**Decomposition temperature** : Not available.

**Viscosity** : Not applicable.

**Flow time (ISO 2431)** : Not available.

**Molecular weight** : 44.01 g/mole

## Section 10. Stability and reactivity

**Reactivity** : No specific test data related to reactivity available for this product or its ingredients.

**Chemical stability** : The product is stable.

**Possibility of hazardous reactions** : Under normal conditions of storage and use, hazardous reactions will not occur.

**Conditions to avoid** : No specific data.

**Incompatible materials** : No specific data.

**Hazardous decomposition products** : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

**Hazardous polymerization** : Under normal conditions of storage and use, hazardous polymerization will not occur.

## Section 11. Toxicological information

### Information on toxicological effects

#### Acute toxicity

Not available.

#### Irritation/Corrosion

Not available.

#### Sensitization

Not available.

#### Mutagenicity

Not available.

#### Carcinogenicity

Not available.

#### Reproductive toxicity

Not available.

#### Teratogenicity

Not available.

#### Specific target organ toxicity (single exposure)

Not available.

#### Specific target organ toxicity (repeated exposure)

Not available.

#### Aspiration hazard

Not available.

**Information on the likely routes of exposure** : Not available.

### Potential acute health effects

**Eye contact** : No known significant effects or critical hazards.

**Inhalation** : No known significant effects or critical hazards.

**Skin contact** : No known significant effects or critical hazards.

# Section 11. Toxicological information

**Ingestion** : As this product is a gas, refer to the inhalation section.

## Symptoms related to the physical, chemical and toxicological characteristics

**Eye contact** : No specific data.

**Inhalation** : No specific data.

**Skin contact** : No specific data.

**Ingestion** : No specific data.

## Delayed and immediate effects and also chronic effects from short and long term exposure

### Short term exposure

**Potential immediate effects** : Not available.

**Potential delayed effects** : Not available.

### Long term exposure

**Potential immediate effects** : Not available.

**Potential delayed effects** : Not available.

### Potential chronic health effects

Not available.

**General** : No known significant effects or critical hazards.

**Carcinogenicity** : No known significant effects or critical hazards.

**Mutagenicity** : No known significant effects or critical hazards.

**Teratogenicity** : No known significant effects or critical hazards.

**Developmental effects** : No known significant effects or critical hazards.

**Fertility effects** : No known significant effects or critical hazards.

## Numerical measures of toxicity

### Acute toxicity estimates

Not available.

# Section 12. Ecological information

## Toxicity

Not available.

## Persistence and degradability

Not available.

## Bioaccumulative potential

| Product/ingredient name | $LogP_{ow}$ | BCF | Potential |
|---|---|---|---|
| Carbon Dioxide | 0.83 | - | low |

## Mobility in soil

**Soil/water partition coefficient ($K_{OC}$)** : Not available.

**Other adverse effects** : No known significant effects or critical hazards.

## Section 13. Disposal considerations

**Disposal methods** : The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Empty Airgas-owned pressure vessels should be returned to Airgas. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Empty containers or liners may retain some product residues. Do not puncture or incinerate container.

## Section 14. Transport information

| | DOT | TDG | Mexico | IMDG | IATA |
|---|---|---|---|---|---|
| **UN number** | UN1013 | UN1013 | UN1013 | UN1013 | UN1013 |
| **UN proper shipping name** | CARBON DIOXIDE | CARBON DIOXIDE | CARBON DIOXIDE | CARBON DIOXIDE | CARBON DIOXIDE |
| **Transport hazard class(es)** | 2.2  | 2.2  | 2.2  | 2.2  | 2.2  |
| **Packing group** | - | - | - | - | - |
| **Environmental hazards** | No. | No. | No. | No. | No. |

**"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."**

**Additional information**

**DOT Classification** : **Limited quantity** Yes.
**Quantity limitation** Passenger aircraft/rail: 75 kg. Cargo aircraft: 150 kg.

**TDG Classification** : Product classified as per the following sections of the Transportation of Dangerous Goods Regulations: 2.13-2.17 (Class 2).
**Explosive Limit and Limited Quantity Index** 0.125
**Passenger Carrying Road or Rail Index** 75

**IATA** : **Quantity limitation** Passenger and Cargo Aircraft: 75 kg. Cargo Aircraft Only: 150 kg.

**Special precautions for user** : **Transport within user's premises:** always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.

**Transport in bulk according to Annex II of MARPOL and the IBC Code** : Not available.

## Section 15. Regulatory information

**U.S. Federal regulations** : **TSCA 8(a) CDR Exempt/Partial exemption**: This material is listed or exempted.

**Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs)** : Not listed

# Section 15. Regulatory information

**Clean Air Act Section 602 Class I Substances** : Not listed

**Clean Air Act Section 602 Class II Substances** : Not listed

**DEA List I Chemicals (Precursor Chemicals)** : Not listed

**DEA List II Chemicals (Essential Chemicals)** : Not listed

## SARA 302/304

### Composition/information on ingredients

No products were found.

**SARA 304 RQ** : Not applicable.

## SARA 311/312

**Classification** : Refer to Section 2: Hazards Identification of this SDS for classification of substance.

## State regulations

**Massachusetts** : This material is listed.

**New York** : This material is not listed.

**New Jersey** : This material is listed.

**Pennsylvania** : This material is listed.

## International regulations

### Chemical Weapon Convention List Schedules I, II & III Chemicals

Not listed.

### Montreal Protocol (Annexes A, B, C, E)

Not listed.

### Stockholm Convention on Persistent Organic Pollutants

Not listed.

### Rotterdam Convention on Prior Informed Consent (PIC)

Not listed.

### UNECE Aarhus Protocol on POPs and Heavy Metals

Not listed.

## Inventory list

**Australia** : This material is listed or exempted.

**Canada** : This material is listed or exempted.

**China** : This material is listed or exempted.

**Europe** : This material is listed or exempted.

**Japan** : **Japan inventory (ENCS)**: This material is listed or exempted.
**Japan inventory (ISHL)**: This material is listed or exempted.

**Malaysia** : Not determined.

**New Zealand** : This material is listed or exempted.

**Philippines** : This material is listed or exempted.

**Republic of Korea** : This material is listed or exempted.

**Taiwan** : This material is listed or exempted.

**Thailand** : Not determined.

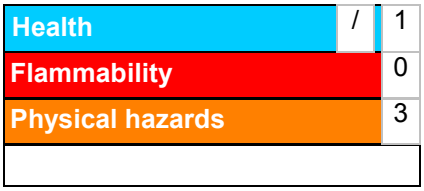
**Turkey** : This material is listed or exempted.

**United States** : This material is listed or exempted.

**Viet Nam** : Not determined.

# Section 16. Other information

## Hazardous Material Information System (U.S.A.)




**Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings and the associated label are not required on SDSs or products leaving a facility under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered trademark and service mark of the American Coatings Association, Inc.**

**The customer is responsible for determining the PPE code for this material. For more information on HMIS® Personal Protective Equipment (PPE) codes, consult the HMIS® Implementation Manual.**

## National Fire Protection Association (U.S.A.)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**Copyright ©2001, National Fire Protection Association, Quincy, MA 02269. This warning system is intended to be interpreted and applied only by properly trained individuals to identify fire, health and reactivity hazards of chemicals. The user is referred to certain limited number of chemicals with recommended classifications in NFPA 49 and NFPA 325, which would be used as a guideline only. Whether the chemicals are classified by NFPA or not, anyone using the 704 systems to classify chemicals does so at their own risk.**

## Procedure used to derive the classification

| Classification | Justification |
|---|---|
| GASES UNDER PRESSURE - Liquefied gas | Expert judgment |

## History

**Date of printing** : 2/12/2018

**Date of issue/Date of revision** : 2/12/2018

**Date of previous issue** : 4/25/2017

**Version** : 0.03

**Key to abbreviations** : ATE = Acute Toxicity Estimate
BCF = Bioconcentration Factor
GHS = Globally Harmonized System of Classification and Labelling of Chemicals
IATA = International Air Transport Association
IBC = Intermediate Bulk Container
IMDG = International Maritime Dangerous Goods
LogPow = logarithm of the octanol/water partition coefficient
MARPOL = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution)
UN = United Nations

**References** : Not available.

## Notice to reader

# Section 16. Other information

**To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.**

**Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.**

# Appendix E

# Research information about the substance

*Plant and Soil* **238:** 325–333, 2002.



# The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species

Matthias C. Rillig[1,4], Sara F. Wright[2] & Valerie T. Eviner[3]
[1]*Microbial Ecology Program, Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA;* [2]*USDA-ARS-SMSL, BARC-W, 10300 Baltimore Ave., Beltsville, MD 20705, USA;* [3]*Department of Integrative Biology, University of California, Berkeley, CA 94720, USA;* [4]*Corresponding author**

Received 5 December 2000. Accepted in revised form 1 November 2001

*Key words:* Grassland, hyphae, path analysis, water-stable aggregates

## Abstract

Soil aggregation and soil structure are fundamental properties of natural and managed ecosystems. However, most of our knowledge on the role of plant species in soil aggregation is derived from work in agroecosystems or with agriculturally important plants. Here we examined the effects of five plant species on soil aggregate water stability. The five species (three grasses, one forb, and a legume) were from the same natural grassland, and were grown in monoculture plots in the field. Our first goal was to test if productivity-related or species-specific factors would prevail in determining soil aggregation. We also tested what the relative importance of the soil protein glomalin (produced by arbuscular mycorrhizal fungi, AMF) in soil aggregation is, compared to other factors, including AMF hyphal and root length and percent plant cover. We found significant differences in soil aggregate water stability (1–2 mm size class) for the five plant species examined, and corresponding differences in plant cover, root weight and length, AMF soil hyphal length, and glomalin concentrations. A structural equation modeling approach (path analysis) was used to distinguish direct from indirect effects of factors on soil aggregation based on covariance structures. Root length, soil glomalin, and percent cover contributed equally strong paths to water-stable aggregation. The direct effect of glomalin was much stronger than the direct effect of AMF hyphae themselves, suggesting that this protein is involved in a very important hypha-mediated mechanism of soil aggregate stabilization, at least for the 1–2-mm size class of aggregates.

## Introduction

Soil structure is central to soil and ecosystem functioning as it controls fluxes of water, gases and nutrients. Soils also serve as large repositories for carbon, with carbon storage capacity greatly depending on soil structure. The vast majority of knowledge about soil structure in general, and on the influence of organisms (e.g. plants) on soil aggregation is derived from experiments and observations made in agroecosystems or using agriculturally important plants (Angers and Caron 1998; Haynes and Beare 1997). While many of these observations will also be transferable to natural ecosystems as general principles, it is important to realize that effects on soil structure can perhaps not always be separated from management practices, like tillage and fertilization, associated with agricultural cultivation (Angers and Caron, 1998). Few studies have addressed biological factors involved in soil aggregation in natural ecosystems (e.g., Jastrow et al., 1998; Miller and Jastrow, 1990). The focus of this study was to elucidate contributions of mycorrhizal fungi and other biological factors on soil aggregation using native plants from a grassland ecosystem.

Given constant abiotic factors, fungal hyphae are among the most important, if not the most important agents in soil aggregate stabilization among the soil biota (Degens, 1997), although effects of roots, soil bacteria and fauna are clearly significant as well (Degens,

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1997; Oades, 1984). Among the fungi, arbuscular mycorrhizal fungi (AMF) appear to be the most important mediators of soil aggregation for three reasons. The extraradical hyphae of AMF represent a substantial, often dominant component of soil microbial biomass (e.g. Allen, 1991; Miller et al., 1995; Rillig et al., 1999). By directly tapping into carbon resources of the plant, they are independent of the limiting carbon supply in bulk soil on which saprobic fungi depend (Smith and Read, 1997). Additionally, since grazers prefer saprobic hyphae over AMF hyphae (Klironomos and Kendrick, 1996), AMF hyphae appear to have a longer residence time in soil, allowing for a less transient contribution to soil aggregate stabilization than saprobic hyphae. As a consequence, AMF hyphae were one of the most important components in a path analysis model describing biotic influences on soil aggregation compared to numerous other biological factors (Jastrow et al., 1998).

Recently, a new factor of presumably great importance in soil aggregation was discovered: glomalin (Wright and Upadhyaya, 1996). Glomalin is a glycoprotein, produced by AMF, and its concentration in aggregates (Wright and Upadhyaya, 1998) and soil (Rillig et al., 2001) correlates with the percentage of water-stable aggregates (WSA). We were interested in examining the relative importance of glomalin in comparison with other biological factors in explaining the proportion of WSA in soil. Specifically, we were interested in separating the direct effects of hyphae from those caused by glomalin, and how plant species can alter these effects.

Plant species can influence soil aggregation (Reid and Goss, 1981; Scott, 1998, Tisdall and Oades, 1979) through a number of mechanisms, including: Root structure and distribution, quality of carbon inputs, quantity of carbon inputs, effects on soil microclimate, and influences on microbial communities and their activities (e.g. bacterial extracullular polysaccharide production, mycorrhizae). In prairies, water stable aggregates >0.2 and >2 mm diameter have been associated with the presence of grass species more than with any other vegetation group (Jastrow, 1987). We examined soil aggregation associated with different grassland species to test if species-specific factors or more general, productivity-related factors could be held responsible for observed differences in water stability of aggregates. There is a scarcity of information on the effects of co-occurring plant species from a natural ecosystem on soil aggregation (Angers and Caron, 1998), although examples from agroecosystems are available (e.g. Degens et al., 1994).

## Materials and methods

### *Field experiment*

This research took place at the University of California Hopland Research and Extension Center, located in Mendocino County, California (39°00′ N latitude, 123°04′ W longitude). The ecosystem is a California annual grassland in the coastal range of northern California. The area experiences a Mediterranean climate, with wet, cool winters, and hot, dry summers. The soil was a fine-loamy over clayey, mixed, mesic Ultic Haploxeralf (Sutherlin series).

In the summer of 1997, a 30 m × 60 m area was mowed, and litter was removed and subsequently autoclaved to kill the seeds in the litter. In order to minimize the existing seedbank, the area was irrigated (5 cm of water), and the resident seedbank was allowed to establish, as it would in a typical fall germinating rain. After irrigation, the germinated vegetation was killed using glyphosate (Roundup). This process was repeated once more to almost eliminate the pre-existing seedbank. We established 1-$m^2$ plots of different plant monocultures, with 9 replicates of each species treatment (seven of which were used for this study). These were layed out in a randomized block design. Seeds of each species were planted at a density planned to achieve constant end of season biomass among the species (based on preliminary greenhouse data; V. Eviner, unpublished). Seeds were raked into the top cm of soil, and then the autoclaved litter was placed back into the plots. The seeds were allowed to germinate naturally with the fall germinating rains. Species composition was maintained through weeding. Percentage plant cover and density were representative of surrounding area of grassland (V. Eviner, unpublished).

The plant species used for this experiment were: 3 grass species [*Avena barbata* (slender wild oats $n$ = 6), *Aegilops triuncialis* (barbed goatgrass; $n$ = 6), *Taeniatherum caput-medusae* (medusa head; $n$ = 7)]; 1 forb species, *Amsinckia douglasiana* (fiddleneck; $n$ = 6); and 1 legume, *Trifolium microcephalum* (maiden clover $n$ = 7). In April of 1999. after 2 growing seasons, samples were extracted from experimental species plots with a 2 cm diameter corer to a depth of 15cm. Three cores per plot were taken, spaced approximately equidistantly in the center area of the plot

(to minimize edge effects), and pooled. Soil samples were air-dried and stored in paper bags until analysis.

*Water-stable aggregates in the 1–2-mm size class ($WSA_{1-2\text{-}mm}$)*

All soils had been stored as air-dried samples >4 months. We concentrated on macro-aggregates of 1–2 mm diameter, since the amounts of these aggregates are sensitive to short term (< 2 yr) management and treatment of soils (Kemper and Rosenau, 1986); also most effects of glomalin have been observed for this size class. Replicate 4 g samples of soil aggregates were moistened by capillary action for 10 min. Water-stability of aggregates was then measured with a wet-sieving method using the apparatus and procedure described in Kemper and Rosenau (1986). Percentage of water-stable aggregates ($WSA_{1-2\text{-}mm}$) is calculated using the mass of aggregated soil remaining after wet sieving and the total mass of aggregates at the beginning. The initial and final weights of aggregates were corrected for the weight of coarse particles (> 0.25 mm).

