THESIS

DRIP IRRIGATION OF PLASTIC MULCHED STRAWBERRY USING CARBONATED WATER - A GREENHOUSE STUDY

Submitted by

Margaret L. Shore

Department of Horticulture

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 1994

COLORADO STATE UNIVERSITY

September 21, 1994

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MARGARET LOUISE SHORE ENTITLED DRIP IRRIGATION OF PLASTIC MULCHED STRAWBERRY USING CARBONATED WATER - A GREENHOUSE STUDY BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

D. L. Smith

Frank D. Moore III

Harrison Hughes

Advisor K. M. Brunk

Department Head

ABSTRACT OF THESIS

DRIP IRRIGATION OF PLASTIC MULCHED STRAWBERRY USING CARBONATED WATER - A GREENHOUSE STUDY

Carbonated water irrigation enhanced yields of tomato, however, little is known about the mechanism of this response. Objectives were: 1.) determine if strawberry responds to irrigation with carbonated water and 2.) determine if yield increase, should it occur, is due to short-term soil pH optimization or air-soil atmospheric enrichment with CO_2. Two different soils (2:1, perlite:soil) were used: a calcareous soil (5% $CaCO_3$, pH 8.0), with a Zn content of 0.9 μg/g and a non-calcareous soil (< 1% $CaCO_3$, pH 6.4) with a Zn content of 8.4 μg/g.

The carbonated water temporarily lowered the pH of the calcareous soil to 6.7 and the non-calcareous soil to 5.9, at both extremes of the optimal range (5.2-6.4) for strawberry. There was significant increase in above ground (1 cm) CO_2 during irrigation. Also, a significant increase in soil CO_2 was observed in the calcareous soil, carbonated water treatment over the noncalcareous, carbonated water treatment, which suggests carbonic acid played a role in lowering the surface pH of the calcareous soil from 8.0 to 6.7 shortly after each irrigation event.

Application of carbonated water increased production of buds and open flowers at the $P<0.05$ significance level. Carbonated water increased the production of marketable fruit ($P<0.10$) as compared to the noncarbonated water considering both soils. In addition, there was greater crown dry weight and higher leaf chlorophyll content ($P<0.05$) observed in plants irrigated with carbonated water. The magnitude of the response to carbonated water was similar for each soil. The noncalcareous soil had significantly greater accumulation of Zn in leaf tissue as compared to calcareous soil, considering both irrigation treatments. However, the calcareous soil, carbonated water irrigation treatment had a slight increase in the uptake of Zn over the calcareous, noncarbonated water treatment. Also, there was no significant difference in the uptake of Fe, Mn, or Cu in regard to irrigation treatments or soil type.

Margaret Louise Shore
Horticulture Department
Colorado State University
Fort Collins, CO 80523
Fall, 1994

ACKNOWLEDGMENTS

I would like to take this opportunity to thank the numerous people who have helped me throughout my graduate work at Colorado State University.

Without the help spiritually and monetarily of my family, all this would not have been possible.

Thanks to Dr. Kenneth M. Brink, Head, Department of Horticulture for accepting me as a teaching assistant to manage the Horticulture Department's teaching greenhouses.

I wish to thank the members of my graduate committee who have been a great deal of help with regards to my research. I thank Dr. Dan Smith for his help with the CO_2 infra-red analysis and for guiding my thinking on the theoretical and practical aspects of my research and Dr. Frank Moore, who has been like my father, giving me advice on mathematics and statistics. I would also like to thank my doctor father, Dr. Harrison Hughes, for his advice on strawberries and tireless effort in helping me with writing.

The employees of the Plant Environmental Research Center (PERC) have been a tremendous asset with regards to providing greenhouse space and assistance while conducting my research during 1992 to 1994. In particular, I would like to acknowledge Dean Dushak, David Hantz and Dr. Douglas Hopper, greenhouse director.

My sincere thanks to John Waddell with the National Seed Storage Laboratory, Fort Collins, CO, for the use of his lab equipment and guidance on laboratory procedures; for Shelia Blackman and Bill Prange and their practical advise.

I would like to acknowledge the secretaries in the Department of Horticulture, who have done a great deal of work to help me finish my thesis. In particular, Judy Croissant, Gretchen Deweese, Bonnie Schilling, and Dee Bailey. Ann McSay has also given a great deal of assistance with running the computer software on the third floor of the Shepardson building.

The assistance of Julie Moore and Kieth Albers was invaluable in helping me record and take data.

My fellow graduate students have been a of source inspiration and advice throughout the last three years. In particular, thanks to Jan Stevens, Floyd Wright, Masooma Ali-Ahmed, Scott Reeves, Scott Ried, Mike Weissmann and his wife Racheal, David Hanna and Erik Ervin.

In closing, I wish to thank those friends who are outside of academia for their help and support. In particular, LaRue Wentz who assisted with formatting the thesis; Pat Taylor for her advice and caring interest in my well being, Tony Kraushaar for his faithful support and Rajdev L. Basudev for his help in proof reading and objective interest in my research.

I am most grateful to the Yantorno family, my future employers, for being flexible in allowing me time to finish my thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The strawberry is an important fruit crop with both high yield and cash value on a per plant basis in relation to other small fruit crops. California is the center of production in the United States with an average yield of 20 tons per acre. In many production areas of California as well as in the Great Plains, high light levels throughout the growing season, due to clear skies, are common. However, fruit yield in these areas may be impacted by high soil pH.

Commercial strawberry production in California, where 80% of this country's crop is grown (Wilhelm and Sagen, 1974), may benefit from irrigation with carbonated water. Commercial growers already use drip irrigation under plastic mulch to retain moisture and warm soils during the winter season (Darrow, 1966; Wilhelm and Sagen, 1974). Furthermore, the majority of the production areas have high pH soil and many bright, cloudless days. Since most calcareous soils (prevalent in California and the Great Plains) are between pH 7.3 - 8.5 (Lindsay, 1979) and the optimum soil pH for strawberry is 5.2 to 6.4 (Murphy, 1982), a reduction in yield may occur. Carbonated water may serve to both lower soil pH and increase ambient CO_2 concentration.

Carbon dioxide enrichment has been the subject of many greenhouse studies in the past, particularly with roses in Colorado (Hanan et al., 1978). Aerial CO_2 enrichment has been used to enhance production, as well as prolong

shelf life. More recently, carbonated water has been used in both greenhouse and field to enhance plant growth.

Strawberry has been used in several studies involving the application of CO_2. All studies in closed environments have shown positive growth responses using CO_2 aerial enrichment (Cummings and Jones, 1918; Enoch et al., 1976 and Cahn, 1989). Cahn (1989), in a growth chamber study, demonstrated earlier fruit ripening and a greater number of ripe fruit with elevated aerial CO_2. In a field study, Cahn (1989) measured an increase in CO_2 concentration from 342 (normal atmospheric CO_2 concentrations) to 492 μL L^{-1} CO_2, at a 20 cm height, within mulched (0.025 mm-1.0 mil black plastic mulch) strawberry plots, during drip irrigation using carbonated water.

Carbonated water has the potential to influence plant growth in two ways. It lowers the pH of calcareous soils thus facilitating greater uptake of phosphorus and micronutrients (Enoch and Olesen, 1993; Novero, 1991). It may also, directly or indirectly, result in additional available carbon for photosynthesis (Enoch and Olesen, 1993).

The objective of this research was to determine if strawberry responds to irrigation with carbonated water in a greenhouse and to determine if yield increase, should it occur, is due to short-term soil pH optimization or air-soil atmospheric enrichment with CO_2.

LITERATURE REVIEW

Atmospheric CO_2 is found at approximately 340 μL L^{l-1}. At this level, efficient diffusion through stomates allow for normal photosynthesis (Salisbury and Ross, 1985). However, additional CO_2 within the plant environment should enhance photosynthesis (Enoch and Olesen, 1993).

A wide variety of crops have been studied with regards to aerial enrichment of the greenhouse environment and these studies have been reviewed extensively (Enoch and Kimball, 1986; Porter and Grodzinski, 1985). Greenhouse experiments demonstrated that growth enhancement can occur with aerial application of CO_2. Tomato and lily are examples of crops which showed significant yield increase with the addition of 1000-1500 μL L^{-1} of CO_2. The tomato yield was achieved by greater fruit numbers and higher fruit weight (Van Berkel, 1986). Van Berkel (1986) reported a study in which lilies showed sturdier growth, greener leaves and reduced bud blasting under the influence of elevated CO_2, thus indicating an enhanced photosynthesis apparatus.