*Percent cover and aboveground biomass*

In early June of 1999, a visual percent plant cover estimation was made for each of the plots. This cover estimation is substantially lower than the actual growing season% cover values for *Amsinckia* and *Trifolium*, since these measurements incorporate high gopher activity on these plots that occurred between early April and the time of sampling. At the time of belowground sampling, measurements of aboveground biomass were made by harvesting plant material in a 10.16-cm-diameter ring , and drying, and weighing it.

*Soil AM fungal hyphae, non-mycorrhizal fungal hyphae, and roots*

Hyphae were extracted from a 4 g soil subsample by an aqueous extraction and membrane filter technique modified after Jakobsen et al. (1992), as described in Rillig et al. (1999). Soil samples were mixed and suspended in 100 mL of deionized water, to which 12 mL of a sodiumhexametaphosphate solution (35 g $L^{-1}$) was added. The soil suspensions were shaken for 30 s (end-over-end), left on the bench for 30 min, and then decanted quantitatively through a 38 $\mu$m sieve to retain hyphae, roots and organic matter. The material on the sieve was sprayed gently with deionized water to remove clay particles, and then transferred into a 250 mL Erlenmeyer flask with 200 mL of deionized water. The flask was shaken vigorously by hand for 5 s, left on the bench for 1 min, and then a 2 mL aliquot was taken and pipetted onto 25 mm Millipore filters. The material on the filter was stained with 0.05% Trypan Blue in lactoglycerol and transferred to microscope slides. Hyphal length was measured with a grid-line intersect method at 200 × magnification, distinguishing hyphae into mycorrhizal and non-mycorrhizal hyphae according to Rillig et al. (1999).

Roots were extracted from 10-g soil samples by floatation and wet-sieving. Soils were suspended in 1 L of water in a beaker and stirred vigorously; the floating roots were decanted onto a 0.50 mm sieve, rinsed, and picked with forceps. This process was repeated until no further roots were retained on the sieve. Root weight was measured after drying over night at 105 °C. Root lengths were measured using the WinRhizo V 3.10B root image analysis system (Régent Instruments Inc, Québec, Canada).

*Glomalin*

Glomalin extractions from whole-soil subsamples were carried out as described by Wright and Updahyaya (1998). Easily-extractable glomalin (EEG) was extracted with 20 mm citrate, pH 7.0 at 121 °C for 30 min. Total glomalin (TG) was extracted with 50 mm citrate, pH 8.0 at 121 °C in rounds of 60 min each. For the sequential extractions, the supernatant was removed by centrifugation at 5000 × *g* for 20 min. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin. Extracts from each replicate were pooled and then analyzed. After extraction cycles were completed, samples were centrifuged at 10000 × *g* to remove soil particles, and protein in the supernatant was determined by ELISA using the monoclonal antibody MAb 32B11 (Wright and Upadhyaya, 1998). Immunoreactive fractions of glomalin are designated by the prefix IR, hence we obtained the two glomalin fractions IREEG and IRTG. We also measured glomalin using a Bradford assay (Wright and Upadhyaya 1998), yielding the two fractions called TG and EEG (without the IR prefix). Concentrations of glomalin were extrapolated to mg/g for all four measured fractions (EEG, TG, IREEG, IRTG) by correcting for the dry weight of coarse fragments (> 0.25 mm) included in the extraction of soil.

*Statistical methods*

Means were compared using analysis of variance, with the factor plant species used as a fixed effect. We also tested for a block effect. Residuals were examined for normality (Shapiro-Wilks W test), and homogeneity of variances was tested (Levene's test).

Path analysis (structural equation modeling) has been used previously to test causal relationships among interacting biological factors on aggregate stability (Jastrow et al., 1998). We used the AMOS 4.01 software package (SmallWaters Corporation, Chicago, IL., USA) to design the model, and calculate path coefficients, squared multiple correlations, and model fit. To test for collinearity among independent variables we used the ridge regression procedure of the NCSS 2000 software package (NCSS Statistical Software, Kaysville, UT, USA).

## Results

*Effects of plant species*

The block effect was never significant and was, therefore, dropped from the analysis. The proportion of $WSA_{1-2\text{-}mm}$ differed significantly ($F$ = 4.54, $P$ = 0.006) in the soils underneath the five plant species (Figure 1), ranging from 72 to 85% stable aggregates in this size class. Percent plant cover ($F$ = 10.3; $P < 0.0001$) and aboveground plant biomass ($F$ = 13.7; $P < 0.0001$) both were significantly different between plant species (Figure 2a and b). The latter result indicates that the goal to achieve similar plant biomass was not achieved in the field. Root weight ($F$ = 9.00; $P < 0.0001$) and length ($F$ = 9.39, $P < 0.0001$) were both significantly different as well for the five species, the pattern closely following that for water-stable aggregates (Figure 3). AMF soil hyphal length ($F$ = 14.89; $P < 0.0001$) and non-mycorrhizal fungal hyphal length ($F$ = 6.91; $P$ = 0.0006) differed in the soils under the five plant species as well, but NM hyphal length exhibited quite a different pattern than AMF (Figure 4). AMF hyphal length was also always higher than NM fungal hyphal length. We only found a significant difference among the five plant species for the immunoreactive easily-extractable glomalin fraction (IREEG; $F$ = 7.94; $P$ = 0.0002)), not for the total immunoreactive glomalin pool (IRTG; $F$ = 1.55; $P$ = 0.21) (Figure 5). This pattern also held true for the protein fractions measured using the Bradford assay (EEG and TG) (Figure 6).

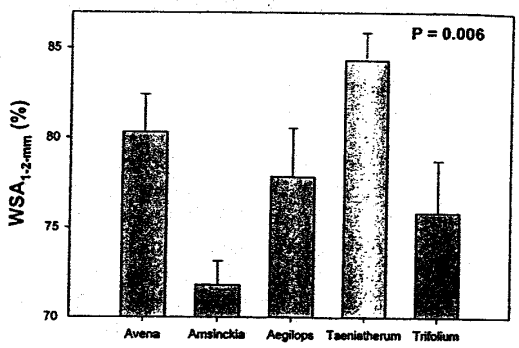


*Figure 1.* Effect of plant species on the proportion of water stable aggregates ($WSA_{1-2\text{-}mm}$) in the 1–2 mm size class. Plant species are (number of replicates): *Avena barbata* ($n$ = 6), *Amsinckia douglasiana* ($n$ = 6), *Aegilops triuncialis* ($n$ = 6), *Taeniatherum caput-medusae* ($n$ = 7), *Trifolium microcephalum* ($n$ = 7). Error bars are standard errors of the mean ($P$-value from ANOVA).

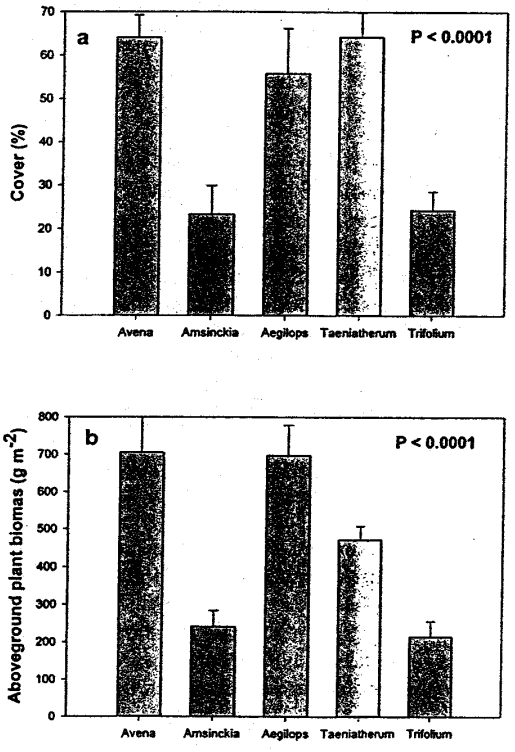


*Figure 2.* Effect of plant species on percent plant cover (a) and aboveground plant biomass (b). Error bars are standard errors of the mean ($P$-values from ANOVA). For plant species names and number of replicates see Figure 1.

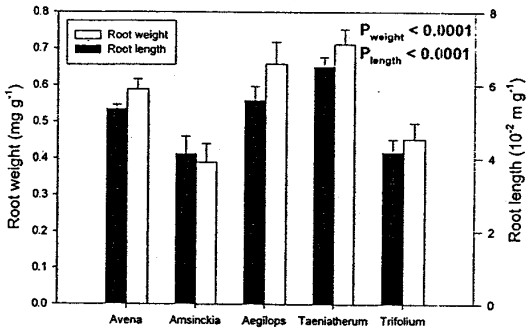


*Figure 3.* Effect of plant species on root weight (mg $g^{-1}$ soil) and length (cm $g^{-1}$ soil). Error bars are standard errors of the mean (*P*-values from ANOVA). For plant species names and number of replicates see Figure 1.

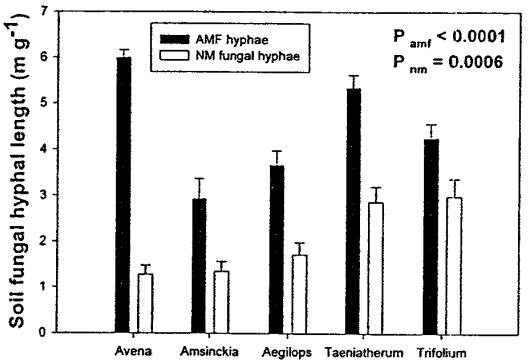


*Figure 4.* Effect of plant species on arbuscular mycorrhizal fungal (AMF; black bars) and non-mycorrhizal fungal (white bars) hyphal lengths (m $g^{-1}$ soil). Error bars are standard errors of the mean (P-values from ANOVA). For plant species names and number of replicates see Figure 1.

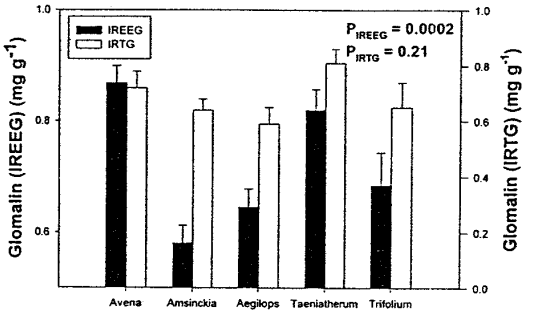


*Figure 5.* Effects of plant species on soil concentrations (mg $g^{-1}$ soil) of glomalin. IREEG (black bars) is the easily extractable glomalin fraction: IRTG (white bars) is the total glomalin fraction (IR indicates that glomalin was measured by *i*mmuno-*r*eactivity). Error bars are standard errors of the mean (*P*-values from ANOVA). For plant species names and number of replicates see Figure 1.

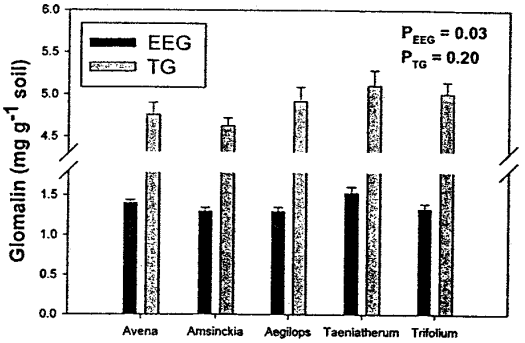


*Figure 6.* Effects of plant species on soil concentrations (mg $g^{-1}$ soil) of glomalin. EEG (black bars) is the easily extractable glomalin fraction; TG (white bars) is the total glomalin fraction (these fractions were measured using the Bradford assay). Error bars are standard errors of the mean (*P*-values from ANOVA). For plant species names and number of replicates see Figure 1.

### *Construction of path model*

Root weight and root length were highly correlated, as there was no change in specific root length among the plant species (data not shown; $F = 1.97$; $P = 0.13$). Previous studies have found a better model fit with root length (Jastrow et al., 1998), and this was also the case here; we therefore used root length in the model. Root length was causally linked with hyphal length and directly with percent $WSA_{1-2\text{-}mm}$. Percent plant cover was linked with root length and $WSA_{1-2\text{-}mm}$. The latter was included since protection of the soil surface by plant material could lessen the impact of rainwater, which could cause aggregate disintegration (e.g. Angers and Caron, 1998). We constructed a direct and an indirect path (via glomalin) from hyphal length to $WSA_{1-2\text{-}mm}$. We did not include length of non-AMF fungal hyphae in the model (Figure 4). Initially, we also included labile carbon pools in the model (carbon respired after 48 or 120 hours of incubation; data not shown), but this did not improve model fit or the multiple correlation coefficient for $WSA_{1-2\text{-}mm}$; we therefore dropped these variables from the model.

### *Path analysis*

Almost 50% of the variability in water-stable aggregates in the 1–2 mm size class was explained by the variables hypothesized to have an effect on aggregation, as included in the path diagram (Figure 7). Product-moment correlations between the variables included in the path model are shown in Table 1.

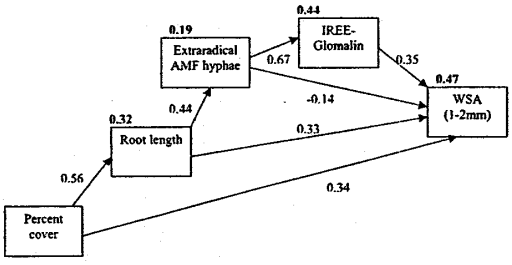


*Figure 7.* Path model depicting the hypothesized causal relationship of dependent and independent variables. Numbers on arrows are standardized path coefficients. Numbers in bold are estimates of the proportion of total variance explained (squared multiple correlations) for each dependent variable (i.e. all except root length). Each arrow signifies a hypothesized direct causal relationship in the direction of the arrow. Indirect causal effects occur if one variable is linked to another via other, intermediate variables. The model fit was significant ($\chi^2 = 6.61$; df = 3; $P > 0.05$).

Decomposition of correlations into direct, indirect and total effects and their statistical significance (from bootstrap analyses given by the Amos software) are shown in Table 2 for all variables in the model. Root length, percent plant cover, and IREE-Glomalin had about equally strong direct paths to $WSA_{1-2\text{-}mm}$, with 0.33, 0.34 and 0.35, respectively (all $P < 0.1$; see Table 2 for details). The direct path from extraradical hyphal length was weak (and not statistically significant). There was a strong path from hyphal length to IREE-Glomalin. Root length contributed a strong path to hyphal length (0.67). The total effect of root length on $WSA_{1-2\text{-}mm}$ is composed of the direct effect, and the sum of all the indirect effects (each obtained by multiplying the path coefficients along the direction of a causation path). The total effect of root length was 0.37. The total effect of percent plant cover on $WSA_{1-2\text{-}mm}$ was 0.55. While the direct effect of hyphal length on $WSA_{1-2\text{-}mm}$ was −0.14, the total effect, including the indirect effect via glomalin, was +0.10. The indirect path of hyphae via glomalin (0.23) was hence greater than the direct effect of hyphae on $WSA_{1-2\text{-}mm}$.

Using maximum likelihood estimation, we obtained a $\chi^2$ of 6.61 for this model (df = 3; $P$ = 0.09). The goodness-of-fit ($\chi^2$) test tests the null hypothesis that the covariance matrix implied by the model (expected) reproduces the observed covariance matrix. Failure to reject that null hypothesis ($P > 0.05$) therefore indicates that the model was a good fit. We also calculated a c.f.i. (comparative fit index, ranging from 0 to 1) of 0.93, and a Tucker-Lewis Index (an index that appears least affected by sample size, ranging mostly from 0 to 1) of 0.79, further supporting that an acceptable fit of the model to the data was achieved. The model-implied and observed correlation coefficients were, as a consequence of model fit, well correlated ($r^2 = 0.75$). The independent model (observed variables are assumed to be uncorrelated with each other) fit was non-significant ($\chi^2 = 66.4$; df =10; $P < 0.0001$). We examined normality of the data by Mahalanobis distances ($d^2$) of individual data points from the multivariate centroid of the data set, in addition to examining kurtosis and skewness. Based on these tests, we excluded three observations from the final analysis (this lead to a reduction of n for three plant species from 7 to 6). Multicollinearity in the data set was not a problem, as examined by calculating variance inflation factors (all < 10) and condition numbers (based on the eigenvalues of the correlation matrix; all < 100).

## Discussion

Our results show that plant species from the same grassland can affect soil aggregate water stability, perhaps primarily via their different biomass and percent cover. As has been found previously, grass species in our study were associated with higher aggregation than other plant types (Jastrow, 1987, Tisdall and Oades, 1979), while differences among grass species were not statistically significant (Scott, 1998). These differences among plant species provided us with an opportunity to examine which factors were responsible for bringing about these aggregation changes. Particularly, we focused on plant species differences in productivity, *versus* potential species-specific mechanisms.

### *Species-specific versus productivity-related mechanisms*

All of the examined plant species were host plants for AMF (Rillig, unpublished). While AM fungi are mostly believed to be non-host specific, there is a preferential association of mycobionts with certain plant hosts (Bever et al., 1996). AMF can differ in a variety of physiological and ecological traits, for example in hyphal production (Giovanetti and Gianinazzi-Pearson, 1994), production of glomalin per hyphal length (Wright et al., 1996), and promotion of aggregate stability (Schreiner and Bethlenfalvay,

*Table 1.* Pearson's product-moment correlations between variables included in the path model (Figure 7)

| | Cover (%) | Root length | AMF hyphal length | IREEG | WSA (%) |
|---|---|---|---|---|---|
| Cover (%) | 1.00 | | | | |
| Root length | 0.56 | 1.00 | | | |
| AMF hyphal length | 0.42 | 0.44 | 1.00 | | |
| IREEG | 0.48 | 0.52 | 0.66 | 1.00 | |
| WSA (%) | 0.60 | 0.61 | 0.36 | 0.57 | 1.00 |

1995). Therefore it is conceivable that the different host plants, being colonized by different subsets of the AMF community, could give rise to species-specific changes in aggregate stability. However, species-specific mechanisms determining aggregation did not appear to be important in our study. Although these species differed in C inputs (data not shown), our path analysis indicates that these did not contribute to the aggregation patterns. The five plant species did also not differ significantly in our estimate of gross root architecture, i.e. specific root length; however, they have been shown to be associated with very different root growth rates and root distributions (Peters, 1994).

The alternative hypothesis is that the identity of the species does not matter, but rather their relative productivity. This hypothesis seems to be supported by our data. For example, root length was a good predictor of hyphal length, which, in turn, was a good predictor of glomalin concentration. In a species-specific model, we would have expected to see deviations from a pattern where responses scale linearly with root biomass or length. For instance, a plant species associated with an AMF community with higher average hyphal production or glomalin production should have given rise to significantly lower path coefficients for the root length/ hyphal length – glomalin paths. There was also a relatively strong path from percent cover to $WSA_{1-2\text{-}mm}$, and cover had the strongest total effect on $WSA_{1-2\text{-}mm}$. This is to be expected if the model were essentially productivity-driven.

*The path model*

Our path model differs somewhat in structure from other models with a similar goal. Whereas our data came from a comparison of different plant species, data for the Jastrow et al. (1998) model was derived from the study of a chronosequence of prairie restoration. For example, we have chosen to include percent plant cover of plots as a predictor variable (to test for productivity-related effects). Jastrow et al. (1998) obtained comparable multiple correlations for aggregate stability to our study in some aggregates size classes they studied (e.g. $r^2 = 0.39$ or 0.69 for the 0.212–1.00 mm and 1.00–2.00 mm size classes, respectively). However, they achieved a higher $r^2$ in their consideration of macroaggregates as a whole ($r^2 = 0.88$). Their model also included soil carbon pools: soil organic carbon, microbial biomass carbon, and hot water soluble CHO carbon. The paths from these carbon pools were generally weak (the highest being 0.14 from microbial biomass carbon). Including a labile carbon pool in our path model (carbon respired after 48 or 120 hrs of incubation) did not increase model fit in our path model or the multiple correlation coefficient for aggregate stability.

*The role of glomalin*

We used the path modeling approach to attempt to separate, based on covariance structures, the effects of glomalin from that of the hyphae of AMF themselves (Figure 7). Glomalin, once extracted (i.e. solubilized) from soil, is clearly no longer in its native state. Hence, it is problematic to extract glomalin and simply add it back into soil to study its effect on aggregation separately from that of hyphae. Furthermore, it has been proposed that one of the modes of action of glomalin could be to lead to the formation of a 'sticky' string-bag of hyphae (Jastrow and Miller, 1997) that would stabilize aggregates. It would hence be experimentally difficult to separate the effects of hyphae from those of glomalin associated with their surfaces. Our path model suggests that the indirect effects of hyphae via the production of glomalin were stronger than the direct effect of hyphae, which, according to our model, were rather weak (and as direct effects, not significant).

*Table 2.* Decomposition of correlations into (standardized) direct, indirect and total effects, and their statistical significance (*P*-value; Monte Carlo analysis). Total effects are the sum of indirect and direct effects (due to rounding to three significant digits, indirect and direct effects do not exactly add up in this table). IREEG only has a direct effect. Direct effects are equal to the path coefficients shown in Figure 7

| | Effects on WSA | | |
|---|---|---|---|
| | Direct | Indirect | Total |
| Cover | 0.336 (0.049) | 0.213 (0.044) | 0.548 (0.003) |
| Root length | 0.335 (0.062) | 0.042 (0.590) | 0.372 (0.044) |
| Hyphal length | −0.139 (0.512) | 0.234 (0.089) | 0.096 (0.592) |
| IREEG | 0.352 (0.089) | – | 0.352 (0.089) |

This does certainly not contradict earlier studies that have attributed a strong effect to hyphae (e.g. Jastrow et al., 1998; Miller and Jastrow 1990; Tisdall and Oades 1982), since the total effect of hyphae would include the glomalin effect. This result does, however, highlight the need for further research into the functioning of this abundant soil protein. We have only examined these relationships for one aggregate size ($WSA_{1-2\text{-}mm}$). It should be the subject of further study to test if the importance (and possibly the function) of glomalin differs at other positions in the aggregate hierarchy (Tisdall and Oades, 1982).

## Acknowledgements

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# Influence of enhanced $CO_2$ concentration and irrigation on sudangrass digestibility

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**Abstract**

An experimental line of sudangrass (*Sorghum bicolor* L. Moench) was included in the free-air $CO_2$ enrichment (FACE) project in 1991 at the University of Arizona Maricopa Agricultural Center to evaluate the effect of ambient (approximately 370 $\mu$mol mol$^{-1}$) and enriched (550 $\mu$mol mol$^{-1}$) $CO_2$ in well-watered or water-stressed plots. Our specific objective was to determine modifications caused by these environmental effects on the percentages of morphological parts and the fiber components, and on the in vitro digestibility in vegetative and mature harvests. Enrichment with $CO_2$ did not ($P > 0.05$) change the percentages of morphological parts or fiber components, or the digestibility of any of the morphological components. Protein levels tended to be lower in $CO_2$-enriched plants. However, water-stressed plants tended to have a higher proportion of leaves (blades and sheaths) and a lower proportion of stems, were more digestible, and had lower amounts of anti-quality, aromatic compounds within the plant cell. Stems had the highest digestibility of all morphological components (about 75% in vegetative plants) despite the lowest levels of protein. Stems also showed the greatest changes caused by all treatments, including a 20% decline in digestibility from vegetative to mature samples. The results indicate that enriching $CO_2$ to 550 $\mu$mol mol$^{-1}$ did not reduce digestibility of sudangrass.

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## 1. Introduction

Studies indicate that atmospheric concentrations of $CO_2$ are steadily increasing (Bacastow et al., 1985). Some reports suggest that current concentrations of about 350 $\mu$mol mol$^{-1}$ might double during the next century (Houghton et al., 1990). Increased concentrations of $CO_2$ could have a dramatic effect on plants and, consequently, studies have been conducted in recent years to assess this effect on plant growth and physiology, agricultural yield, and economic consequences (reviewed by Kimball (1983) and Allen (1990)). One probable advantage of increased atmospheric concentrations of $CO_2$ is that plant yield will be significantly increased. For example, Kimball (1983), in reviewing several studies of many agricultural crops, reported that overall yield may increase by 33% with a doubling of atmospheric $CO_2$ concentrations.

Despite the advantage of increased yield, little is known about the quality of agricultural commodities that will be produced in an environment of increased $CO_2$ levels. No results are available on changes in quality of forage for ruminants. The importance of forages to the economy is indicated by statistics, compiled for the USA in 1989, that reported hay production of 131 Mg (tonnes) with an economic value of over $11 billion (US Department of Agriculture, 1990). Further, changes in quality are important, as indicated by evaluations of bermudagrass (*Cynodon dactylon* L. Pers.) cultivars, which showed that a 12% increase in its digestibility resulted in a 30% increase in mean animal weight gain (Lowrey et al., 1968).

Sudangrass is a $C_4$, drought-tolerant, high-yielding warm season annual grass that provides high-quality pasture, hay, and silage (Ball et al., 1991). Our specific objective was to evaluate changes in quality as a result of $CO_2$ enrichment for sudangrass grown under wet and dry conditions. We assessed changes in the proportions of morphological parts and their in vitro digestibilities and chemical characteristics related to quality.

## 2. Materials and methods

### 2.1. Overall design and procedures

This study was conducted as part of the free-air $CO_2$ enrichment (FACE) joint project of the US Department of Energy and the US Department of Agriculture, carried out at the University of Arizona Maricopa Agricultural Center in 1991 (Lewin et al., 1994). This design permitted plant growth under specific modifications of parameters in an otherwise natural environment, rather than growth in artificial laboratory conditions. Enriched $CO_2$ was maintained at 550 $\mu$mol mol$^{-1}$ within four circular plots (22 m diameter) from 05:00 to 19:00 h by computer-controlled instrumentation. Four replicate control plots had ambient concentrations of $CO_2$ at about 370 $\mu$mol mol$^{-1}$. Each of the main $CO_2$ plots was split with regard to irrigation. Half of each plot was well-watered ('wet'), receiving a total of 701 mm water on a 3–5 day schedule during the 16 week growth period of sudangrass. This amount of

irrigation was sufficient to replace the full consumptive use of crops. The other half of each plot was water-stressed ('dry'), receiving a total of 456 mm water over the same 16 week period. The water was applied using a drip irrigation system with the tubes buried about 0.1 m under the rows. Equal applications of urea (about 15 kg $ha^{-1}$ N) were added through the irrigation system to all plots at approximately weekly intervals. The major crop for the study was cotton (*Gossypium hirsutum* L.), and the sudangrass was included in designated areas of each plot. Thus, there were 16 semi-circular plots with two levels of $CO_2$, two levels of irrigation, and four replicates of each treatment combination.

### 2.2. Forage samples

An experimental line of dwarf forage sudangrass (1985, RDC) was developed and supplied by W.W. Hanna (Coastal Plain Experiment Station, ARS–USDA, Tifton, GA). Sudangrass seeds were planted about 10 cm apart in two 1 m sections of row in each plot on 29 May, 1991, after young cotton plants were pulled from the rows. Plants were destructively harvested (i.e. all plant material was collected, including roots) twice. In the early harvest, on 22 July, at approximately 8 weeks of age, every third plant within the rows was collected. Most of these plants were vegetative, with only a few having immature seed heads. In the late harvest, on 16 September, at approximately 16 weeks of age, the remaining plants within the rows were collected. These plants were mature, having fully developed seed heads. After harvest, intact plants were placed in glasshouses and air-dried for several weeks. Morphological parts of the dried plants were separated, freeze-dried, and weighed. Roots were washed to remove the soil before freeze-drying. Leaf blades, leaf sheaths, and stems were ground in a Wiley mill to pass a 1 mm screen for fiber and protein determinations and for in vitro evaluation of digestibility.

### 2.3. Analyses

Single samples from each replicate were analyzed for neutral detergent fiber (NDF) (Van Soest and Wine, 1967), acid detergent fiber (ADF), acid detergent lignin (ADL) (Van Soest, 1963), permanganate lignin (PML) (Van Soest and Wine, 1968), and protein (Association of Official Analytical Chemists (AOAC), 1980). Feed analyses, based on a series of detergent extractions, are used to assess the various chemical fractions of the forage and, thereby, to estimate forage quality and especially digestibility by rumen microorganisms (Van Soest, 1967; Barton et al., 1976). The NDF treatment separates the soluble components from the fiber, thereby providing an estimate of the cell wall content (Van Soest and Wine, 1967). The NDF fraction contains potentially digestible, structural carbohydrates (e.g. cellulose and much of the hemicellulose) and the nondigestible aromatic components (e.g. lignins). Phenolic compounds limit digestibility of the potentially degradable polysaccharides through association with condensed, polymeric aromatics (i.e. lignins) and also through covalently linked phenolic acids. The ADF and ADL treatments remove the less tightly associated structural carbohydrates of the cell wall, thus providing an

assessment of the most refractory carbohydrates bound with the aromatic constituents in the cell wall (Akin et al., 1975). The treatments to estimate lignin (i.e. ADL and PML) do not provide a precise characterization of the chemical nature of the aromatic compounds, which probably differ for the two procedures (Van Soest and Wine, 1967).

For in vitro digestibility, the Tilley and Terry (1963) two-stage procedure was used to analyze duplicate samples per replicate. 'Summative digestibilities' were calculated to estimate the digestibility of the shoots. For these results, the percentage of each morphological component was calculated from the total weight of leaf blades, leaf sheaths, and stems. This percentage was multiplied by the digestibility coefficient for each component, and the products for blades, sheaths and stems were summed.

### 2.4. Statistics

Data were analyzed using the procedure of the Statistical Analysis System Intitute Inc. (1985) for a split plot experiment with repeated measurements. Error term (a), to test the main effect (enriched $CO_2$ treatment vs ambient), was the residual sum of squares from treatment × ring, with three degrees of freedom. Error term (b) in the subunit analysis was the residual sum of squares.

## 3. Results

The percentages of total dry weight partitioned among the various morphological

Table 1
Percentage of total dry weight in various morphological components of sudangrass grown in control (ambient — about 370 $\mu$mol mol$^{-1}$) and FACE (enriched to 550 $\mu$mol mol$^{-1}$) $CO_2$ concentrations and with well-watered (wet) and water-stressed (dry) levels of irrigation

| $CO_2$ Treatment | Irrigation | Percentage morphological components[d] | | | | |
|---|---|---|---|---|---|---|
| | | Leaf blades | Leaf sheath | Stems | Flowers | Roots |
| *Early harvest* | | | | | | |
| Control | Wet | $40.8 \pm 3.8^{ab}$ | $16.1 \pm 1.3^{a}$ | $31.1 \pm 3.2^{a}$ | $0.40 \pm 0.37^{a}$ | $11.6 \pm 2.4^{a}$ |
| Control | Dry | $46.8 \pm 2.3^{c}$ | $18.0 \pm 0.4^{b}$ | $22.5 \pm 3.2^{b}$ | $0.03 \pm 0.05^{b}$ | $12.7 \pm 1.3^{a}$ |
| FACE | Wet | $38.8 \pm 2.3^{b}$ | $16.1 \pm 1.2^{a}$ | $31.6 \pm 3.3^{a}$ | $0.08 \pm 0.15^{b}$ | $13.4 \pm 1.3^{a}$ |
| FACE | Dry | $44.9 \pm 2.4^{ac}$ | $17.3 \pm 0.9^{ab}$ | $25.7 \pm 2.5^{b}$ | $0^{b}$ | $12.1 \pm 0.9^{a}$ |
| *Late harvest* | | | | | | |
| Control | Wet | $21.5 \pm 3.3^{ab}$ | $11.8 \pm 0.5^{a}$ | $55.0 \pm 7.6^{a}$ | $6.8 \pm 2.2^{a}$ | $5.0 \pm 4.7^{a}$ |
| Control | Dry | $22.3 \pm 1.7^{a}$ | $10.8 \pm 1.9^{a}$ | $50.5 \pm 5.7^{a}$ | $9.8 \pm 5.2^{a}$ | $7.0 \pm 4.2^{a}$ |
| FACE | Wet | $17.8 \pm 1.0^{b}$ | $10.3 \pm 1.0^{a}$ | $55.0 \pm 6.3^{a}$ | $12.8 \pm 3.8^{a}$ | $4.5 \pm 6.4^{a}$ |
| FACE | Dry | $21.5 \pm 3.0^{ab}$ | $11.0 \pm 2.4^{a}$ | $51.5 \pm 8.2^{a}$ | $10.0 \pm 4.3^{a}$ | $5.8 \pm 5.9^{a}$ |

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leq 0.05$.

parts (Table 1) were not different ($P > 0.05$) as a result of $CO_2$ treatment, but differences occurred between irrigation treatments. Leaf blades and sheaths were greater ($P \leqslant 0.05$), or tended to be greater, in plants grown under water stress in the early harvest; stems contributed a higher proportion of biomass for well-watered plants in both harvests. In the early harvest, plants were generally pre-flowering, but water stress tended to delay maturity, as shown by a lower proportion of plants having flowers.