Other crops in which CO_2 enrichment has been beneficial include roses with stronger stems and increased stem length, chrysanthemums with increased lateral branching and *Saintpaulia* sp., *Nephrolepis* and *Begonia* with greater leaf numbers (Moe, 1986). Carnation yield was increased up to 38% and the growth time required to produce flowers was reduced by two weeks

with added CO_2 (Nelson, 1978). Fall snapdragons were reported to have flowered 13 days earlier, while geranium cuttings were shown to root easier and subsequent plants were sturdier with higher numbers of branches (Nelson, 1978). Holley observed a 33% yield increase in rose flowers 32 weeks after aerial injection of 2000 ppm of CO_2 with high ventilation and aerial injection of 500 ppm CO_2 in conjunction with lower ventilation (Hanan, et al., 1978).

The greenhouse is an ideal environment for CO_2 enrichment due to the increased air movement associated with tube fans, which cause increased wind speeds. Increased wind speed reduces the resistance to CO_2 diffusion from the bulk air to the leaf. Thus, additional CO_2 can potentially be diffused through the boundary layer next to the leaf. There is a direct relationship to increased photosynthesis and wind-speed (Hanan et al., 1978) in the greenhouse.

Additional aerial CO_2 can overcome diurnal fluxuations which occurs in enclosed environments, including the plant canopy, bringing a constant supply of CO_2 to the stomates (Porter and Grodzinski, 1985).

Aerial application of carbon dioxide was shown to increase root length (110%) and weight (143%), along with increased nitrogen uptake in a recent study conducted by Rogers et al., 1992 on soybean (*Glycine max* L.) They also noted a significant increase in root to shoot dry weight. An increase in leaf area and dry weight were noted, as well.

Cahn (1989) demonstrated that increased levels of CO_2 (600 μL L^{-1} and 900 μL L^{-1}) resulted in a trend toward earlier anthesis, fruit set, and ripe fruit as well as significantly larger fruit of strawberry in a growth chamber. Leaf

production did not appear to be altered by enrichment, but the enriched plants produced thicker leaves than control plants indicating greater photosynthetic activity and greater photosynthetic potential in response to higher CO_2 concentrations. Enriched plants yielded significantly greater root, shoot, and crown dry weight than the unenriched plants.

In a field experiment using wheat (*Triticum aestivum* L.) and trickle irrigation, a 20% yield increase was observed by Nakayama and Bucks (1980) when irrigating with carbonated water, the equivalent of 1900 ppm in tap water.

Mauney and Hendrix (1988) irrigated cotton (*Gossypium hirsutm* L.) with carbonated water in a greenhouse, which resulted in an increase in yield, CO_2 exchange rate (CER), leaf chlorophyll and starch content. They found that leaf tissue zinc (Zn) and manganese (Mn) increased significantly over the control. Since both Zn and Mn are important in photosynthesis, Mauney and Hendrix concluded that the increased amounts of those micronutrients supported a more robust photosynthetic apparatus. As a result, increased photosynthetic activity and significantly higher yields were observed. Mauney and Hendrix (1988) concluded that carbonated irrigation water increased the availability of the two metal elements, possibly due to the shift of soil pH as the result of using carbonated water.

In potato (*Solanum tuberosum* L. var Russett Burbank), CO_2 enrichment of the root zone increased dry matter content and enhanced tuberization (Arteca et al., 1979). Similarly, eggplant (*Solanum melongena* L.) grown under

long days, warm temperatures and elevated CO_2 concentration in the root zone developed a significant increase in dry weight, leaf area, and stem diameter (Baron and Gorski, 1986).

Zornbach and Schickerdanz, as reported by the Tansely Review in 1993, showed both cyclamen and poinsettia had an increase in fresh and dry weights due to a rise in aerial CO_2 concentration when both crops were irrigated with carbonated water.

Hartz and Holt (1991) showed results contrary to previously sited literature relative to advantages of using carbonated water. They used carbonated water that was injected continuously into the irrigation stream at rates of 1.0 $g \cdot liter^{-1}$ or 0.5 $g \cdot liter^{-1}$ and dropped the soil pH from 7.3 to 5.3. They observed an actual decrease in yield using carbonated water in both tomatoes and cucumber or no increase at all.

Terrestrial plants are known to take up CO_3^{-2} as well as HCO_3^- though their root system (Livingston and Beal, 1934; Bedri el al., 1960; Geisler, 1963; and Baron and Gorski, 1986). In Germany, Schafer (1988) applied radioactively labeled carbonic acid ($H_2{}^{14}CO_3$) to pots of spring wheat. The soil surface of the pots was sealed at the shoot level. The results showed a maximum of 1.21% of total carbon found in shoots came from the carbonic acid carbon (H_2CO_3), while one-third of that applied remained in the soil solution, presumably available to the roots as CO_2 and HCO_3^- (original not seen).

Novero et al. (1991) reported, in a field experiment using trickle irrigation with carbonated water and black polyethylene mulch, an increase in marketable

tomato yield, as well as an increase in Zn uptake. The mulching alone increased yield by 8%. When carbonated water was applied, yield was increased by an additional 7-23%. Furthermore, the plots using carbonated water showed a significant increase in fruit size which contributed to the increased yield. In 1993, Arienzo et al. reported that carbonated water increased the zinc, copper, iron and manganese content of tomato leaves in both early and later growth stages.

The general growth habit of the strawberry is a rosette, with trifoliolate leaves born out of a short thickened stem, called a crown (Darrow, 1966; Fletcher, 1917). This low growth habit with leaf blades held horizontally may facilitate the trapping of CO_2 evolving from the soil. The degassing process that occurs in carbonated water frees CO_2 which may collect beneath the plastic mulch. There is a possibility this gas then passes through the hole associated with the plant and is finally trapped beneath the plant canopy which may enhance photosynthesis (Moore, 1990).

When CO_2 is injected under pressure into water, it creates carbonated water and a very dilute solution of a weak acid, carbonic acid. This acidifying solution, with a pH of 4.0-5.0, undoubtably generates CO_2 from the calcium carbonate of the calcareous soils found in the western United States. Since calcium bicarbonate (HCO_3^-) is formed in the process, the bicarbonate ion becomes available to plant roots. When carbonated water diffuses within the soil, CO_2 as well as the carbonate ion (CO_3^{-2}), which depends on the system pH, would be available (Moore, 1990) to the canopy and roots for uptake

(Arteca and Poovaiah, 1982, Baron and Gorski, 1986; Schafer, 1988; Enoch and Olesen, 1993).

Our hypothesis is that carbon dioxide lowers the pH of tap water. This may result in greater uptake of phosphorus and micronutrients by plants. Carbonated water may also result in greater carbon availability for enhancement of photosynthesis.

MATERIALS AND METHODS

The experiment recorded in this thesis was preformed in the spring of 1993 at the Plant Environmental Research Laboratory (PERC) at Colorado State University.

Plant establishment

Two soil types were used in this experiment. One was a Weld loam (Ca of <1%, O. M. 1.8%, pH 6.4) classified as a fine montmorillonitic, mesic, Ardic, Paleustoll, collected from a Colorado farm near Bennet, in May of 1992. The second soil type was a Nunn clay loam (Ca 5%, O. M. 1.9%, pH 8.0) classified as a fine, montmorillonitic, mesic, Ardic, Arguitstol. The latter was obtained from the former site of the Colorado State University Agronomy farm on Drake and Timberline Road, Fort Collins. The two soils were tested at the Colorado State Soil Testing Laboratory in May 1992 shortly after collection.

A soil medium of 2:1, perlite:soil, by volume was mixed and pasteurized. The perlite is an inert volcanic material and was chosen to facilitate drainage and because it will not add CO_2 gas or any essential nutrients. Potassium nitrate and super phosphate were each added at a rate of .454 kg per .0283 m^3 (Boodley, 1981). Initial micronutrients levels were determined by analysis of the Colorado State Soil Testing Laboratory (Table 1).

Table 1. Initial soil analysis for Zn, Fe, Mn and Cu.

Soil Type	Zn μg/g	Fe μg/g	Mn μg/g	Cu μg/g
Noncalcareous Soil	8.4	19.6	1.3	2.9
Calcareous Soil	0.9	9.0	4.9	2.9

A total of 200 bare root strawberry plants, day neutral cultivar (*Fragaria* x *anannasa* Duch cv 'Miur') obtained from a nursery in northern California, were potted in nursery containers (15.2 cm diameter by 17.2 cm high, #1 plastic nursery containers) with 2.2 liters of soil mix on April 7, 1993. Each crown had two to three growing points. Prior to potting the plants were soaked approximately 30 minutes in a solution containing 15 mg Benlate per 3.78 L of tap water.

To establish the plants, they were watered with tap water for three weeks before the experiment began. The plants began to bud after two weeks and flowered during the third week of irrigation with city water. The most vigorous 120 plants (120 out of 200) were chosen for the experiment. A black plastic mulch (0.025 mm-1 mil) was cut to fit the inside diameter of the soil surface of the nursery container with a 2.5-cm hole cut into the center through which the plant protruded. The mulch served as a diffusion barrier channeling CO_2 through the hole and into the strawberry canopy while allowing some oxygen exchange.