In vitro digestibilities for the various morphological parts (Table 2) were not different ($P > 0.05$) with $CO_2$ treatments, but consistent trends occurred with the irrigation treatments. Leaves and stems grown under water stress were more digested ($P \leqslant 0.05$), or tended to be more digested, than those of plants grown with ample water, and this phenomenon was significant ($P \leqslant 0.05$) in late-harvested stems for both $CO_2$ levels. The order of digestibility was stems > leaf blades > leaf sheaths for both harvests. When digestibilities of morphological parts were considered for all four treatments, the decline in digestibility from early to late harvest was 20%, 9%, and 18% for stems, blades, and sheaths, respectively.

Fiber and protein concentrations for the various morphological parts of sudangrass were generally not different ($P > 0.05$) with $CO_2$ treatment (Table 3). Two exceptions were that $CO_2$ enrichment increased ($P \leqslant 0.05$) NDF in early-harvested leaf blades and decreased ($P \leqslant 0.05$) stem protein in both harvests. Trends, however, were apparent between irrigation treatments, but these varied for the chemical components and for the harvests. For all morphological parts, early-harvested and water-stressed plants had lower ($P \leqslant 0.05$) amounts of ADF, and stems also had lower ($P \leqslant 0.05$) NDF amounts. In the late-harvested plants, the most consistent trend

Table 2
In vitro digestibility of morphological parts of sudangrass grown in control (ambient — about 370 $\mu$mol·mol$^{-1}$) or FACE (enriched to 550 $\mu$mol mol$^{-1}$) $CO_2$ concentrations and with well-watered (wet) or water-stressed (dry) levels of irrigation

| $CO_2$ Treatment | Irrigation | Percentage in vitro digestibility[d] | | |
|---|---|---|---|---|
| | | Leaf blade | Leaf sheath | Stem |
| *Early harvest* | | | | |
| Control | Wet | $64.9 \pm 2.0^{ab}$ | $60.6 \pm 1.1^{ab}$ | $74.2 \pm 3.3^{a}$ |
| Control | Dry | $65.8 \pm 2.9^{a}$ | $62.1 \pm 1.6^{ac}$ | $77.4 \pm 3.4^{a}$ |
| FACE | Wet | $64.7 \pm 1.5^{ab}$ | $59.7 \pm 0.8^{b}$ | $76.3 \pm 2.2^{a}$ |
| FACE | Dry | $62.5 \pm 0.9^{b}$ | $63.9 \pm 1.7^{c}$ | $77.6 \pm 1.7^{a}$ |
| *Late harvest* | | | | |
| Control | Wet | $56.6 \pm 2.3^{a}$ | $51.3 \pm 2.4^{a}$ | $59.0 \pm 2.6^{a}$ |
| Control | Dry | $59.4 \pm 3.0^{a}$ | $50.9 \pm 1.7^{a}$ | $63.8 \pm 2.2^{b}$ |
| FACE | Wet | $58.6 \pm 1.7^{a}$ | $50.2 \pm 3.3^{a}$ | $59.1 \pm 1.2^{a}$ |
| FACE | Dry | $59.8 \pm 1.9^{a}$ | $50.9 \pm 2.9^{a}$ | $63.0 \pm 3.3^{b}$ |

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

Table 3
Chemical composition of morphological components of sudangrass grown in control (ambient — about 370 $\mu$mol mol$^{-1}$) or FACE (enriched to 550 $\mu$mol mol$^{-1}$) $CO_2$ concentrations and with well-watered (wet) or water-stressed (dry) levels of irrigation[d]

| $CO_2$ Treatment | Irrigation | Percentage components in early harvest | | | | |
|---|---|---|---|---|---|---|
| | | Neutral detergent fiber | Acid detergent fiber | Acid detergent lignin | Permanganate lignin | Protein |
| *Leaf blade* | | | | | | |
| Control | Wet | $60.7 \pm 1.2^{a}$ | $26.4 \pm 1.3^{ab}$ | $3.1 \pm 0.1^{a}$ | $5.9 \pm 1.6^{a}$ | $19.9 \pm 1.0^{a}$ |
| Control | Dry | $60.3 \pm 1.3^{a}$ | $24.4 \pm 0.5^{c}$ | $3.1 \pm 0.2^{a}$ | $4.9 \pm 0.2^{a}$ | $18.6 \pm 0.8^{a}$ |
| FACE | Wet | $62.9 \pm 1.1^{b}$ | $27.3 \pm 0.8^{a}$ | $3.3 \pm 0.3^{a}$ | $5.8 \pm 0.5^{a}$ | $18.9 \pm 1.4^{a}$ |
| FACE | Dry | $61.6 \pm 0.6^{ab}$ | $25.7 \pm 1.3^{bc}$ | $3.2 \pm 0.2^{a}$ | $5.8 \pm 1.7^{a}$ | $19.1 \pm 0.7^{a}$ |
| *Leaf Sheath* | | | | | | |
| Control | Wet | $65.4 \pm 0.7^{a}$ | $35.7 \pm 0.8^{a}$ | $4.1 \pm 0.2^{ab}$ | $7.5 \pm 1.0^{a}$ | $6.3 \pm 0.2^{a}$ |
| Control | Dry | $62.7 \pm 0.3^{b}$ | $31.6 \pm 0.7^{b}$ | $3.9 \pm 0.2^{b}$ | $6.2 \pm 0.3^{a}$ | $6.2 \pm 1.7^{a}$ |
| FACE | Wet | $64.7 \pm 1.9^{ac}$ | $35.4 \pm 0.3^{a}$ | $4.9 \pm 1.3^{a}$ | $7.0 \pm 0.1^{a}$ | $5.6 \pm 0.3^{a}$ |
| FACE | Dry | $63.4 \pm 0.6^{bc}$ | $32.5 \pm 1.5^{b}$ | $3.9 \pm 0.1^{b}$ | $7.1 \pm 1.4^{a}$ | $6.2 \pm 0.5^{a}$ |
| *Stem* | | | | | | |
| Control | Wet | $54.5 \pm 1.5^{a}$ | $31.0 \pm 0.6^{a}$ | $6.0 \pm 5.0^{a}$ | $6.1 \pm 0.5^{a}$ | $5.1 \pm 0.3^{ab}$ |
| Control | Dry | $48.6 \pm 4.2^{b}$ | $25.5 \pm 2.5^{b}$ | $4.9 \pm 3.8^{a}$ | $4.7 \pm 0.4^{b}$ | $8.3 \pm 0.8^{c}$ |
| FACE | Wet | $54.2 \pm 2.0^{a}$ | $31.3 \pm 1.5^{a}$ | $3.4 \pm 0.5^{a}$ | $5.9 \pm 0.3^{a}$ | $4.5 \pm 0.6^{b}$ |
| FACE | Dry | $50.6 \pm 1.9^{ab}$ | $27.5 \pm 1.9^{b}$ | $3.1 \pm 0.4^{a}$ | $5.7 \pm 0.5^{a}$ | $6.0 \pm 0.8^{a}$ |

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

was in the concentrations of ADF, ADL, and PML, which were lower ($P \leqslant 0.05$), or tended to be lower, for plants grown under water stress. Generally, the NDF and lignin concentrations increased from early-harvested to late-harvested plants for all morphological parts. Although generally not significant ($P > 0.05$), protein concentrations (Table 3) showed a trend for lower values with $CO_2$ enrichment, and values were lower ($P \leqslant 0.05$) for protein in FACE stems. Protein amounts were lowest in stems, despite the highest digestibility for this part, and amounts were significantly higher ($P \leqslant 0.05$) in water-stressed plants, regardless of $CO_2$ treatment.

'Summative digestibilities' (Table 4) indicated that $CO_2$ enrichment did not alter the overall digestibility of plant shoots. With ample water, the percentage decrease in digestibility for early-harvested and late-harvested shoots was 15%, whereas the decrease was only 10% with water-stressed plants, regardless of $CO_2$ concentration.

## 4. Discussion

Forages, such as the sudangrass used in the present study, are composed of plant cell walls (i.e. fiber) and soluble components inside the cells. Soluble components (e.g.

Table 4
Summative digestibilities of sudangrass plants grown in control (ambient — about 370 $\mu$mol mol$^{-1}$) and FACE (enriched to 550 $\mu$mol mol$^{-1}$) $CO_2$ concentrations and with well-watered (wet) or water-stressed (dry) levels of irrigation

| $CO_2$ Treatment | Irrigation | Percentage in vitro digestibility[d] | |
|---|---|---|---|
| | | Early harvest | Late harvest |
| Control | Wet | $67.5 \pm 2.0^{a}$ | $57.6 \pm 1.6^{a}$ |
| Control | Dry | $68.1 \pm 1.1^{a}$ | $61.2 \pm 1.9^{b}$ |
| FACE | Wet | $67.9 \pm 1.1^{a}$ | $57.9 \pm 0.8^{a}$ |
| FACE | Dry | $67.2 \pm 0.8^{a}$ | $60.5 \pm 2.9^{ab}$ |

[d] Average ± standard deviation for four replicates.
[abc] Different superscripts for values within a plant component within a harvest indicate significant differences, $P \leqslant 0.05$.

sugars and proteins) are virtually 100% digestible, but plant cell walls vary in bioavailability (Van Soest, 1967). Although the amount of cell walls per se can influence digestibility, the greatest limitation to plant digestibility is the association of structural carbohydrates of the cell wall (e.g. hemicellulose) with phenolic compounds (Akin and Chesson, 1989; Hartley and Ford, 1989). Therefore, the amount and type of fiber has a dramatic influence on forage digestibility (Akin, 1989).

The $CO_2$ treatment did not alter the in vitro digestibility or the chemical components (i.e. aromatics) most limiting cell wall digestibility for sudangrass. Irrigation treatment often altered digestibility, with water-stressed plants tending to have a higher digestibility and lower amounts of phenolic compounds in the fiber. This effect was more pronounced in the stem component. In addition to the fiber components, protein amounts in stems were higher in water-stressed plants, but this component did not appear to have a substantial influence in the in vitro procedure, where N was not limiting. Our data are in agreement with those of Wilson (1982), who, based on studies in the literature assessing environment and digestibility, concluded that light to moderate drought stress increased digestibility compared with that of well-watered plants. The lower contents of stem, lignin, and flowers support the contention of Wilson (1982) that plant aging may be slower in plants receiving less water.

The treatments influenced the digestibility of the stem component more than that of the other components of sudangrass. Although stems are often lower in digestibility than leaves (Pritchard et al., 1963; Twidwell et al., 1991), this component of sudangrass in the present study had the highest digestibility. Other work (D.E. Akin, unpublished data, 1992) indicated that both pith and rind of sudangrass are potentially highly digestible in young stages. Plant cell walls have been reported in general to decline in digestibility with increased maturity (Van Soest, 1967). The steep decline in stem digestibility with maturity found in this study is in agreement with other data showing that maturity reduces stem digestibility to a greater extent than that of leaves (Pritchard et al., 1963).

We are not aware of any previously published work on the effect of $CO_2$ enrich-

ment on forage digestibility. However, unpublished results (D.E. Akin and H.B. Johnson, 1992) indicated that leaves and stems of wheat (*Triticum aestivum* L.) did not differ in digestibility when produced under ambient (350 $\mu$mol mol$^{-1}$) or sub-ambient (220 and 250 $\mu$mol mol$^{-1}$) concentrations of $CO_2$; water stress resulted in improved digestibility of stems, regardless of $CO_2$ concentration. Similarly, preliminary work (D.E. Akin, unpublished data, 1993), with wheat grown in a similar FACE project to that described above, indicated that enriched $CO_2$ concentrations have little effect on digestibility. A previous study (Kimball et al., 1987), involving beet armyworm herbivory of cotton, led to the conclusion that cotton grown under $CO_2$ enrichment (650 $\mu$mol mol$^{-1}$) was of lower nutritive value to insects than plants grown under ambient $CO_2$ concentrations. Further, some plants respond to enhanced $CO_2$ with increased starch grains and alterations in plant anatomy (Thomas and Harvey, 1983; Vu et al., 1989), both characteristics which potentially influence biodegradability (Akin and Burdick, 1977; Akin, 1989). The present study of sudangrass, using the mixed rumen microbial population that includes microorganisms potentially able to catabolize all carbohydrates within the plant, indicated that the digestibility of this forage was not altered by $CO_2$ enrichment.

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# Soil Life & Carbon

## Answers to Global Warming in Our 'Root Cellar'

*by Mike Amaranthus, Ph.D.*

*Root system of a rye crop inoculated with compost tea and mycorrhizal fungi — this area is rich in feeder roots, soil organisms and soil carbon.*

From the food we eat, to the air we breath, to the clothes we wear, humans depend on the thin covering of the earth's surface we call soil. Arguably this thin and fragile layer of living topsoil is the Earth's most critical natural resource. Soil is literally the "root cellar" for the planet, a storage area that feeds us and protects us in emergencies. It nurtures life in both forest and field and carves intricate paths that link the health of the land, sea and atmosphere.

Lately there has been tremendous attention given to carbon sequestration. Five to ten years ago, few had heard of or cared about the concept. Carbon sequestration has suddenly become a hot topic because carbon in the air combines with oxygen to become carbon dioxide, a greenhouse gas that contributes to global warming.

Soils are key players in the process of storing (sequestering) and recycling carbon. According to Canada's Department of Agriculture and the Environment, soils contain more than all the carbon in the atmosphere and three times more than is stored in all the Earth's vegetation. Soil microbes break down decaying plant and animal matter in the process of creating fertile soils, and healthy soils containing billions of beneficial microorganisms and vigorous root systems have become an important carbon sink, binding up carbon that might otherwise enter the atmosphere.

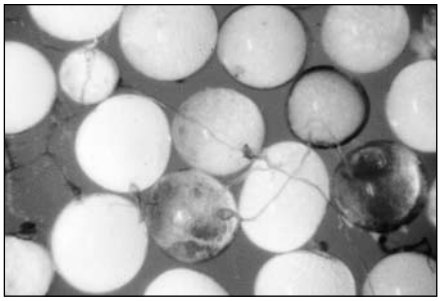
The carbon absorbed from the atmosphere by plants and animals can take several paths before it re-enters the air as carbon dioxide. When a plant or animal dies, it is broken down by soil microorganisms. As the microorganisms consume the organic matter, they release some of the carbon into the atmosphere in the form of carbon dioxide. Some is destined for longer-term storage in roots and in the bodies of plant-eating or carnivorous animals. Animals then return more of the carbon to the atmosphere as $CO_2$ through respiration, although some will be stored within their bodies until they die and decompose. Finally, as plants and animals decay, instead of escaping as carbon dioxide, a significant portion of their carbon becomes part of the organic component in soils through the activities of essential soil organisms. These beneficial microorganisms work to produce a substance known as humus, a stable, rich component of soil that is the color of dark chocolate and loaded with carbon.

### HISTORY LESSON

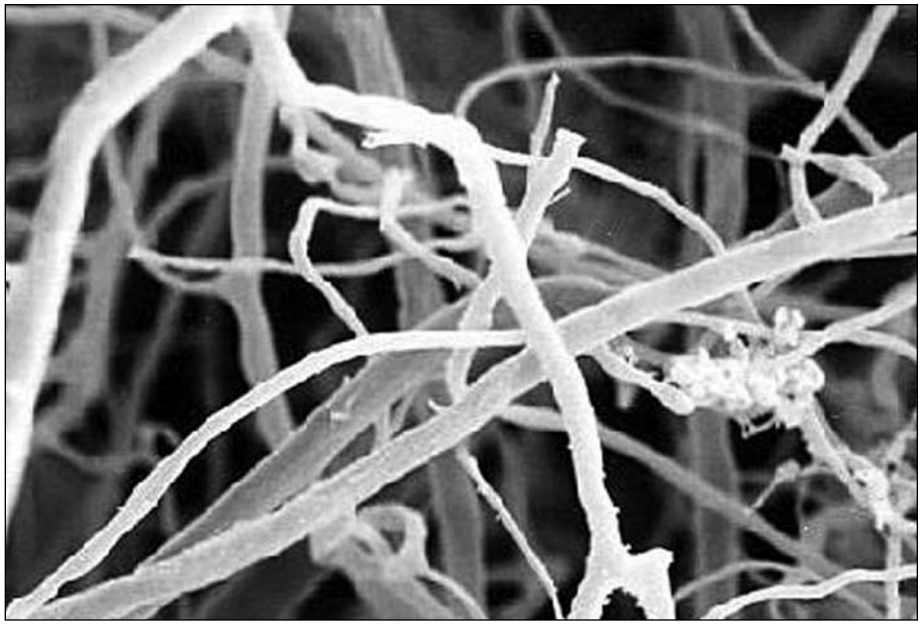
When frequent tillage is introduced, long chains of carbon that are the essence of humus are converted into carbon dioxide, which releases into the atmosphere. Soil depleted of the humic fraction is more prone to erosion, loss of microbial diversity and a breakdown of structure and can support fewer animals and plants. "Organic matter is the elixir of microbial life in the soil," explains Dr. Dave Perry, professor and ecologist at Oregon State University. "It holds water, preventing drought and floods, it supports the living soil organisms that hold the key to sustainable plant growth, and it is a reservoir of carbon that plays a key role in global climate change."

Soils can contain a wide range of organic matter. Most topsoils range from 1 to 20 percent organic matter. The best agricultural lands have loamy topsoil in which there is a high concentration of organic matter. Some of the richest in the world were found in the Great Plains of the central United States, where perennial grasses, their roots systems and associated soil organisms over thousands of years built up deep layers of carbon-laden topsoil. They form continuously, but very slowly. Only about one inch of soil is formed every 500 to 1,000 years, so loss of good topsoil is a serious issue that has led to the rise and fall of civilizations.

The great early civilizations of Mesopotamia, for example, arose because of the richness of their soils, and collapsed because of declines in soil quality. Poor



*Endomycorrhizal spores such as these are deposited beneath the soil surface and do not rapidly recolonize agricultural sites once they have been lost.*



*Mycorrhizal filaments in the soil extract nutrients and water and leave deposits of carbon-rich glomalin.*

land management and excessive irrigation caused soils to become increasingly degraded and unable to support the Fertile Crescent civilizations. Ancient Greece suffered a similar fate. The philosopher Plato, writing around 360 B.C., attributed the demise of Greek dominance to soil degradation: "In earlier days Attica yielded far more abundant produce. In comparison of what then was, there are remaining only the bones of the wasted body; all the richer and softer parts of the soil having fallen away, and the mere skeleton of the land being left." What Plato likely did not recognize is how much carbon had washed away from these Greek soils.

In the New World, similar processes were unfolding. Harvard Professor Sylvanus Morley concluded back in the 1930s that the great Mayan Civilization of Mesoamerica collapsed because they overshot the carrying capacity of the land. Deforestation and erosion exhausted their resource base. Mayans died of starvation and thirst in mass, and others fled once-great cities, leaving them as silent warnings for generations to come.

UCLA professor Jared Diamond, author of the books *Guns, Germs and Steel* and *Collapse*, argues that most inhabitants of Easter Island in the Pacific died because of deforestation, erosion and soil depletion. In Iceland, farming and human activities caused about 50 percent of the soil to end up in the sea, explains Diamond, concluding, "Icelandic society survived only through a drastically lower standard of living." Not surprisingly, the practice of destroying soils by torching watersheds or salting farms and fields has been employed by armies in warfare from the time of Alexander the Great to Napoleon.

Today, we are facing many of the same issues: removal of native vegetation, over-harvest, dwindling supplies of fresh water, overworked soils and sprawling population growth. Our poor management of the land has resulted in serious warning signs. Widespread agricultural pollution of lands and seas, accelerated topsoil loss, damage to fish and aquatic life, pesticide buildup in our bodies, and rapidly declining nutritional value of food have become environmental problems of immense importance that are directly related to soil. Now is the time to bring attention to the critical role our management of soil plays in another environmental issue of great significance: global climate change.

## FIRST LESSON

How do we stop the degradation of our soils? The answers can be found in nature below the soil surface in our "root cellar." A favorite habitat of microbes is near and in the roots of plants. Although many of them live throughout the soil, up to 100 times more live close to the roots of plants.

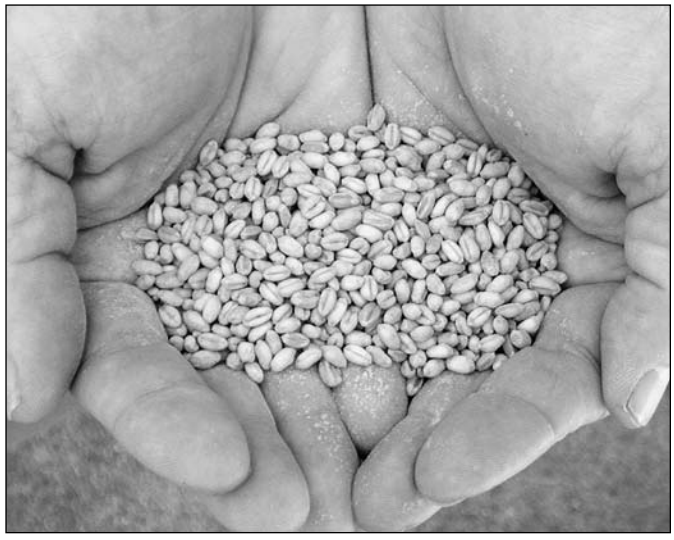
This area near the roots is called the rhizosphere, the thin layer of soil surrounding the roots. Some microbes have such a close relationship with plants that they actually live *inside* the plant, such as beneficial mycorrhizal fungi. Their threads penetrate into the root and secure sugars provided by the plant to fuel their growth. In exchange, these same filaments radiate out from the root into the surrounding soil where they capture nutrients and water and transport these materials back to the plant. It is estimated that mycorrhizal fungal filaments explore hundreds to thousands of times more soil volume than roots alone.

Endomycorrhizae, also known as arbuscular mycorrhizae, are the symbiotic association of fungus and root that occur on more plant species than all other types of mycorrhizae combined. They have been observed in the roots of more than 1,000 genera of plants representing some 200 families. It has been estimated that more than 85 to 90 percent of the estimated 400,000 species of vascular plants in the world form arbuscular mycorrhizae. These include most grains, vegetables, fruit and nut trees, vines and turf grasses.

Benefits of mycorrhizae include:

- Improved nutrient and water uptake;
- Improved root growth;
- Improved plant growth and yield;
- Reduced transplant shock;
- Reduced drought stress.

Some modern agricultural practices reduce the biological activity in soil.



*A granular mycorrhizal inoculant (left) and a mycorrhizal inoculant coating on wheat seed.*

Certain pesticides, chemical fertilizers, intensive cultivation, compaction, organic matter loss, and erosion adversely effect beneficial mycorrhizal fungi. An extensive body of laboratory testing indicates that the majority of intensively managed agricultural lands lack adequate populations of mycorrhizal fungi. Farming widespread areas affects the plant/mycorrhizal relationship in two fundamental ways. First, it isolates the plant from beneficial mycorrhizal fungi available in natural settings. Second, it increases a healthy crop's need for water, nutrients and soil structure.

Once lost from a farm, endomycorrhizal populations are slow to recolonize unless there is close access to natural areas that can act as a source of mycorrhizal spores. Endomycorrhizal fungi do not disperse their spores in the wind, but must grow from root to root or be dispersed by animals, so close proximity to healthy and undisturbed natural sites may be necessary. Normally though, farmers seldom have the opportunity to grow their crops immediately adjacent to undisturbed natural ecosystems.

Inoculating farmland soils with mycorrhizal fungi before, during or following planting can improve crop establishment, growth, yield and carbon sequestration. Mycorrhizal inoculants are available in liquid, powder and granular forms and can be sprinkled onto roots during transplanting, banded beneath seed, used as a seed coating or watered in via existing irrigation systems. The goal is to create physical contact between the mycorrhizal inoculant and the crop roots, and the type of application depends upon the farmer's equipment and needs. Inoculants that are concentrated and contain several species of mycorrhizal fungi produce the best results. The cost of inoculation generally ranges from $7 to $17 per acre.

## CARBON-RICH SUPERGLUE

Mycorrhizae also perform another service for the ecosystem that has only recently come to light. The USDA published a report by Don Comis on work by Sara F. Wright and Kristine A. Nichols that suggests a substance called glomalin, discovered by Wright in 1996, does indeed "glom" onto a large amount of carbon. The glomalin molecule is made up of 30-40 percent carbon and represents up to 30 percent of the carbon in soil. It is a natural superglue that binds organic matter to mineral particles in soil. It also forms soil clumps — aggregates — that improve soil structure and keep other soil carbon from escaping. It is in fact glomalin that gives soil its tilth — a subtle texture that enables experienced farmers to identify great soil by feeling for the smooth granules as they flow through their fingers. Glomalin is relatively stable in soils, lasting anywhere from seven to 42 years.

Endomycorrhizae form with nearly all the important agricultural plants (with the exception of the brassicas). Glomalin (produced by the endomycorrhizal fungal group *Glomus*, hence the name) is produced by endomycorrhizal fungi established on a plant's roots. The fungi produce glomalin from carbon they trade for other nutrients and water, apparently to seal themselves and gain enough rigidity to carry materials across the air spaces between soil particles. Sara F. Wright's discovery of glomalin is causing a complete reexamination of soil organic matter. It is increasingly being included in studies of carbon storage and soil quality.

## $CO_2$ & GLOMALIN

In an earlier study, Wright and scientists from the University of California at Riverside and Stanford University showed that higher $CO_2$ levels in the atmosphere stimulate the fungi to produce more glomalin. A three-year study was done on semiarid shrub land, and a six-year study was conducted on grasslands in San Diego County, California, using outdoor chambers with controlled $CO_2$ levels. When atmospheric $CO_2$ reached 670 parts per million — the level predicted for the middle to late 21st century — mycorrhizal fungal filaments (hyphae) grew three times as long and produced five times as much glomalin as fungi on plants growing with today's ambient level of 370 ppm.

Longer hyphae help plants reach more water and nutrients, which could

help plants face drought in a warmer climate. The increase in glomalin production helps soil build defenses against degradation and erosion and boosts its productivity. Wright says all these benefits can also come from good tillage and soil management techniques rather than higher atmospheric $CO_2$. "You can still raise glomalin levels, improve soil structure, and increase carbon storage," she notes.

Forests, croplands and grasslands around the world are potentially valuable for offsetting carbon dioxide emissions from industry and vehicles. In fact, some private markets have already started offering carbon credits for sale by owners of such land. Industry could buy the credits as offsets for their emissions. The expectation is that these credits would be traded just as pollution credits are currently traded worldwide. Although such plans risk abuse by industrial polluters and are thus controversial, the importance of our crops, forests and grasslands in offsetting the environmental damage caused by human technology is unquestionable.

## SECOND LESSON

Today most human food comes from legumes, oilseed crops and cereal grains. It is estimated that 80 percent of agricultural land is occupied by these crops. These human staples are relatively high in protein and calories and easy to store and transport, thus making them attractive to both consumers and producers. However, these annual crops must be grown from seed every year, generally using fossil-fuel intensive cultivation and fertilization methods. To maintain annual yields, farmers are faced with growing input costs for seed, fuel, fertilizer, pesticides and herbicides. All these practices, including tillage, consume or release large amounts of carbon dioxide into the atmosphere. In addition, erosion and runoff from these intensively cultivated lands can pollute freshwater supplies and degrade the soil.

Data from the Rodale Institute's long-running comparison of organic and conventional cropping systems confirms that organic methods are far more effective at removing carbon dioxide from the atmosphere and fixing it as beneficial organic matter in the soil. Data from 23 years of continuous research in side-by-side fields is conclusive: the organic system has shown an increase in soil carbon of 15-28 percent, compared to *no increase* in the non-organic system. Dr. David Douds of the Agricultural Research Service suggests that healthy mycorrhizal fungi populations in organic systems are key to the increase in soil carbon. In addition, a recent study of energy inputs conducted by Dr. David Pimentel of Cornell University found that organic farming systems use just 63 percent of the energy required by conventional farming systems, largely because of the massive amounts of energy required to synthesize nitrogen fertilizer.

Compared to perennial grasses, annual crops such as wheat, corn, sunflowers and sorghum have relatively shallow root systems. The vast majority of annual roots are confined to the top foot of soil. These root systems die after harvest, leaving non-vegetated soil exposed to erosion of precious topsoil. Perennial root systems, on the other hand, commonly exceed 6 feet in depth and maintain this living tissue year-round. This allows perennial grasses to be resilient in the face of extremes of environment and diseases were almost nonexistent. Over time prairie soils built and maintained deep and carbon-rich productive topsoil. It is a soil legacy that helped make America prosperous.

*A glomalin-rich soil inoculated with beneficial soil organisms.*

This is big news. Organic farming with help from mycorrhizal fungi can take massive amounts of carbon dioxide out of the air. If all 160 million acres of corn and soybeans in the United States were converted to organic production, the reduction in atmospheric $CO_2$ could translate to:

- 57.7 million cars off the road (25 percent of nation's cars!);
- 773 billion car miles not driven.

Let's look at nature's "root cellar" as an example of how the system works — for example, a native tall-grass prairie in the Midwest. These prairie systems were productive year after year and needed no fertilizers, pesticides or herbicides. Pests and to sprout into action when warm temperatures, water and nutrients become available. Deep perennial grass-root systems and associated mycorrhizal fungi reduce fertilizer losses, conserve water, and boost the soil's storage of carbon. Roots and mycorrhizal fungi pump carbon-rich plant sugars such as glomalin into the soil, feeding beneficial soil organism that conserve and access soil nutrients.

Perennial roots themselves become a root cellar of stored carbon. Deep root systems capture and utilize more rainwater than shallow root systems, thus reducing off-site movement of water and nutrients. In addition, perennial grasses

do not have to be planted every year, thus reducing consumption of fuel by farm machinery. Perennial root systems and associated mycorrhizal fungi tie up soil resources, discouraging invasions of weeds. Pesticide, herbicide and fertilizer use is greatly diminished, which again lowers the amount of fossil fuels needed on the farm. Greater root depths, longer growing seasons for roots and mycorrhizal fungi let perennials sequester carbon at a rate 50 percent higher than an annually cropped field.

For all of these reasons, plant breeders both in the United States and internationally have initiated breeding programs to develop wheat, sunflower, sorghum and intermediate wheatgrass as perennial grain crops. While still in the early stages, plant geneticists such as Wes Jackson in Kansas are making progress. The Land Institute, a nonprofit founded by Jackson, has discovered that of the 13 most widely grown grain and oil seed crops, 10 are capable of hybridization with perennial relatives. The widespread production of high-yield perennial grain crops, if successful, could have a major positive impact on both the environment and the sequestration of carbon in the root cellar.

## CONCLUSIONS

Hidden underground in our planet's root cellar, nature has given us a template to help us resolve a variety of serious environmental issues, including global warming. Often overlooked and underappreciated, the living soil holds the key to the future. Vigorous long-lasting root systems and associated tiny fungal threads can accumulate and store vast amounts of carbon. Are we destined to relive the mistakes of previous civilizations or are we wise enough to learn from natural systems? It's time to examine our root cellar for solutions.