Plants were arranged in a randomized complete block design in a 2 x 3 factorial arrangement (two soils and three water treatments, counting the control) with each replication containing six combinations of soil type and irrigation water pH. There were 20 blocks in all. An experimental unit consisted of one plant.

Irrigation system

Polyvinylchloride (PVC) irrigation pipe, 1.8 cm in diameter, was used to deliver noncarbonated (city water) and to established plants. The noncarbonated water and carbonated water treatments used 15 cm diameter "ring type" drip emitter. Connected to the emitters, was a 1 meter long, 3 mm diameter "spaghetti" tube attached to the PVC irrigation tubing. Both irrigation systems had their own set of PVC pipes. Gate valves sealed off both treatments to prevent the possibility of cross contamination of irrigation water. Medical grade CO_2 gas was injected at a rate of 4.22 kg per m^2 into a restaurant carbonator, Cornelius VA13 Carbonator, (Cornelius Company, 1055 West Main Street, Anoka, Minnesota, Cahn, 1989) and mixed with tap water at a rate of 3.52 kg per m^2. The resulting mixture was then piped into its own set of irrigation pipes.

Carbonation decreased the tap water pH from an average of 6.7 to 4.0 with an approximate 1600 ppm of carbon dioxide gas (Cahn, 1989). Concentrated sulfuric acid (H_2SO_4) was added to distilled water to bring the pH of the acidified irrigation water pH down to 2.0. This was applied by hand as a dilute aqueous solution. Irrigation amounts were determined before each irrigation event by weighing randomly selected pots of each treatment. From that weight, a specific amount of water was determined to bring the pots up to field capacity. These ranged from 400 ml to 650 ml depending on previous temperature and level of irradience in the greenhouse.

Irrigation treatments began on May 1, 1993 and continued for 12 weeks with treatments applied at three to four day intervals on a normal watering schedule at 8:00-8:30 am. The temperatures in the greenhouse ranged from 25 ± 3 C° during the day to 16 ± 2 C° at night. Temperatures were recorded on a hygrothermograph, model 594, made by the Bendix Aviation Corporation.

Experimental Observations

Data collection began on May 13, 1993 during the second week of treatments and continued weekly until the 12th week for a total of 10 weeks. By the tenth week there were few buds and flowers and no fruit to report due in part to nutrient deficiencies detected by typical deficiency symptoms.

The data collected consisted of total number of buds, open flowers, and marketable fruit, fruit fresh and dry weights, and average number of leaves produced. For these observations data were taken each week for each plant with the exception of leaf numbers, which were recorded every three weeks. Final plant measurements included leaf area, leaf dry weight, specific leaf area and weight, plant and crown dry weights, and chlorophyll analysis. Air samples were taken for CO_2 analysis on May 30 and continued weekly until July 7, 1993. Samples of soil pH were taken intermittently from May 24 to July 23, 1993.

On three separate dates, May 21, June 4 and July 23, leaves were collected for diurnal starch tests.

On May 21, the first fully expanded leaves of two growing points were excised from three randomly selected replications. The leaves were taken in the

morning from 7:00-8:00 am and another set of leaves were taken from three different replications in the evening from 3:00-4:00 pm. On June 4 and July 23rd the same procedure was used but with six replications for am and pm collection times. On each occasion the midribs and large veins were excised. The leaves were placed into coin envelopes and dropped into liquid nitrogen within one minute of removal from the plant.

Frozen leaf samples were freeze-dried using a drier manufactured by Virtus Research Equipment, Gardiner, New York. The leaves were then ground in a mortar and pestle and sieved through a 40 mesh screen. A 50 mg (± 1 mg) sample was weighed and recorded.

Three ml of 80% ethanol was added to each sample and then vortexed and placed in 40 C° H_2O for 30 minutes to extract ethanol soluble sugars and chlorophyll. After the liquid was removed, the test tubes were then vortexed and centrifuged for 20 minutes. The previous two steps were repeated two more times, collecting and disposing of the supernatant each time. The remaining tissue was air dried under a fume hood over night.

Starch pellets were used in following glucose oxidase assay for starch (Sigma kit #510-DA Glucose Oxidase).

The color reagent supplied in the kit was prepared according to package directions. A 0.1 M (pH 4.5) acetic acid buffer was made using 6.0 mls per 1 liter of distilled water with the pH adjusted with sodium hydroxide to 4.5. A solution of amyloglucosidase (Sigma catalog #a-7420) containing 50 units/ml of acetate buffer, the equivalent of 1 mg/ml buffer or 50 units/ml was prepared

fresh for each assay. A standard glucose curve was generated from the stock solution supplied by the glucose oxidase kit.

The starch pellets were prepared in the following manner. The pellets were hydrolyzed with 2 ml of acetate buffer which was added to each pellet left after the ethanol extraction. Marbles were placed on top of each test tube and they were placed in a boiling water bath for one hour. Samples were allowed to cool and 0.2ml of the amyloglucosidase enzyme was added to each tube. The test tubes were incubated for 16 hours in a 45 C° water bath. The samples were boiled for 20 minutes to terminate the reactions and centrifuged for 20 minutes. The remaining liquid was used as undiluted or diluted samples up to 2.5 times the undiluted liquid. Aliquots of samples were taken and brought to final volume of 0.25 ml using distilled water. The aliquotes ranged from 0.10 to 0.025ml of undiluted or diluted sample. An aliquot of 2.5 ml of the prepared coloring reagent was added to the 0.25 ml preparation and developed for 20 minutes in a water bath at 37 C°.

The absorbance was measured with a Hewlet Package Diode Array Spectrophotometer, model 8450A (Hewlet Packard, Corp., Ft Collins, CO) at 440 nm as compared to a distilled water zero. The absorbance was below 1 and within the range of the standards. The ug of glucose was determined from the standard curve equation.

The mg of starch was determined by:

$$\mu g \text{ glucose} \times \frac{\text{ml diluted final volume}}{\text{ml aliquot for assay}} \times \frac{\text{2.2 ml original solution}}{\text{ml taken for dilution}}$$

$$\times \frac{\text{0.9 ug starch}}{1\ \mu g \text{ glucose}} \times \frac{\text{0.001 mg starch}}{1\ \mu g \text{ glucose}} = \text{mg starch from sample}$$

Leaf starch content was then expressed on a mg per gram basis.

The flower buds were observed each week on a per plant basis. There was no distinction between bud size noted when the data was collected. The buds were green and tightly closed or just beginning to show white petal color (Darrow, 1966).

The open flowers were recorded on a per plant basis. These flowers were fully open with all flower petals intact.

Fruit numbers were recorded once per week. Fruit that were bright red with no white areas near the calyx or the basil tip were counted. Those approximately 18-25 mm in diameter were reported as marketable fruit (International Standardization of Fruit and Vegetables, 1979). Fruit began to ripen during the third week of treatments (May 20) and continued until after the seventh week when the number of developing fruit dropped off dramatically.

Fruit collection continued until the ninth week. Nutrient deficiency symptoms began during the sixth week and likely effected the amount of fruit produced by the seventh week. Some pistils were not well formed resulting in misshapened fruit. These fruit were not counted in the total number of marketable fruit. The greatest amount of ripe fruit was recorded during the

fourth week of treatments (May 27). The marketable fruit were weighed immediately after picking on a OHAUS (model GT 4800) gram scale and the weight was recorded. Total fresh weight was obtained by adding all fruit harvested and weighed during each week, i.e. fruit fresh weight was cumulative.

After the fresh weight was recorded, fruit were carefully placed in paper bags and the top of bags were sealed with staples to prevent any spillage. The fruit was then dried at 70 C° in a Despatch series V forced air drier for 48 hours. Dry weights were recorded on the same OHAUS scale.

At three week intervals, the total number of leaves present was recorded on a per plant basis.

At the end of the experiment, on July 23, the third fully expanded leaf of two growing points was taken from four randomly selected replications. The leaf area was recorded within 45 minutes after taking the leaf samples using the LI-COR, model 3100, leaf area meter (LI-COR, Inc., Lincoln, NE)

After drying at 70 C in a Despatch series V forced air drier for 48 hours, the leaves were ground with a mortar and pestle to pass through a 40 mesh screen. The leaf dry weight was obtained and used in calculation of specific leaf area and specific leaf weight. Both calculations are ratios. Specific leaf area is leaf area divided by the dry weight while the specific leaf weight is leaf dry weight divided by leaf area.

The ground leaves used for the leaf dry weight measurement were placed in a Waring blender along with 100 ml of 80% acetone and homogenized for

five minutes. The homogenate was filtered with Whatman 541 filter paper and the filtrate solution was brought to a constant volume with 80% acetone. The absorbance of the filtrate at 650 nm was determined using a Baush and Lomb spectrometer, Spectronic model 20. Absorbance values were used to determine chlorophyll concentration of extracts. The path length used was 1 cm. Chlorophyll was then expressed in a projected leaf weight basis (Burinsma, 1963).