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# $CO_2$ Enrichment Can Boost Yields and Help Mitigate Climate Change

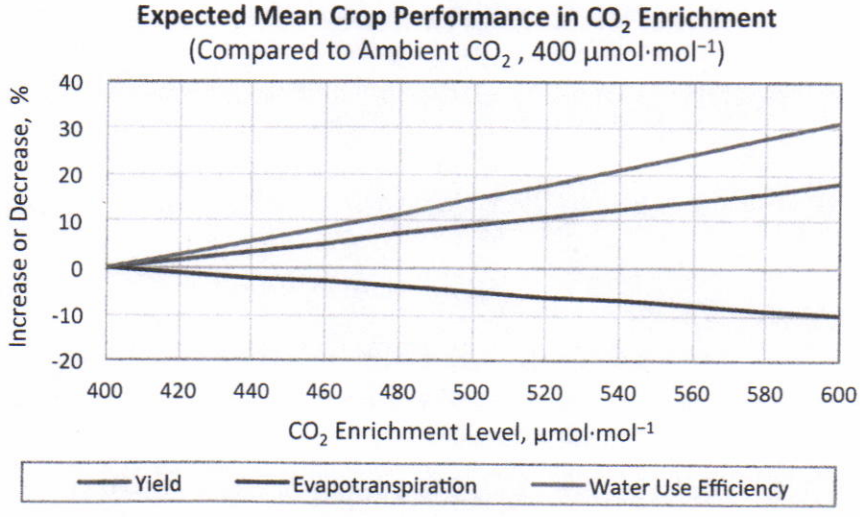
Shawn Ashkan and David Zoldoske

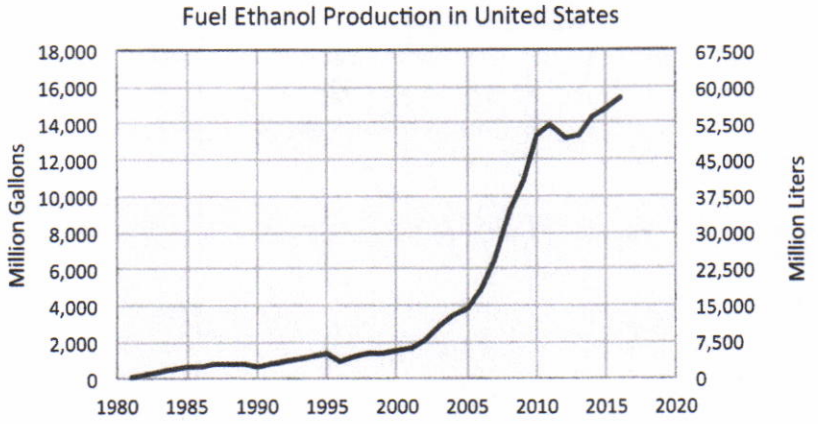
Since the 1990s, global climate change has emerged as an important national and international concern. Climate change is linked to increased greenhouse gas (GHG) emissions, driven largely by carbon dioxide ($CO_2$) and primarily due to fossil fuel consumption. To combat climate change, many governments are developing policies to reduce $CO_2$ emissions. Uncertainties still exist concerning the long-term environmental effects of rising $CO_2$ levels. However, $CO_2$ is essential for photosynthesis and is the main input for crop growth. Increases in the $CO_2$ concentration around plant canopies can increase photosynthesis and reduce stomatal conductance, thus increasing crop yields while reducing crop water use.

## Crop responses to $CO_2$

Crop responses to $CO_2$ are well documented. Plants open their stomata to capture and sequester $CO_2$. The stomata also control the transpiration of water from plants. With elevated ambient $CO_2$ levels, and therefore a greater concentration gradient from the $CO_2$-enriched air to the sub-stomatal cavity, plants reduce their stomatal openings. The resulting tradeoff between carbon gain and water loss means that elevated $CO_2$ levels around the plant canopy can reduce plant water loss per unit of carbon gain.

The crop water production function, or the relationship between crop yield and water consumption, is highly linear for a given species. With other variables held constant, it's difficult to improve the production function because the stomata control the carbon gain and water loss simultaneously. However, additional $CO_2$ can alter the carbon gain/water loss relationship and enhance both the crop productivity and the water use efficiency (WUE). $CO_2$-induced






stomatal closure also reduces the entry of ground-level ozone into the leaves. This can further enhance crop productivity, as ground-level ozone reduces crop growth and yield.

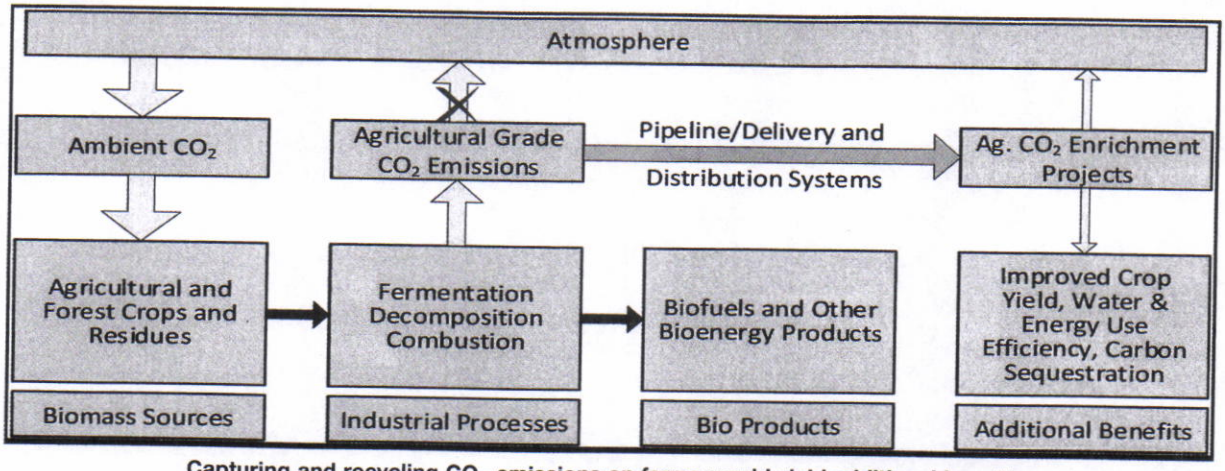
## $CO_2$ from ethanol production

In the U.S., fuel ethanol production has increased substantially, from about 300 million liters (80 million gallons) in 1981 to more than 57 billion liters (15 billion gallons) today. Ethanol is primarily used in the production of transportation fuels and is blended up to 15% with gasoline. With the expected growth in gasoline consumption and higher renewable fuel standards that encourage cleaner, low-carbon fuels to reduce the U.S. carbon footprint, ethanol production is expected to increase until carbon-neutral alternatives are found.

California is developing solutions to reduce GHG emissions, particularly $CO_2$ emissions. According to the California Global Warming Solutions Act of 2006 and its subsequent amendments, California is planning a GHG emissions reduction target for the year 2030 that is 40% below 1990 levels. Because transportation accounts for about 40% of GHG emissions in the state, one of the ways California plans to reduce GHG emissions is large-scale production of biomass-based fuels. Currently, fuel ethanol is produced mainly from fermented corn in the U.S., but ethanol feedstocks can include a variety of other sugar and starch based crops, such as sugarcane, sugarbeets, sorghum, and grains, as well as cellulosic materials such as grasses and ag/forestry residues such straw, sawdust, and wood chips.

Unfortunately, fuel ethanol production also generates $CO_2$ emissions. When biomass is fermented to produce ethanol, $CO_2$ is released as a byproduct of this biological process. About 720 grams of $CO_2$ is produced per liter of ethanol (or six pounds of $CO_2$ per gallon of ethanol). If these $CO_2$ emissions are not captured, then large amounts of $CO_2$ can be released into the atmosphere from ethanol facilities. Ethanol could be carbon-neutral if it were produced in a carbon-neutral fashion. The $CO_2$ that is absorbed by photosynthesis during growth of the feedstock crop can offset the crop's biogenic emissions, which are due to the natural carbon cycle. Similarly, capturing the $CO_2$ emissions at an ethanol facility and using those emissions for beneficial purposes could offset the facility's emissions.


Ethanol plant.




**Capturing and recycling $CO_2$ emissions on farms would yield additional benefits.**

## Recycling $CO_2$ back to the farm

Beneficial use of the waste $CO_2$from an ethanol facility could include recycling the captured $CO_2$ back to the local farms to enhance crop production and achieve carbon sequestration. In California, ethanol facilities are often located in rural areas and surrounded by orchards, such as almonds and pistachios, as well as vineyards. The waste $CO_2$ captured at ethanol facilities could provide aerial $CO_2$ enrichment for these high-value crops to improve their resource use efficiency and sequester carbon in the soil and plants.

Aerial $CO_2$ enrichment increases the growth and yield of most plant species. In the last several decades, many studies in greenhouses and controlled environment chambers, combined with numerous large-scale Free-Air $CO_2$ Enrichment (FACE; https://facedata.ornl.gov) experiments in the U.S. and abroad, have shown that elevated $CO_2$ concentrations around plant canopies reduce transpiration in all species and increase yields of crops with C3 photosynthetic pathways, which include most trees, vegetables, and field crops. Elevated $CO_2$ concentrations also increase yields of crops with C4 pathways, such as corn and sorghum, when water is limited.

Generally, woody perennials are more responsive to elevated $CO_2$ concentrations than herbaceous crops. In addition to yield enhancement with elevated $CO_2$, trees can produce more and deeper roots that sequester carbon in the soil. $CO_2$-induced stomatal closure can also result in other environmental and economic benefits, such as improvement in WUE. This is particularly beneficial in view of the growing demand for water in urban areas, which will likely lead to declining availability of irrigation water for agriculture.

Over the last ten years, the Center for Irrigation Technology at California State University, Fresno, has conducted several field-scale $CO_2$-enrichment projects with different crops, such as tomatoes and sugarbeets, achieving yield enhancements of 20% to 50%. California is an advanced agricultural area with high regional WUE, and opportunities are limited for significant WUE improvements. The main objective of these research projects was to examine the technical and economic feasibility of recycling $CO_2$ emissions from ethanol facilities to agricultural fields in the San Joaquin Valley.

Large-scale implementation of aerial $CO_2$ enrichment would require simple, efficient, and low-cost techniques for capturing, storing, and transporting $CO_2$. Various scheduling, delivery, and control systems have been tested using drip irrigation equipment. As a gas, $CO_2$ can be delivered to plant canopies using available irrigation systems with excellent uniformity of application.

## Summing it up

The positive benefits of $CO_2$ enrichment for crop production and WUE have been reported for a wide range of plant species, particularly trees, with their deep sequestration of carbon in the soil. Meanwhile, ethanol production is increasing, and millions of tons of $CO_2$ emissions from ethanol facilities could be used annually for these agricultural benefits and to help mitigate GHG emissions. $CO_2$ can be readily transported to agricultural fields, where it can be distributed to plant canopies using drip emission systems. As the next step, incentive policies are needed to help implement this productivity-enhancing technology, to help meet the growing global demand for food and water.

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## Further Reading

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# Tansley Review No. 54
# Plant response to irrigation with water enriched with carbon dioxide

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CONTENTS



SUMMARY

The influence of irrigation with $CO_2$-enriched water on plant development and yield is reviewed. The reason for irrigation with $CO_2$-enriched water was – in most cases – to increase yield. The present evaluation considers results from over a hundred studies performed since the first experiment in 1866. Special emphasis is given to the comparison of 85 experiments made by Mitscherlich in 1910 with 358 irrigation experiments made in the last 80 years. In a statistical analysis of these experiments, the measured plant parameter (often growth and/or gas exchange rates) showed a highly significant mean increase of 2·9 % in plants irrigated with $CO_2$-enriched water as compared with control.

Evidence of five mechanisms was found. The subterranean carbon dioxide concentration influences: (*a*) the rate of nitrification and hence of nitrogen availability; (*b*) the rate of weathering and pH, and hence the availability of other plant nutrients; (*c*) the $CO_2$ uptake via roots into the transpiration stream, contributing to the rate of leaf photosynthesis; (*d*) the hormone levels in the plant; and (*e*) the rate of pesticide decomposition in soils.

After examining the available evidence we found that (*a*) and (*b*) in some experiments are important to plant growth, since they change the physiochemical environment of the roots. On the other hand, while (*c*) could theoretically contribute up to 5 % of plant carbon assimilation, it usually contributes less than 1 %, while (*d*) contributes most of the observed effects of $CO_2$-enriched water on plants. In addition, pesticide decomposition in soils can be delayed by supra- or sub-optimal $CO_2$ concentrations.

Key words: Carbon dioxide enrichment, watering with $CO_2$, $CO_2$ as plant hormone, soil air, root environment.

## I. INTRODUCTION

Some topics in plant and crop physiology have been extensively investigated for so long that early investigations are unknown to all but a few contemporary scholars. Consequently, knowledge once common is forgotten, and modern experiments are performed which basically repeat earlier ones.

The topic of the present investigation represents a case in point. The effect of irrigation with $CO_2$-

enriched water had been studied for 50 years before Mitscherlich (1910) reported the results of his 85 experiments on oat plants, which pretty well covered most aspects studied by subsequent research. Mitscherlich's report, written in German, was soon forgotten and when Russian and later Anglo-Saxon writers repeated the same type of experiment after the Second World War, and especially from 1980 onward, work done at the end of the last and the beginning of this century was not mentioned.

In this presentation we have tried to evaluate not only recent work from the last decade but also the earlier investigations from 1866 onwards. Some experiments showed growth enhancement, while other studies displayed no effect or a detrimental effect. Attempts will be made to explain mechanisms and processes influenced by irrigation with $CO_2$-enriched water.

## II. HISTORICAL BACKGROUND

From about the mid-nineteenth century it has been known that most terrestrial plants derive most, if not all of their carbon from the above-ground atmospheric air, as reported by Boussingault (1844). Unger (1855), however, thought that $CO_2$ absorption from the bulk atmosphere could not account for all the carbon in the plant.

The earliest irrigation experiments with $CO_2$-enriched water, by Birner & Lucanus (1866), showed that the first effect of irrigating oat plants with a $CO_2$-enriched solution was a noticeable injury to the plants. However, with a continued addition of $CO_2$ these plants showed better development and higher dry weight than control plants grown in the same nutrient solution but without $CO_2$. The author concluded that the extra supply of $CO_2$ in the solution had a small favourable influence on the production of organic material, but considered it a moot question whether the additional $CO_2$ was absorbed by the roots and transported to the leaves, or was diffused from the solution into the air and then absorbed by the leaves. The conclusion of Birner & Lucanus is relevant for all subsequent work and holds true for most studies performed up till now.

Moll (1877), Vines (1882), Pfeffer (1881) and Sachs (1882) learned from their experiments that only insignificant amounts of $CO_2$ were absorbed via the roots. Mitscherlich (1910) grew oat plants in various soils watered with tap water saturated with $CO_2$ at different plant nutrient levels, and found no better yield from 85 experiments with these plants than from controls. He argued with other authors who had found increased growth in their $CO_2$-enriched-water application. In his view the discrepancy might be due to increased solubility of soil materials, and since his soil already possessed an abundance of $CO_2$, further addition did not improve growth.

Mitscherlich's comprehensive study did not discourage others from investigating the subject. Noyes (1914) passed $CO_2$ through soil, causing corn and tomato to wilt and stop their growth. Free (1917) reported that bubbling a stream of $CO_2$ through a nutrient solution in which buckwheat plants were growing caused injury and death to the plants. Partial recovery was ensured if, after the first day, the stream of $CO_2$ was replaced by a stream of atmospheric air. But the treated plants remained smaller than controls. Cannon (1925) found that high $CO_2$ levels applied to the root zone inhibited growth of all their test plants if treatments were extended. The sensitivity of the different species varied. Growth was restored when the $CO_2$ mixture was replaced by atmospheric air.

In spite of the overwhelming evidence in support of the viewpoint that only insignificant amounts of $CO_2$ are absorbed via the roots of most terrestrial plants, early publications sometimes claimed the opposite. Stoklasa (1927, 1929) claimed that a considerable amount of the $CO_2$ assimilated in the leaves originated from roots and stem. Barbieri (1930) and Miller (1931) were of the same opinion, the latter stating: '*There thus appears to be good evidence that a green plant may absorb carbon dioxide from the soil and thus supplement its supply from the air.*' However, Livingston & Beall (1934) pointed out that most of the literature quoted by Miller (1931) did not furnish any evidence bearing on the question.

Thus, after half a century of research, the consensus was in the 1930s that the overwhelming majority of the assimilated $CO_2$ came from the atmosphere and only minor amounts ($< 5\,\%$) via the roots. Moreover, several of the early works showed that very high levels of $CO_2$ concentration in the root zone inhibited plant growth.

In our literature survey of the subject we have found only one trustworthy reference (Keeley, Osmond & Raven, 1984) to a vascular terrestrial plant that receives most of its carbon through its roots. It is a 2–4 cm high plant without stomata, *Stylites andicola*, found in the high Andes (at over 4000 m above sea level) in Peru. The plant possesses some of the characteristics found in plants with Crassulacean Acid Metabolism (CAM).

In the present review newer (mostly after 1980) published as well as unpublished experiments with $CO_2$-enriched irrigation of plants were evaluated statistically. From these and earlier experiments we attempted to formulate different hypotheses concerning the mechanisms through which $CO_2$-enriched irrigation water may influence plant yield. Through our cooperating with the producer of equipment for mixing $CO_2$ into irrigation water (Carborain, produced by Danfoss in Nordborg,

Denmark) we received numerous reports, letters and other written information about the experiments, trials and testing of $CO_2$-enriched water for irrigation of crops. This material, in addition to published research papers, constitutes the basis for the evaluation reported below.

## III. METHODS FOR COMPARING EXPERIMENTAL DATA

A number of theoretical approaches exist on how to deal with apparently conflicting research results (Light & Smith, 1971; Rosenthal, 1978, 1979) and how to combine results of many independent randomized controlled trials (Sacks *et al.*, 1987). There are even methods for estimating how many unpublished results containing statistically non-significant results or statistically significant results with opposing views are needed to negate the conclusion reached by summarizing the published results.

The aim of this study was to evaluate all (i.e. both published and unpublished) results pertaining to irrigation with $CO_2$-enriched water. We therefore wanted to take into account experiments with $CO_2$-enriched water which we knew had never been published, presumably because they were inconclusive or gave negative results (Heij, 1985; Mortensen, 1987, personal communication).

The problem of how to deal with unreported results exists in all disciplines of science. Rosenthal (1979) writes on the subject of '*the file drawer problem: the extreme view is, that journals are filled with the 5 % of studies that have type 1 errors (i.e. accepting a hypothesis on the basis of statistical evidence when in fact it is wrong) while the file drawers are filled with the 95 % of the studies that show non-significant results (or type 2 errors, i.e. rejecting a hypothesis when in fact it is correct).*'

Rosenthal (1979) calculated that in most meta-analysis studies the number of unpublished studies has to be very much larger than that of the published ones. As we had access to an estimated 95 % of all reports of $CO_2$-enriched water experiments we do not expect that unpublished material, filed in various drawers, will change our conclusions. We could not use the meta-analysis techniques described by Rosenthal (1979) as the results evaluated by us did not compare the same plant parameter. Our analysis is limited to a more simplistic estimate of the various experiments as suggested by Kimball (1983). He computed the relative increases in growth or yield due to $CO_2$ enrichment and normalized the data by calculating the logarithm of the ratio of $CO_2$-enriched plant yield to control yield.

If $CO_2$ enrichment is hypothesized to have no significant effect on yield, one would expect the logarithms of the ratios to be normally distributed over a mean of zero. The means of the logarithm of the yield ratios of experiments by Mitscherlich in 1910 and of 358 experiments carried out later were computed, and their antilogarithms presented.

## IV. ANALYSIS OF YIELD RATIOS

The effects of $CO_2$-enriched water were evaluated statistically. The 85 experiments with oat plants by Mitscherlich in 1910 ('old tests') were compared with 358 made since then, including 150 in the last decade ('new tests'). The 358 observations of the yield or biomass productions were extracted from the publications quoted and from reports of trials made with the commercial equipment referred to earlier. In the last decade 10 agricultural, 10 horticultural and 9 other species were treated with $CO_2$-enriched water.

Most experiments (observations) in this analysis produced one yield ratio value. Some reported yields were actually means of observations from several replicates, but since only the mean was reported, only one value was available for further analysis. The mean of the logarithms of the yield ratios were computed and the value of the antilog presented. Thanks to our access to most unpublished reports from the last decade and to personal communications with most scientists currently engaged in research in this field, we are reasonably certain that we have succeeded in including the vast majority ($> 95$ %) of results produced in $CO_2$-enriched water experiments in recent years.

The means and standard deviations of the ratios of yield using $CO_2$-enriched water:yield of control, are $0{\cdot}976 \pm 0{\cdot}184$ ($N = 85$, range: 0·516–1·545) and $1{\cdot}029 \pm 0{\cdot}120$ ($N = 358$, range: 0·107–1·500) for old and new tests, respectively. Both new and old data are normally distributed (Kolmogorov–Smirnov test, $z = 0{\cdot}894, P = 0{\cdot}37$; $z = 0{\cdot}447, P = 0{\cdot}65$ respectively). The increase in yield of 2·9 % with $CO_2$ in new experiments is highly statistically significant (Wilcoxon's signed ranks tests, $z = -6{\cdot}983$, $P = 0{\cdot}0001$). The reduced yield of 2·4 % after treatment in old experiments is, however, not significant (Wilcoxon's signed-ranks test, $z = -0{\cdot}838$, $P = 0{\cdot}40$).

An approximate $t$ test of equality of the means of the two samples of experiments (before and after Mitscherlich) whose variances are unequal was used, because the variances of the two samples were highly significantly different ($F_s = 2{\cdot}3511$, $P < 0{\cdot}01$). The $t$ test showed that the two means were significantly different ($t'_s = 2{\cdot}531 > t'_{0{\cdot}05} = 1{\cdot}989$, $0{\cdot}01 < P < 0{\cdot}05$). Thus results from experiments with $CO_2$-enriched irrigation water before and after Mitscherlich differed from each other.

Based on our analysis we conclude that an application of $CO_2$-enriched water in agriculture (new tests after 1910) increases yield by 2·9 % ($P <$

0·05). No statistically significant variation in effect was detected between years, localities, crops and soil types as far as those data were available from the different test reports. In order to explain this rather small effect, we shall examine the conditions and processes involved.

## V. TEMPORAL AND SPATIAL CHANGES IN THE SOIL ATMOSPHERE DURING AND AFTER IRRIGATION WITH $CO_2$-ENRICHED WATER

### 1. *$CO_2$ content of water*

$CO_2$ is soluble in water, with about 99 % as dissolved gas and 1 % as carbonic acid. The components of inorganic carbon in water are $CO_2$, $H_2CO_3$, $HCO_3^-$, and $CO_3^{2-}$. The amount of $CO_2$ in irrigation water added to the soil and crop equals the amount of irrigation water multiplied by H (Henry's coefficient) and $P_{CO_2}$ (the specific pressure of $CO_2$ in the gas phase in equilibrium with the water). H decreases with temperature and is about 1·0 at 15 °C (Butler, 1982).

The $CO_2$ content of the irrigation water depends on the technical specification of the equipment, hereunder the design of the pump (water pressure), the (spray) nozzle and the location of the water release, which can be above-ground sprinklers or below-ground drip irrigation. One producer gives typical concentrations of 0·5–1·3 g $CO_2$ $l^{-1}$ water at 10 °C.

The methods and frequency of irrigating with $CO_2$-enriched water will affect the temporal and spatial distribution of $CO_2$ in the soil. Irrigation is generally applied in order to restore water loss of plants and soil. During irrigation with $CO_2$-enriched water the soil-air $CO_2$ content will be elevated. During the subsequent drainage and evapotranspiration, the soil air will become depleted of $CO_2$ and enriched with oxygen and nitrogen until a new irrigation event takes place (Gornat, Enoch & Goldberg, 1971). If $CO_2$-enriched water is applied from drip water units, the location directly below the drip joint will be changed most, and points further away will be less affected, thus causing a spatial inhomogeneity in the horizontal plane.

Sprinklers will give a somewhat more homogeneous horizontal $CO_2$-in-soil-water distribution, but will mostly affect a shallow upper layer and will thus cause a vertical inhomogeneity in the root zone. In addition there will be a temporal variation in $CO_2$ concentration caused by the irrigation schedule.

In a drip- or sprinkler-irrigated field we could expect that major changes in soil-air composition would take place about 10 % of the time in about 10 % of the root volume. Thus in many field experiments we estimate that the major environmental changes (high $CO_2$ and low $O_2$) in the root zone, due to irrigation with $CO_2$-enriched water, are present in 10 % of 10 % and thus in 1 % only of the time–space continuum. Consequently, it is not surprising that many field experiments show little or no effect. Only in recirculated hydroponic systems with constant $CO_2$ additions to nutrient solutions are a high percentage of roots located in the modified environment.

### 2. *Unintended atmospheric $CO_2$ enrichment*

When $CO_2$-enriched water is applied to the soil, some of the $CO_2$ escapes into the bulk air surrounding the shoot, as noted over a century ago by Birner & Lucanus (1866). There are several examples of unintended atmospheric $CO_2$ enrichment associated with the recent use of $CO_2$-enriched water for irrigation of the root zone in plant enclosures (growth rooms, greenhouses, etc.).

When $CO_2$-enriched water was applied for 2–3 h $d^{-1}$ to a hydroponic system in a closed greenhouse, Mortensen (1986) reports that the atmospheric $CO_2$ concentration was 800–850 ppm; with vents opened to 10–15 cm it was about 400–500 ppm, and when vents were fully open the $CO_2$ concentration was 335 ppm. In the control greenhouse, the $CO_2$ concentration was between 260 and 320 ppm and thus lower than in the greenhouse irrigated with $CO_2$-enriched water. Mortensen (1986) considers unintended $CO_2$ enrichment to be the main part of the $CO_2$-enriched water effect. Similar observations were made by Zornbach & Schickedanz (1987), who found that cyclamens and poinsettias show an increase in both fresh and dry weight due to a rise in the $CO_2$ concentration of the greenhouse atmosphere when watered with $CO_2$-saturated water. $CO_2$ concentrations when the vents were closed were up to 800 ppm higher than the control and gradually approached the control 4 h after $CO_2$-enriched water application. When vents were open, peak $CO_2$ concentrations were about 500 ppm higher than the control, and remained higher for about 1 h.

Molitor, von Hentif & Fisacher (1986) found that by using $CO_2$-enriched water they inadvertently enriched the bulk air in the greenhouse during ventilation for 4 h daily, by 30–70 ppm relative to the control treatment. In another experiment (Molitor *et al.*, 1986) there were two control plots, one in the same greenhouse as that containing the $CO_2$-enriched plot, the other one in a separate greenhouse. Hydroponically grown *Dracaena marginata*, *Ficus benjamina* and *Saintpaulia inoatha* were treated with $CO_2$-enriched water, which enhanced the above-ground $CO_2$ concentration to 700 ppm between 05.00 and 08.00 h and to about 500 ppm between 12.00 h and 15.00 h. The control plants in the same greenhouse experienced a $CO_2$ concentration increase of 200 ppm in the morning hours and 50 ppm in the afternoon compared with the other set of control plants which were grown in another greenhouse.

In contrast, Marcelis (1986) in a glasshouse experiment did not find that $CO_2$ concentration in the atmosphere close to the pot plants was changed much after watering with $CO_2$-enriched water. There were no significant changes in productivity or size or weight of the test plants (pot chrysanthemums, gloxinias or gerberas), possibly because the aerial $CO_2$ concentration remained unaffected.

The air exchange rate, the size of the greenhouse and the proportion of its area used for $CO_2$-enriched irrigation experiments are likely to determine whether the greenhouse air will be $CO_2$ enriched or not.

When the $CO_2$-enriched water is applied in the open, the bulk atmosphere is only changed very little. Kimball *et al.* (1986) found that in an open-field application of $CO_2$-enriched water to cotton in Arizona the mean bulk-air $CO_2$ concentration increased only from 360 ppm in the control plots to 364 ppm in the plots treated with $CO_2$-enriched water.

Measurements of the $CO_2$ enrichment of the air surrounding shoots of the control plants are important for an evaluation of experiments from greenhouse and growth chambers. Where atmospheric bulk-air $CO_2$ concentrations of plant enclosures are not measured in the treated and in the control plots, we cannot be sure that the $CO_2$ concentrations around the shoots are the same. In our further evaluation of experiments we should bear in mind that $CO_2$-enriched water often influences only a minor part of the soil time–space continuum, but could affect the bulk atmosphere of plants grown in plant-enclosures such as greenhouses, growth chambers, etc. for extended periods.

Where atmospheric $CO_2$ was increased thanks to the use of $CO_2$-enriched water, we must assume that enhanced growth is caused mostly or partly by aerial $CO_2$ enrichment of the above-ground shoots. Though the amount of carbon added to a crop through irrigation with $CO_2$-enriched water is negligible compared to the photosynthesis of the crop, it is possible that short periods with elevated $CO_2$ have a trigger effect (as proposed by Enoch, 1990).

## VI. MECHANISMS THROUGH WHICH IRRIGATION WITH $CO_2$-ENRICHED WATER INFLUENCES YIELD

We suggest that the following mechanisms explain how $CO_2$-enriched irrigation water influences growth, development and yield of plants.

### 1. *Influence of $CO_2$ in soil on nitrification*

Nitrification is the oxidation process in which nitrite is formed from ammonium and by further oxidation is transformed into nitrate. Soil nitrification is primarily mediated by Nitrobacteriaceae. Rettger (1927) investigated over 100 different bacteria genera and found that there was a threshold $CO_2$ concentration below which normal growth rates of bacteria could not be maintained. Lowe & Evans (1962) found that, in five species of *Rhizobium*, $CO_2$-free air is a suboptimal environment for nodulation, and that plant-available nitrogen is limited when $CO_2$ is suboptimal. The $CO_2$ concentration in soil has generally been regarded as exceeding the biological demand of the nitrifying bacteria (Alexander, 1965). However, Buyanovsky & Wagner (1983) reported soil $CO_2$ concentrations in the field which ranged between $< 1{\cdot}0$ and 70 ml $l^{-1}$. Clark (1968) measured soil nitrification over a range of $CO_2$ concentrations and found that maximum nitrification rates occurred between 5 and 29 ml $l^{-1}$. Thus, under field conditions $CO_2$ in the soil may be suboptimal or supraoptimal for maximum nitrification rates.

### 2. *Influence of $CO_2$ in soil on pH and nutrient availability*

Hydrogen ions produced through the reaction of $CO_2$ with $H_2O$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$$

interact with some soil minerals in the weathering process and cation-exchange which contribute to mineral solubilization and availability to plants, for example:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \leftrightarrow 2CaHPO_4 + Ca(HCO_3)_2;$$

and

$$Na_2SiO_3 + 2H_2CO_3 \leftrightarrow 2NaHCO_3 + H_2SiO_3$$

(Waksman, 1932; Berner & Robinson, 1991). Adding $CO_2$ to soil could thus initially increase the plant-available phosphate and calcium.

There is conflicting evidence concerning the influence of $CO_2$ in soil on nutrient availability after a prolonged period. Carbonated water is a weak acid, and thus somewhat more potent than ordinary water as a solvent for nutrients in natural soils. Hence, over time soil irrigated with $CO_2$-enriched water may develop plant nutrient deficiencies due to excessive leaching. Kimball *et al.* (1986) found that plots treated with $CO_2$-enriched water showed N and Zn deficiency as compared with control. Uptake of microelements depends on soil type and pH. $CO_2$-enriched water increased the uptake of Zn and Mn by a cotton crop (Moore, 1989), presumably due to the shift in pH. Moore points out that lowering the pH of alkaline soils is beneficial during the short periods when crop irrigation is applied. It is well known that different plant species have different optimal pH demands, which for most cultivated

plants are in the range of 5·5–7·0 (e.g. Ryan, Stroehlein & Miyamoto, 1975; Bailey & Hammer, 1986; Zieslin & Snir, 1989).

In addition to the effect which $CO_2$-enriched water has on Ph, $CO_2$ *per se* in some cases seems to reduce nutrient and water uptake by roots (Chang & Loomis, 1945). Also, manganese uptake by beetroot tissue can be partly inhibited by the presence of $CO_2$ (Skelding, 1957). Treatments consisting of bubbling $CO_2$ through hydrocultures 10 min $h^{-1}$ reduced water uptake and absorption of K, N, P, Ca and Mg. Adding $H_2SO_4$ to controls to bring the pH of the solution to the same value as that caused by $CO_2$ had no effect on water uptake by wheat, corn and rice plants (Chang & Loomis, 1945). Matocha & Mostaghimi (1988) found that when $CO_2$ was injected for 3 h daily into the soil, Fe and P uptake by sorghum plants was drastically reduced. Also the Mg and Zn contents in plant tissues were depressed, hence the soil $CO_2$ enrichment caused a highly significant reduction in dry matter production (Matocha & Mostaghimi, 1988).

Opposing views are presented by Mauney & Hendrix (1988). They found the leaf Zn and Mn were deficient in the cotton plants used in the control treatment but were present in sufficient amounts in plants irrigated with $CO_2$-saturated water. Also, Larsen & Bang (1989) found that following an increase in $CO_2$ the soil pH decreased by about 0·5 units, plant-available P increased slightly and a large increase in available Ca, Mg and K occurred.