The leaf mineral element analysis was preformed on the remaining dried leaf tissue from the samples harvested on June 4, 1993. The tissue was pre-digested and then digested further with nitric and perchloric acids respectively. It was heated and diluted with distilled water (Jones and Case, 1990). The digested tissue was analyzed at the Colorado State Soil Testing Laboratory using ICP analysis of solutions.

Soil pH samples were taken from three blocks with three plants per six treatments for a total of 18 plants. Sampling was taken at a depth of 2 cm in situ at one pre-selected spot within the pot one hour after treatment. Approximately 1 ml of distilled water was added to the selected spot. A Cole Palmer portable pH meter model WD00062400 was used to determine the pH of the solution. Soil pH samples were taken approximately every other week for a total of six observation times.

The pH of the noncarbonated and carbonated treatment solutions was taken five minutes after irrigation was completed. The pH of the acidified distilled water was determined prior to application. One irrigation time was

selected each week over a six week period to collect aerial and soil air CO_2 samples. During each week aerial samples were taken 30 minutes, 24 hours post irrigation and 48 hours at the end of a selected irrigation event. Soil air samples were collected 7 and 48 hours after the same irrigation. All air samples, whether above or below ground, were taken with a 10 cm^3 syringe using a 21 gauge needle in still air. Aerial samples were taken 1 cm above the soil surface near the crown and 20 cm above the soil surface. A 1 cm I.D. open ended pyrex tube, 5 cm in length, was placed at a depth of 10 cm below the soil surface of each pot. One end of the test tube was sealed with a rubber stopper with a 3 mm hole in the middle of the stopper. A nylon tube, 1 mm I.D.x 3 mm O.D. and 15 cm in length were inserted into the hole of the stopper and the other end was heat sealed (Novero, 1991). All syringes were sealed with neoprene stoppers immediately after sample were taken. The CO_2 concentration was determined within 30 minutes after being taken using a pulse injection technique (Clegg et al., 1978) and a Beckman, model 865-25 infrared gas analyzer (Beckman Instruments, Inc, Fullterton, CA).

A standard curve was generated for each run of samples and a regression curve was used to predict the ppm of carbon dioxide per number of chart divisions generated. A four way factorial containing the parameters of irrigation water pH, soil type, position of air sample and observation time was performed to determine the effect of carbonated water on the amount of carbon dioxide that was generated during the irrigation process as compared to the controls.

RESULTS

Carbonation, reduced the irrigation water pH average from 6.7 to 4.0 (Table 2, Figure 1). When the carbonated water was applied to the calcareous soil it temporarily lowered the pH from 8.0 to 6.7, while tap water (pH 6.7) temporarily lowered it to 7.6. The acidified water (pH 1.9) temporarily lowered the pH of the calcareous soil to an average of 4.6. For the noncalcareous soil, the carbonated water lowered the pH of the growing media from 6.4 to 5.9 while the noncarbonated water raised the pH to 6.5 and the acidified water temporarily lowered it to 2.4. This was observed within one hour of irrigation. It was, however, only a temporary drop as the soil pH returned to its previous level within 24 hours.

The relative amount of CO_2 observed in the atmosphere and soil after application of carbonated or tap water showed significant differences among treatments within the soil and at 1 cm above the soil (Table 3, Figure 2). There were no differences at the 20 cm sampling point. The calcareous soil with carbonated water irrigation had significantly higher levels of CO_2, 1904 ppm, as compared to carbonated water irrigation of noncalcareous soil, 1200 ppm. The latter was significantly higher than the two noncarbonated treatments, which were similar to each other. At the sampling height of 1 cm, the calcareous soil with carbonated water was significantly greater, 1204 ppm,

Table 2. Initial soil pH, irrigation water pH and potting mix pH at -2 cm and 1 hour after irrigation.

pH	Calcareous Soil	Noncalcareous Soil
Soil pH	8.0	6.4
Irrigation pH	Carbonated Water 4.0	Noncarbonated Water 6.7
Potting Mix pH	Carbonated Water 6.7	Carbonated Water 5.9
	Noncarbonated Water 7.6	Noncarbonated Water 6.5

Figure 1. Influence of carbonation and soil type on soil pH. Means followed by the same letter are not significant at $P<0.05$. Soil pH of 2, 4 and 7 are approximate and are the result of irrigating with water acidified with H_2SO_4, carbonated water and noncarbonated water, respectively.

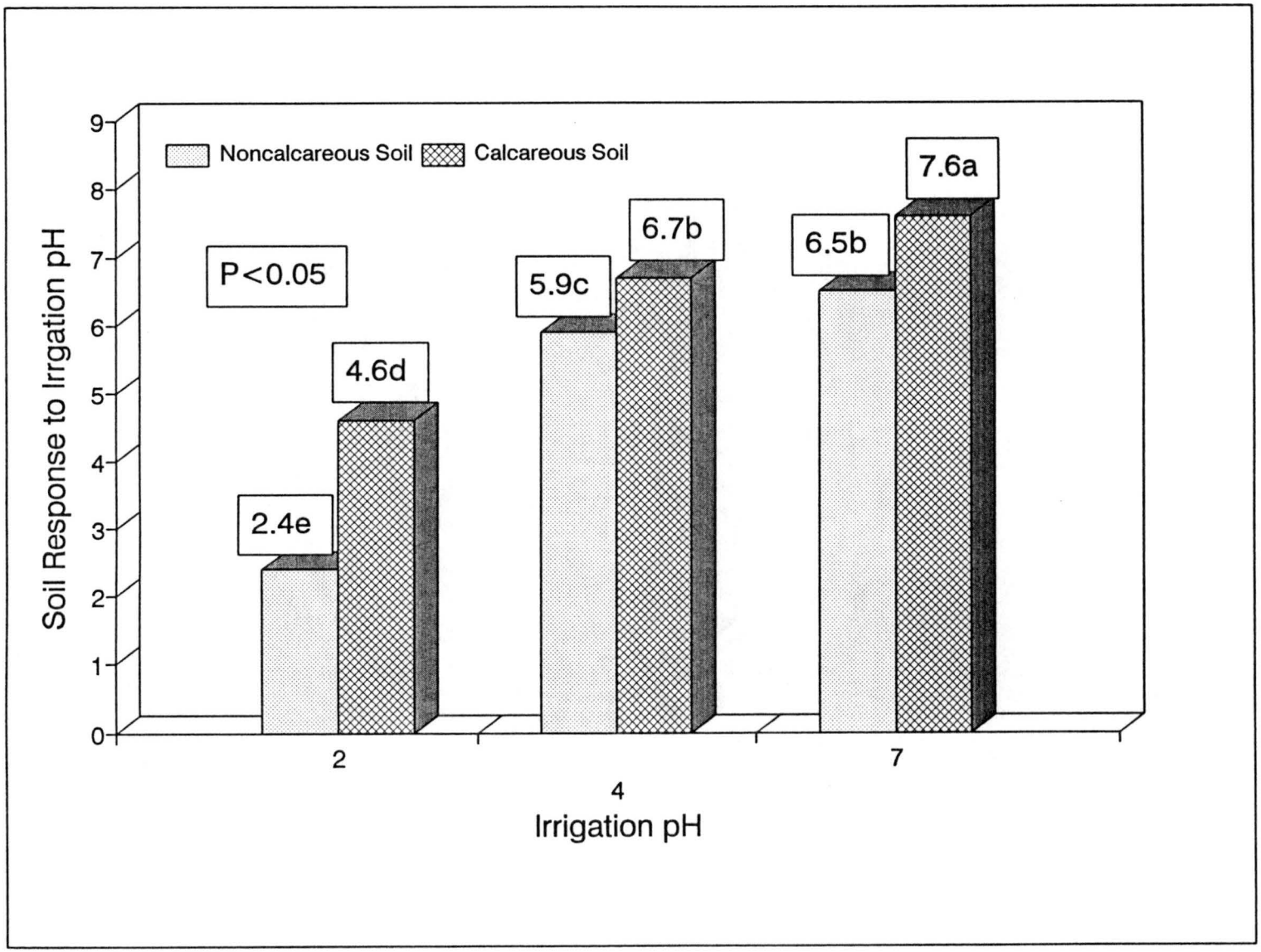
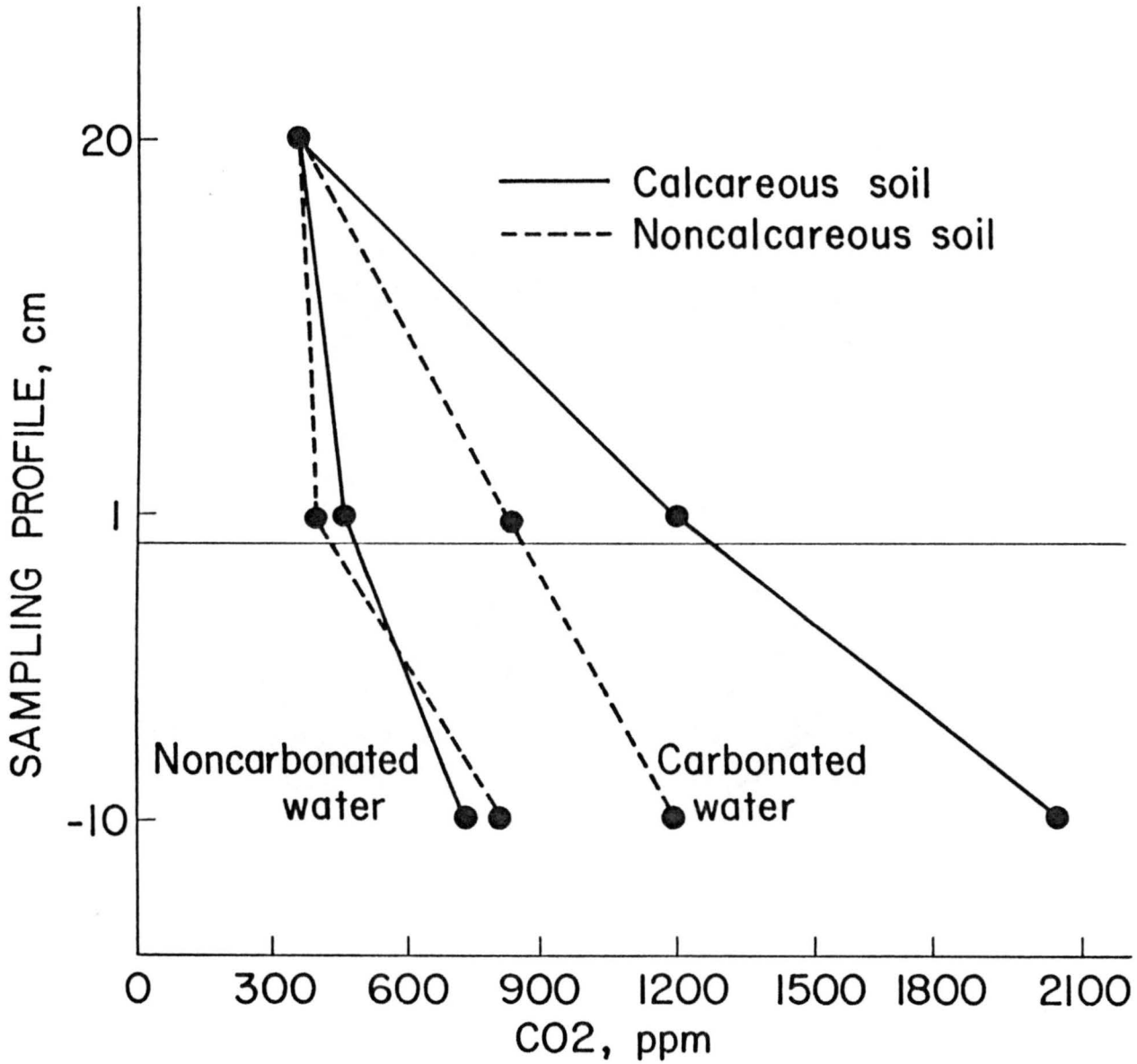

Noncalcareous Soil
Calcareous Soil
P<0.05
2.4e
4.6d
5.9c
6.7b
6.5b
7.6a
Soil Response to Irrgation pH
0
1
2
3
4
5
6
7
8
9
2
4
7
Irrigation pH

Table 3. Carbon dioxide in ppm one half-hour after irrigation.

Water Treatment	Soil Type	Sampling Profile 20 cm	1 cm	-10 cm
Noncarbonated	Noncalcareous	371	406e*	747e*
	Calcareous	385	386e	775e
Carbonated	Noncalcareous	379	811b	1200c
	Calcareous	377	1204a	1904a
Acidified	Noncalcareous	366	447d	860d
	Calcareous	385	537c	1781b

* Means followed by different letters within a column are significant at $P < 0.05$.

Figure 2. Mean CO_2 concentrations above and below ground as influenced by carbonation and soil type.


20
1
-10
SAMPLING PROFILE, cm
Calcareous soil
Noncalcareous soil
Noncarbonated water
Carbonated water
0
300
600
900
1200
1500
1800
2100
CO2, ppm

than the carbonated water treatment of the noncalcareous soil, 811 ppm. The latter was significantly greater than the noncarbonated soil treatments with 386 and 406 ppm.

The acidified water treatment had no significant differences at the 20 cm sampling level (Table 3). At the 1 cm sampling height the calcareous soil had a CO_2 level of 537 ppm which was significantly greater than the noncalcareous soil at 447 ppm. Seven hours after application with acidified water, the calcareous soil had a significantly higher CO_2 concentration of 1781 ppm as compared to 860 ppm for the noncalcareous soil. This treatment was dropped from analysis of its effects on plant growth parameters because the irrigation pH of 2.0 appeared detrimental over the two soil treatments by the end of the experiment. Perhaps, if the irrigation of the acidified distilled water had been once per week, the impact of the low pH would not have been as dramatic. In subsequent experiments, a pH of 4.0, to match the carbonated water irrigation pH should be used.

Analysis of the total number of buds and open flowers showed significant differences for both irrigation and soil treatments (Table 4). The total number of buds per plant counted in the calcareous soil treatment was 15 as compared to 10 with the noncalcareous soil. The carbonated water treatment averaged over both soils had a mean of 14 which was significantly greater than

Table 4. Influence of noncalcareous, calcareous soil and noncarbonated, carbonated water treatments on total buds, open flowers, and marketable fruit per plant over nine weekly observations.

Soil Type	Water Treatment	Buds	Open Flowers	Marketable Fruit
Noncalcareous	Noncarbonated	10	6	6
	Carbonated	12	7	7
Calcareous	Noncarbonated	11	7	7
	Carbonated	17	11	8
Averages				
	Noncarbonated	11	6	6
	Carbonated	14*	9*	7
Noncalcareous		10	6	6
Calcareous		15*	9*	8

ANOVA Source	df
Rep	19
H_2O pH(A)	1
Soil (B)	1
A x B	1
Error	57

*Significant at $P<0.05$

the noncarbonated water treatment of 11. Open flowers per plant from the calcareous soil treatment averaged over the two irrigation treatments was significantly higher with a mean of 9 flowers per plant verses the noncalcareous soil with a mean of 6 flowers per plant. The carbonated water treatment averaged over both soils was significantly higher than the noncarbonated water treatment with a mean of 9 as compared to 6.

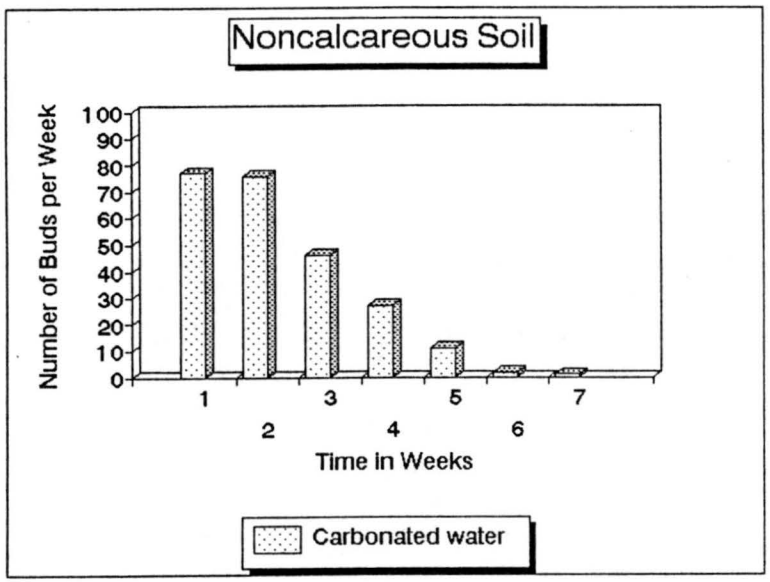
Flower bud numbers per 20 plants over seven weeks showed more buds were produced in the carbonated irrigation treatment over both soils, especially during week 1 and 2 (Figure 3). Open flower numbers were greater during week 2 for the carbonated irrigation, calcareous soil treatment (Figure 4). Marketable fruit were not significantly different for either soil or irrigation treatments at $P < 0.05$ (Figure 5).