A mixed reaction was reported by Labanauskas *et al.* (1971). They found that elevated soil $CO_2$ concentration increased total K and Mg per plant, while N, P, K and B in roots and N, P, Ca, Mg and Mn in the tops were reduced. The $CO_2$ treatment increased dry weight and seedling height of citrus.

Knight *et al.* (1989) found that in soil systems where P availability is governed by the solubility of Ca-phosphate minerals, mycorrhiza may contribute to the P-nutrition of host plants via the $CO_2$-enhanced weathering process mentioned earlier. This mechanism is in addition to the cation-exchanges in the soil–humus matrix.

### 3. *$CO_2$ uptake via roots and transpiration stream*

Some Russian publications (Kursanov, Kuzin & Mamul, 1951; Kuzin, Meronova & Mamul, 1952; D'yakonova, 1970) which claimed that a considerable part of terrestrial plants' $CO_2$ absorption could take place via the roots have not been confirmed by other investigations.

Kuzin *et al.* (1952), using labelled $CO_2$, showed that $^{14}C$ was translocated from the root zone to the leaves. Kursanov, Krykjova & Vartapetyan claimed that the amount of $CO_2$ intake into the root system was not connected directly with the absorption of water by the plant, leading them to state that in soil containing 1 % $CO_2$ about a quarter of the $CO_2$ for photosynthesis may come from the soil. This finding contrasts with their earlier investigation (Kursanov *et al.*, 1951), where they found that nearly all root absorption of $^{14}CO_2$ by bean plants from a nutrient solution took place during the daylight hours when there is transpiration.

D'yakonova (1970) tried to calculate the role played by the soil as a $CO_2$ supplier to plants by comparing the soil's $CO_2$ efflux (soil respiration flux) to the total $CO_2$ uptake by crops. D'yakonova claimed that soil respiration should be able to provide 50–100 % of the $CO_2$ required by plants, thus ignoring the fact that $CO_2$ from soil respiration is the result of degradation of soil organic matter and root respiration (i.e. recycled $CO_2$), first assimilated by the plant from the free bulk atmosphere. Furthermore, D'yakonova did not take into account that the efficiency of the canopy in absorbing soil $CO_2$ from the efflux is limited to daylight hours, and even then most of the $CO_2$ efflux is lost to the bulk atmosphere and not assimilated by the plant leaves, except when plants are growing in airtight systems (hermetically closed growth chambers or greenhouses).

Stringent experiments by Stolwijk & Thimann (1957) led them to conclude that the uptake of $CO_2$ by the roots must be considerably less than 1 % of the amount of $CO_2$ uptake by the leaves in photosynthesis. Further, they found in peas that there was a small and consistent stimulation of root growth by 0·5 % $CO_2$ in the root zone, but that root growth is inhibited by $CO_2$ levels as low as 1·5 %. They estimated that the $CO_2$ content of some soils was already supraoptimal, and therefore that carbonate fertilization would be detrimental to the pea plants. Voznesenski (1958) found that root $CO_2$ uptake is insignificant, and always less than 5 % of total $CO_2$ absorbed by the plant. Bergquist (1964) found that at 0·6 % $CO_2$ in the soil the $CO_2$ uptake was many times less than the amount lost by root respiration. Kick, Sauerbeck & Fuhr (1964, 1965*a*, *b*) found that 0·2 % of total respired carbon originated from root-fixed carbon. They further showed that root-fixed carbon in some experiments reached 1·3 % of total photosynthesized carbon, but under field conditions could not be expected to exceed 0·1 % of carbon fixed by photosynthesis.

Higuchi (1982) and Higuchi, Yoda & Tensho (1984) showed that uptake of $CO_2$ by lowland rice seedlings was 3–4 times larger than that of wheat seedlings. It appears that $CO_2$ absorbed by rice seedlings moves to the shoot in the gaseous phase, whereas in wheat it moves via the transpiration stream. This difference is related to the ecophysiological and anatomical differences between aquatic and terrestrial plants.

Baron & Gorski (1986) showed that the roots of

eggplant contributed to the plant's $CO_2$ uptake. The significance of root-assimilated $CO_2$ to the carbon economy of eggplants was not expressed quantitatively in their study, and no attempt was made to relate the amount of root-absorbed $CO_2$ to foliar-absorbed $CO_2$. However, Schafer (1988), applying $H^{14}CO_3$ to the root systems of summer wheat, found that the root-absorbed fraction was not more than 0·44–1·21 % of total C assimilation.

Heij (1985) showed that even in the best of cases the rate of $CO_2$ uptake with the transpiration stream can only increase the substomatal (intracellular) $CO_2$ concentration by about 10 %, and thus even theoretically the $CO_2$ uptake via roots cannot be a large proportion of photosynthesis. His calculations confirm that the percentage of root-absorbed carbon is always below 5 %, and generally below 1 %, of photosynthesis.

A quantitative estimate of the maximum $CO_2$ absorption from the soil may be obtained from measurements on alfalfa plant growth by Briggs & Schantz (1913). These plants transpired 1068 g of water per g dry matter per season, which is amongst the highest reported. Let us assume (*a*) that all $CO_2$ brought to the leaves by the transpiration stream during daylight periods was assimilated; (*b*) that 75 % of the water lost by transpiration passed through the photosynthesizing tissues in the daylight periods; (*c*) that soil water contains 0·1 g $CO_2$ per litre. For each g of dry matter ($C_6H_{10}O_5$) produced, the $CO_2$ contained in 801 g (75 % of 1068 g) of soil solution should have been assimilated. If the soil solution is supposed to have contained 0·1 g of $CO_2$ per litre (equivalent to 7 % $CO_2$ in soil air), 0·08 g of $CO_2$ should be contained in 801 g of soil solution, and that amount derived from the soil should have been reduced for each g of $(C_6H_{10}O_5)_n$ produced. Since the production of 1 g of $(C_6H_{10}O_5)_n$ requires the reduction of 1·63 g of $CO_2$, 0·08 g of $CO_2$ represents 0·048 g (0·08/1·63) of $(C_6H_{10}O_5)_n$. Consequently, the plants could have received from the soil a maximum of 4·9 % of the $CO_2$ reduced by photosynthesis. Appleman (1927) found that in some instances the atmosphere of Maryland agricultural soils in summer, at a depth of about 10 cm, had a maximum $CO_2$ content as great as 5 %, so the 7 % in the soil air used in the example above is a realistic maximum value.

Thus we may conclude that green plants in general derive 95–99 % of the $CO_2$ reduced by photosynthesis from the free air. The rest is derived from the soil and represents mostly the plant's own reassimilated root respiration.

Only in plants like lowland rice – where transport of root-absorbed $CO_2$ might take place in the gaseous form inside the root, rhizome and above-ground stems – and in unusual plants like *Stylites andicola* (Keeley *et al.*, 1984) may root-absorbed $CO_2$ become quantitatively more important.

### 4. *$CO_2$ as a plant hormone : a hypothesis*

Since the study by Zimmermann & Wilcoxson (1935) it has been known that ethylene ($C_2H_4$) is produced in every plant cell at all times (Abeles, 1985) and interacts with auxin and cytokinins. Ethylene influences several enzyme systems associated with ripening, abscission, senescence and stress (Abeles, 1985). Many physiological effects of ethylene are blocked by $CO_2$. Removal of ethylene or the equivalent blockage by $CO_2$ leads to an instantaneous resumption of root elongation (Chadwick & Burg, 1970) and normal growth (Burg & Burg, 1967; Chadwick & Burg, 1970).

The root elongation of many plant species is enhanced by up to 20 % by exogenous ethylene in the root environment (Jackson, 1985). Optimal root elongation is found at about 0·1 ppm ethylene. Strong inhibition of root extension of between 20 and 80 % is found at ethylene concentrations between 1 and 10 ppm, as summarized by Jackson (1985) from the work of several research teams. Thus it is likely that certain $CO_2$ concentrations in the soil air will reduce the detrimental influence of supraoptimal ethylene concentrations on root extension.

The influence of $CO_2$ on hormone systems has been shown in several species, amongst them potato plants. In 1979 Arteca, Poovaiah & Smith showed responses of potato seedlings to aeration of root zones with 45 % $CO_2$ and 21 % $O_2$ for 12 h leading to higher dry-weight increases for treated plants. A recalculation of their figures gives high relative growth rates (RGR) of 0·178 $d^{-1}$. Arteca & Poovaiah (1982) measured $^{14}CO_2$ transport from the root media into potato plants and reported that between 70 and 80 % of the radioactivity was in the acidic fraction, mainly in the form of malic acid.

Interactions of $CO_2$ with cytokinins and ethylene can be seen in the following studies. Paterson (1975) found that 12 h exposure to 80 % $CO_2$ and 20 % $O_2$ significantly increased the number of tubers and development of stolons in potato plants. Paterson's studies indicate that control of $CO_2$ levels at critical times during the growing season could prevent stolon differentiation into leafy shoots and also increase tuber production in the plant. Palmer & Smith (1969) showed that cytokinins were required for tuberization of isolated stolons in concentrations of about 2·5 mg kinetin $l^{-1}$. Mingo-Castel, Negm & Smith (1974) found stimulating effects of $CO_2$ on tuberization of potato stolons cultured *in vitro*. The stimulatory effect was inhibited by ethylene. The study showed that ethylene inhibits kinetin-induced tuber initiation, and that $CO_2$ and ethylene have antagonist effects on tuberization. Miller (1960) reported in a review that the influence of high $CO_2$ concentrations (> 10 %) with the accompanying high bicarbonate level in the root media often has a

depressive effect on growth, respiration rate, nutrient absorption and translocation by modifying several enzymatic reactions, including the cytochrome oxidase system.

The role of $CO_2$ in the auxin–ethylene interaction affecting the indoleacetic acid-induced inhibition of excised root tips and the role of ethylene in geotropic responses of roots were described by Chadwick & Burg (1970). They also described the influence of 5 and 10 % $CO_2$ on the curvature of intact pea roots and pointed out that $CO_2$ is a competitive inhibitor of ethylene. Root geotropism, mediated by ethylene, is influenced by $CO_2$. Abeles (1985) showed that ethylene can be physically bound to plant tissue, and in those cases the physical binding is not influenced by the inhibitors of ethylene such as $CO_2$ and ($Ag^+$). The dormancy-breaking action of $CO_2$ on seeds of subterranean clover was reported (Ballard, 1961) to be strongly influenced by temperature. At 30 °C the effect almost disappeared, which might suggest that a temperature-sensitive hormone system is involved.

Lin & Molnar (1980) showed examples of treatments with $CO_2$-misting that improved rooting. In their view it is essential for rooting that there is a proper balance between auxins and carbohydrates. As auxins are influenced by ethylene, and ethylene in turn by $CO_2$, it is not surprising that $CO_2$ concentration in the root zone may influence rooting.

In a review by Krizek (1979) it was pointed out that $CO_2$ in exceptional cases has the same effect as ethylene, for instance by acting as a growth promoter in rice and stimulating seed germination of peanuts.

The link between the different plant hormones, auxins, gibberellins and cytokinins is described by Letham (1969). A main feature is that $CO_2$ influences auxins, and since auxin and ethylene are involved in virtually all plant processes, $CO_2$ appears to be involved in the hormone regulation of most plant processes.

It is known (Arteca *et al.*, 1979) that $CO_2$ enrichment changes cytokinin, auxin and abscisic acid levels in roots of potato plants, suggesting that $CO_2$ triggers hormone changes which, in turn, influence dry-matter increase and tuberization.

$CO_2$ also influences seeds. Esashi *et al.* (1986) showed that $CO_2$ enhanced ethylene production in tissue of Cocklebur (*Xanthium strumarium* L.) seeds and thereby inhibited their germination. $CO_2$ enhanced the conversion of ACC to ethylene and the responsiveness of seed tissues by elevating ATP levels.

The morphogenetic influences of $CO_2$ and the biological activity of ethylene which is inhibited by $CO_2$ (Burg & Burg, 1967) and bicarbonate (Geisler, 1963, 1967), together with the other examples quoted above, support the view that in the root zone $CO_2$ acts as a plant hormone, or at the very least influences plant hormone systems. Also, elevated $CO_2$ in the air around the shoots seems to act partly as a plant hormone, as was proposed (Enoch, 1990) in a review paper on crop responses to aerial $CO_2$.

### 5. *Interaction of $CO_2$ with pesticides*

Studying pesticide effects on soil nitrification in closed vessels, Saltzman (1989) observed that nitrification rates were considerably reduced, when soil respiration was measured, using alkaline traps which reduced the soil-air $CO_2$ concentration. Clark (1968) and Singh & Kanehiro (1972) also reported inhibition of nitrification in the presence of alkaline traps. Kinbursky & Saltzman (1990) confirmed the important role of $CO_2$ in nitrification. When $CO_2$ concentration was maintained below 100 $\mu$l $l^{-1}$ by alkaline traps, growth of the $NH_4^-$ oxidizer microbial population and its nitrification activity was reduced.

The concentration of pesticides such as chlorpyrifos, metolachlor, fenamiphos and EPTC can enhance the influence of $CO_2$ on the rate of $NH_4^-$ oxidation by affecting the soil microorganisms (Saltzman, 1989). When pesticides were applied in the recommended concentration of between 1 and 10 ppm at optimal $CO_2$ concentrations they did not influence the $NH_4^-$ oxidation rate significantly. Under $CO_2$ stress conditions (at non-optimal $CO_2$ concentrations for the $NH_4^-$ oxidizer microfloral) the pesticides had a significant inhibitory effect on the oxidation rate of $NH_4^-$. Thus nitrogen availability can become limited by the simultaneous presence of pesticides and sub- or supra-optimal $CO_2$ concentrations such as the ones supplied by $CO_2$-enriched water.

At high application rates of pesticides (100 mg $l^{-1}$), $NH_4^-$ oxidation activity was significantly depressed, both under optimal and $CO_2$ stress conditions. These high pesticide concentrations are not only hypothetical, they occur in the case of non-homogeneous or repeated applications. Without $CO_2$ stress, the maximum reduction of nitrogen production due to pesticides was 37–46 % for a period not exceeding 5 d. Under $CO_2$ stress the maximum reduction was 53–70 % over a period of 13–14 d for the metachlor and EPTC and 59–64 d for chlorpyrifos and fenamiphos (Saltzman, 1989). The existence of a synergistic effect of pesticides and $CO_2$ concentrations (i.e. chemical and environmental stresses) on ammonium oxidation makes the use of $CO_2$-enriched water unfavourable for plant production in those instances.

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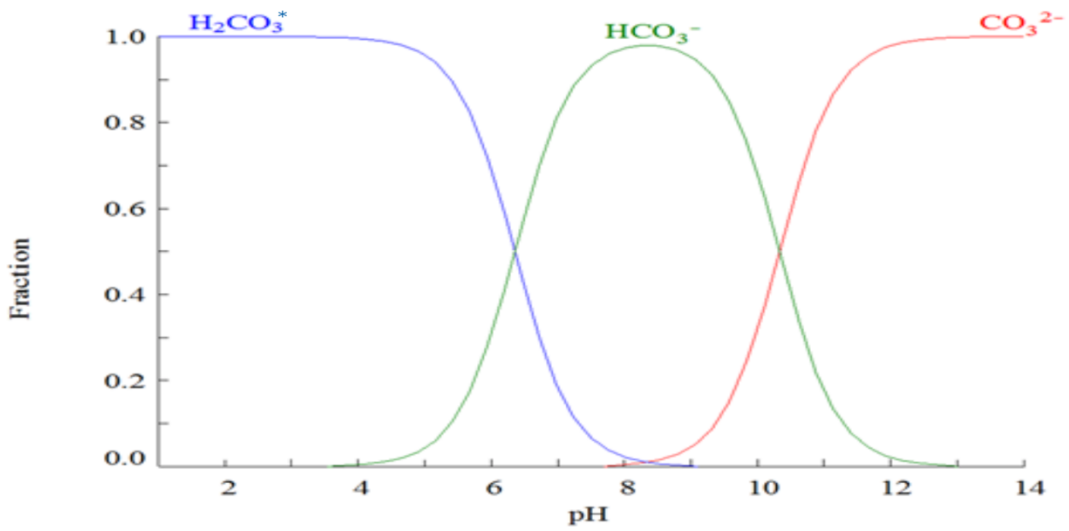
# Appendix F

# Petition Justification Statement




# CARBONIC ACID EXCHANGE THEORY