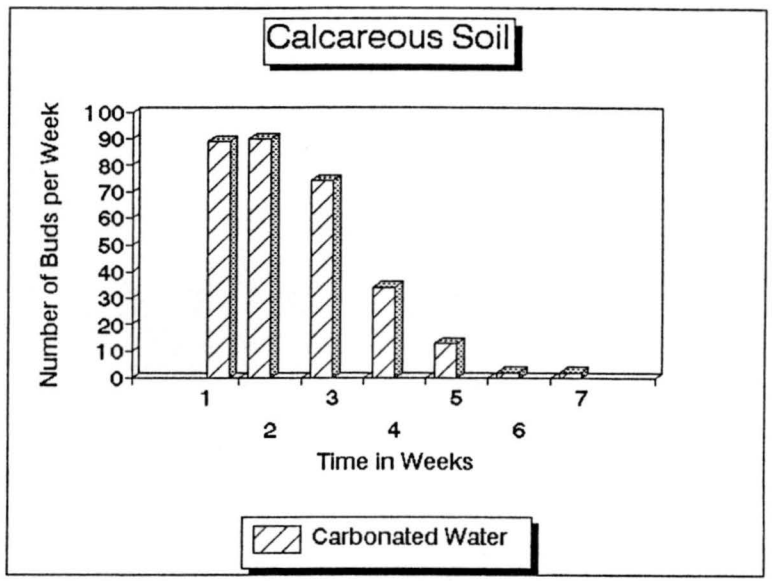
However, carbonated water irrigation produced substantially more marketable fruit than tap water irrigation with a significance at the $P<0.10$ (Table 4, Figure 5).

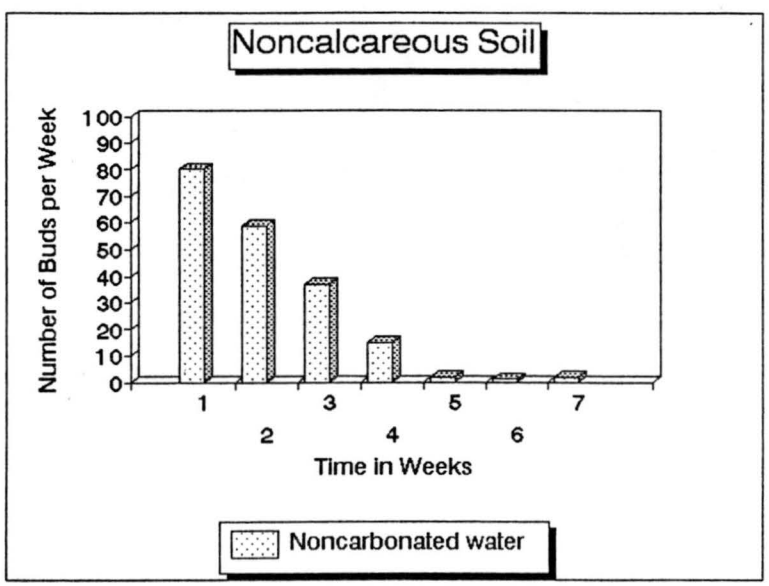
A comparison of total fresh fruit weights, marketable fruit only, indicated that there were no significant differences with either irrigation or soil treatment (Table 5). Nor were there any significant differences in fruit dry weight for both irrigation and soil treatment.

Plant dry weights (including roots) were not significantly different for either irrigation treatment or soil type (Table 5). However, crown dry weight was significantly greater for the carbonated water irrigation treatment over both soils (Table 5).

Figure 3. Number of buds per 20 plants per week in response to carbonation and soil type.


Noncalcareous Soil
Number of Buds per Week
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
Time in Weeks
Carbonated water


Calcareous Soil
Number of Buds per Week
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
Time in Weeks
Carbonated Water


Noncalcareous Soil
Number of Buds per Week
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
Time in Weeks
Noncarbonated water

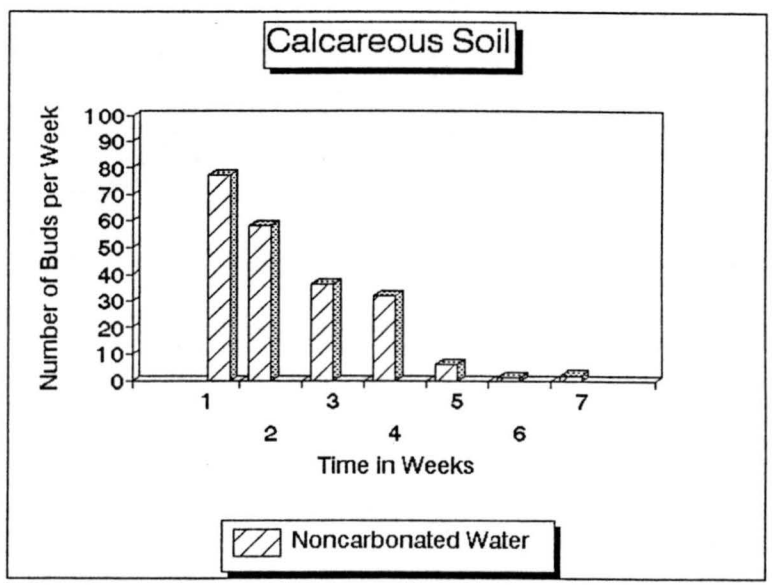
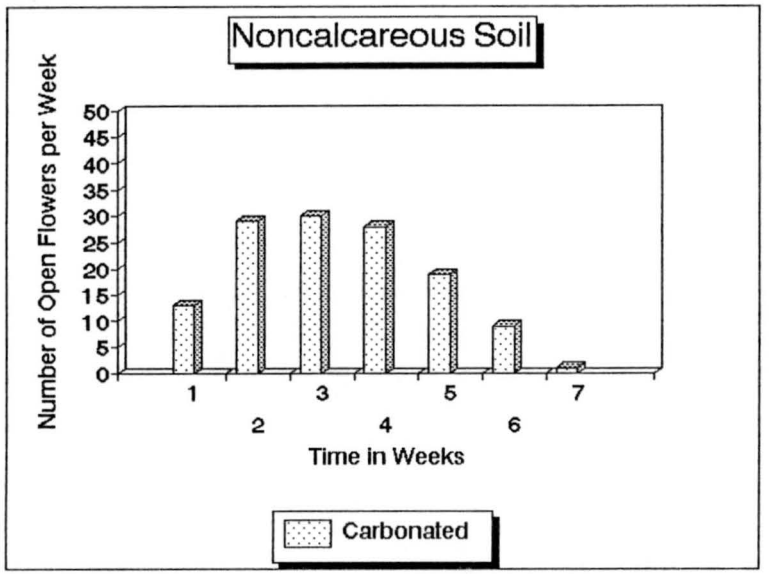
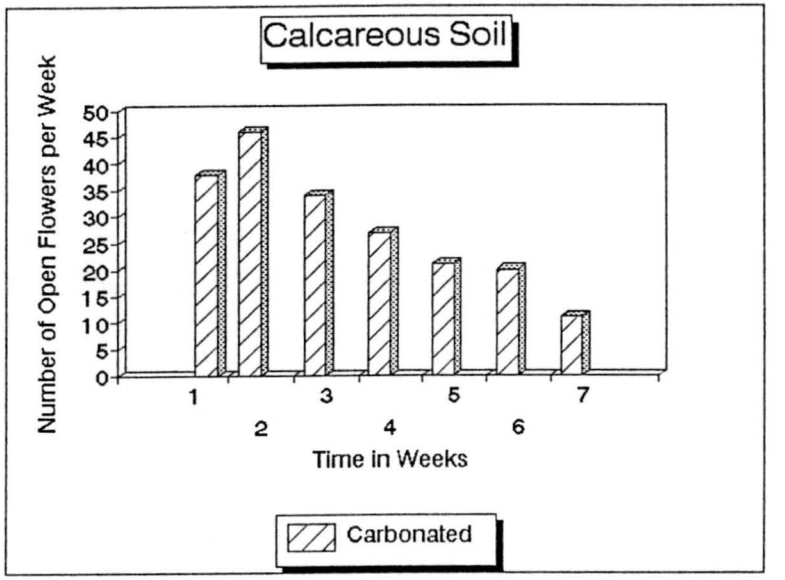
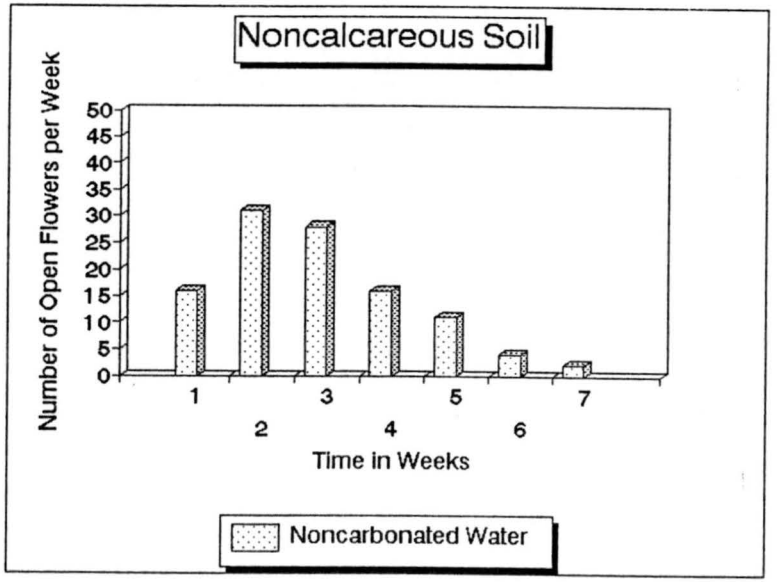

Calcareous Soil
Number of Buds per Week
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
Time in Weeks
Noncarbonated Water

Figure 4. Number of open flowers per 20 plants per week in response to carbonation and soil type.


Noncalcareous Soil
Number of Open Flowers per Week
0
5
10
15
20
25
30
35
40
45
50
1
2
3
4
5
6
7
Time in Weeks
Carbonated


Calcareous Soil
Number of Open Flowers per Week
0
5
10
15
20
25
30
35
40
45
50
1
2
3
4
5
6
7
Time in Weeks
Carbonated


Noncalcareous Soil
Number of Open Flowers per Week
0
5
10
15
20
25
30
35
40
45
50
1
2
3
4
5
6
7
Time in Weeks
Noncarbonated Water

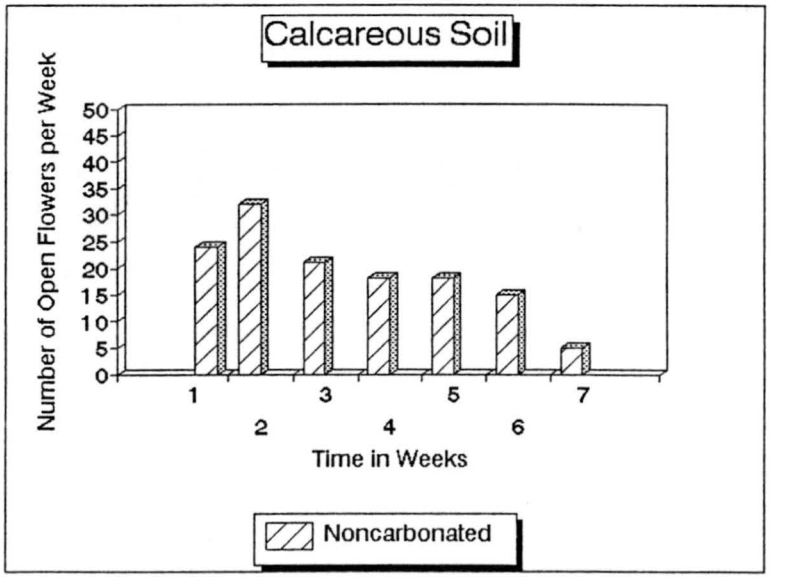

Calcareous Soil
Number of Open Flowers per Week
0
5
10
15
20
25
30
35
40
45
50
1
2
3
4
5
6
7
Time in Weeks
Noncarbonated

Figure 5. Mean marketable fruit totaled over nine weeks as influenced by carbonation, +CO2 (-----) and -CO2 (——), over soil type, calcareous soil (CAL) and noncalcareous soil (NCA).

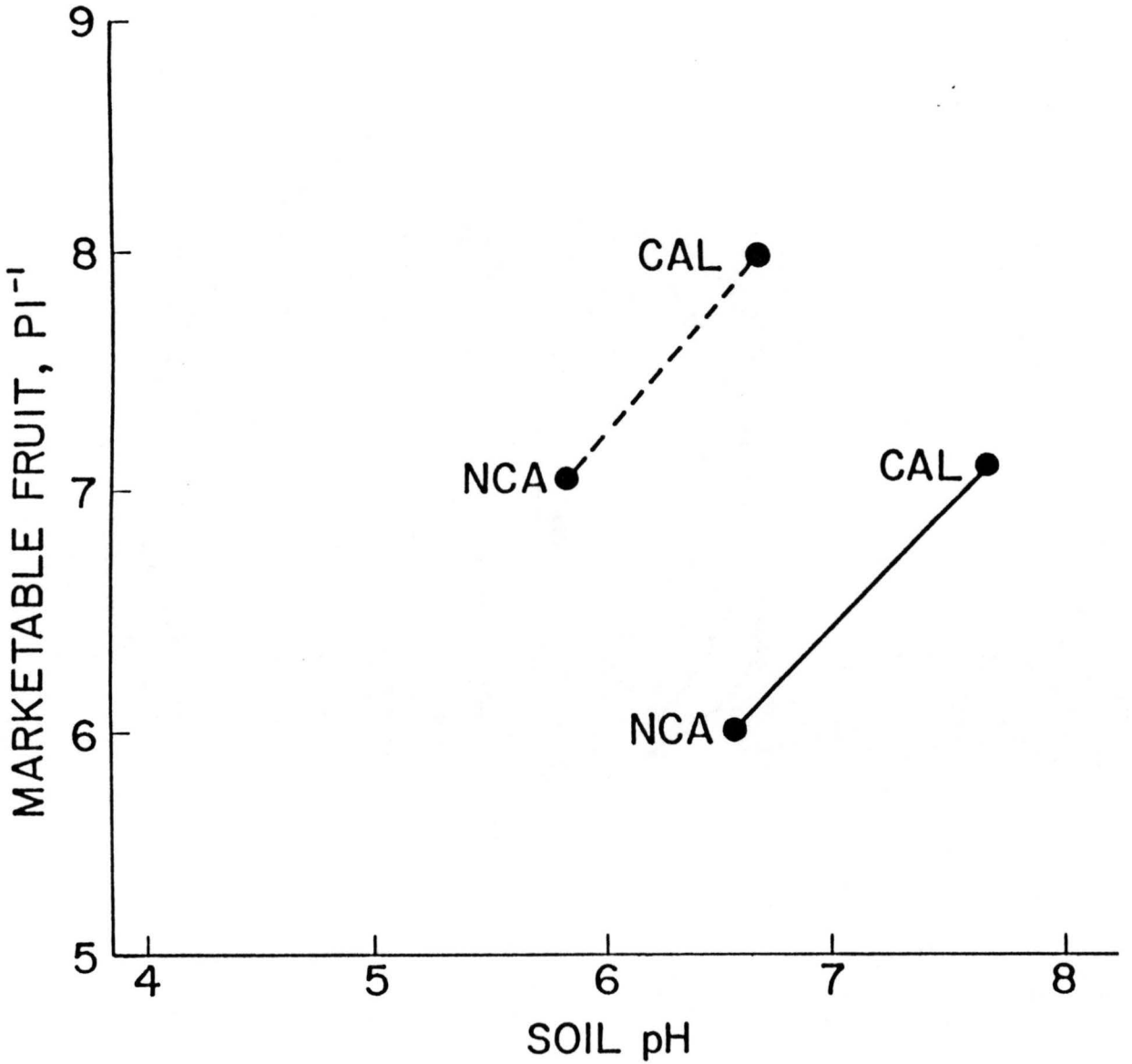

MARKETABLE FRUIT, PI⁻¹
9
8
7
6
5
4
5
6
7
8
SOIL pH
CAL
NCA
CAL
NCA

Table 5. Influence of noncalcareous, calcareous soil and noncarbonated, carbonated water treatments on total fruit fresh and dry weights over nine weekly observations and plant (including roots) and crown dry weights measured in grams.

Soil Type	Water Treatment	Fruit Fresh Weight #/plant	Fruit Dry Weight #/plant	Plant Dry Weight #/plant	Crown Dry Weight #/plant
Noncalcareous	Noncarbonated	39.71	4.33	11.96	1.38
	Carbonated	48.70	4.71	11.62	1.73
Calcareous	Noncarbonated	44.35	4.65	12.76	1.50
	Carbonated	48.84	4.41	12.20	1.71
Averages					
	Noncarbonated	42.03	4.49	12.36	1.44
	Carbonated	48.08	4.41	11.91	1.72*
Noncalcareous		44.20	4.52	11.79	1.55
Calcareous		46.60	4.53	12.48	1.61

ANOVA for Fruit Fresh and Dry Weight

Source	df
Rep	19
H_2O pH (A)	1
Soil	1
A x B	1
Error	9

*Significant at $P<0.05$

The total number of leaves produced over the nine weeks showed no significant differences among treatments (Table 6). Both leaf area and leaf dry weight, however, were significantly greater for the calcareous soil as compared to the noncalcareous soil. The leaf area mean was 159 cm^2 for the calcareous soil as compared to a mean of 115 cm^2 for the noncalcareous soil. Similarly, leaf dry weight was greater for the calcareous soil treatment (1.36 g) when compared to the noncalcareous soil treatment (1.01 g). Although there was a tendency for greater area and leaf dry weight with the use of carbonated water, it was not significant.

Analysis of chlorophyll indicated significant differences for only irrigation treatments (Table 6). The carbonated water treatment mean was 1.39 mg/g verses 0.97 mg/g for the noncarbonated water treatment. There were no significant differences between soils.

Zinc uptake was significantly less ($P<0.05$) in the calcareous soil as compared to the noncalcareous soil (Table 7). The calcareous soil had a mean of 23 μg/g as compared to 33 μg/g for the noncalcareous soil. Manganese, iron and copper showed no differences among treatments. The carbonated water, calcareous soil treatment had a slight increase of Zn (24 μg/g), as compared to the noncarbonated water, calcareous soil (22 μg/g). A similar tendency was observed for Fe and Mn. However, manganese and copper had a tendency ($P<0.10$) to accumulate in higher levels in the noncalcareous soil as compared to the calcareous soil.

There were two leaf collection dates used in the starch analysis, May 21 and June 4, 1993. Starch accumulation showed no significant differences between irrigation treatments and soil types (Table 8). There was a significant difference between morning and evening collection times (Appendix, Tables 29 and 32).

Table 6. Influence of noncalcareous, calcareous soil and noncarbonated, carbonated water treatments on leaf area measured in cm^2, leaf dry weight measured in g, average leaf number per plant over three tri-weekly observations and leaf chlorophyll measured in mg/g.

Soil Type	Water Treatment	Leaf Area	Leaf Dry Wt.	Leaf #/plant	Leaf Chlorophyll
Noncalcareous	Noncarbonated	109	9.60	6	1.02
	Carbonated	120	1.05	7	1.24
Calcareous	Noncarbonated	155	1.25	7	0.93
	Carbonated	164	1.47	7	1.54
Averages					
	Noncarbonated	132	1.11	7	0.97
	Carbonated	142	1.26	7	1.39*
Noncalcareous		115	1.01	7	1.13
Calcareous		159*	1.36*	7	1.23

ANOVA for leaf area, leaf dry weight and leaf chlorophyll

Source	df
Rep	3
H_2O pH (A)	1
Soil (B)	1
A x B	1
Error	9

ANOVA for leaves per plant

Rep	3
H_2O pH (A)	1
Soil (B)	1
A x B	1
Error	9

*Significant at $P<0.05$.

Table 7. Micronutrients present in leaf tissue as a function of soil type and carbonation.

Soil Type	Zn μg/g	Fe μg/g	Mn μg/g	Cu μg/g
Noncalcareous				
Noncarbonated	33	145	490	8.0
Carbonated	33	184	500	7.5
Calcareous				
Noncarbonated	22	85	320	7.1
Carbonated	24	133	430	7.4
Averages				
Noncalcareous	33*	164	495	8.0
Calcareous	23	109	375	7.3

ANOVA

Source	df
Rep	2
H_2O pH (A)	1
Soil (B)	1
A x B	1
Time (C)	
am, pm	1
AC	1
BC	1
ABC	1
Error	14

*Significantly different at $P<0.05$.

Table 8. Leaf starch content in 10^{-3} mg/g for morning (am) and evening (pm) on May 21 and June 4, 1993.

Soil Type	Water Treatment	May 21 am	May 21 pm	June 4 am	June 4 pm
Noncalcareous	Noncarbonated	0.14	4.60	0.67	9.81
	Carbonated	0.16	6.70	0.31	7.87
Calcareous					
	Noncarbonated	0.11	5.70	0.37	8.55
	Carbonated	0.16	5.30	1.54	9.15
Averages					
	Noncarbonated	0.15	5.70	0.49	8.84
	Carbonated	0.13	5.50	0.95	8.85
Noncalcareous		0.12	5.20	0.52	9.18
Calcareous		0.16	6.00	0.92	8.51

ANOVA	May 21	June 4
Source	df	df
Rep	2	5
H_2O pH (A)	1	1
Soil (B)	1	1
A x B	1	1
Error	6	15

DISCUSSION

Carbonic acid, resulting from carbonation, caused a substantial reduction in pH both calcareous soil and noncalcareous soils as compared to noncarbonated water irrigation. This reduction in pH was substantial but lasted for less than 24 hours. Although this did not result in significant differences in uptake of micronutrients, under growing conditions in the greenhouse, there was a tendency for greater uptake of Zn in the calcareous soil with carbonated water, 24 μg/g, as compared to calcareous soil with noncarbonated water, 22 μg/g (Table 7). There may be significantly greater uptake of water in field conditions with more frequent irrigation associated with drying due to wind and greater light intensity. A slight increase in uptake of Zn in tomatoes was reported by Novero et al., (1991). It seems possible that similar results might occur with strawberry grown in the field.

Application of carbonated water did significantly increase CO_2 in the soil as well as 1 cm above the soil surface. It was not significant at the 20 cm level which would likely be associated with ambient air mixing with the elevated CO_2 in the plant canopy. At the time of growing in the greenhouse, there was relatively high solar irradiance which resulted in increased temperatures in the greenhouse. This required frequent ventilation, with fans which resulted in air mixing and an equalization of CO_2 levels in the ambient air.

However, elevated CO_2 beneath the canopy (1 cm level), due to carbonation, did result in a significant increase in some growth factors.

The soil air for the tap water application showed a significantly higher level of CO_2 than aerial samples as expected. Since carbonated water is a weak acid, it reacts with $CaCO_3$ to release CO_2 into the soil atmosphere. The high CO_2 content of the soil air in the acidified water treatment also supports this conclusion (Table 3). Additionally, the pots were mulched and CO_2 from decaying plant tissue would collect under mulched soils (Moore, 1990; Cahn, 1989).

The growing media had less buffering capacity than normal field soil due in part to the 2:1 perlite to soil ratio in the pot. Therefore, the low pH of the acidified water had a detrimental effect over the course of the experiment.

The leaf of the plant is the source of photosynthates generated while the meristem or actively growing point represents the sink where the photosynthates accumulate (Salisbury and Ross, 1985). In the case of strawberry, as flower buds begin to develop they take precedence over leaf production (Kumakura and Shishido, 1994; Salisbury and Ross, 1985). The early flower bud development exhibited in carbonated water treatment, over both soil types, was similar to the growth chamber study preformed by Cahn with strawberry, in 1989. Theoretically, additional CO_2 through the use of carbonated water would then be available to fix more sugars thus creating more energy to drive the development of buds, open flowers and fruit. However,

earlier open flower development was observed only in the carbonated water irrigation in calcareous soil treatment.

In a recent paper, Kumakura and Shishido, 1994, determined that C^{-14} in strawberry was translocated in greater amounts to the terminal inflorescence as they grew and matured to develop fruit. The amount of labelled carbon present in the flower and fruit increased from 25% at anthesis to 60-80% in the developing fruit. This could in part explain the observation in the current study that more buds, open flowers, marketable fruit and higher crown dry weight were found in the carbonated water treatment while leaf number, leaf area and dry weight were not influenced by carbonated water with increased CO_2 level.

The significant increase in bud and open flower development in association with carbonated water irrigation is similar to previous studies. Aerial CO_2 enrichment significantly increased the number of carnation flowers (Nelson, 1978) while the total number of cucumber flowers doubled in a study by Enoch et al., (1976). In the same study using strawberries, Enoch et al. (1976) found an increase in vegetative and reproductive growth. An increase in the number of flowering stems verses blind shoots in rose was reported when plants were grown under increased aerial CO_2 enrichment by Moe (1986).

The increase in crown dry weight was probably associated with greater availability of CO_2 efflux from the degassing process. Cahn (1989) reported that strawberries grown in a growth chamber study in 600 and 900 $\mu L\ L^{-1}$ of

aerial CO_2 had an increase in crown dry weight. This, however, may not have effected fruit production in this experiment. In a previous study by Strik as reported in Cahn (1989), greater crown weight was observed to influence the potential for subsequent yields since greater crown dry weight could lead to higher fruit yields.

The significantly greater leaf area and leaf dry weight observed in the calcareous soil may have been related to the high calcium content of the soil. Calcium (Ca) is known to be continually taken up by young roots before they are suberized and adsorption is blocked (Mengel and Kirkby, 1987). It has an important role in both stabilizing membranes and maintaining membrane integrity which are important in developing leaves and fruit. Thus, the presence of high Ca levels in the calcareous soil would enable greater uptake of Ca and potentially increased leaf area and dry weight.

The significantly increased chlorophyll content of carbonated water treatment supports the idea that high aerial CO_2 levels support a more robust photosynthesis apparatus (Enoch and Zieslin, 1988; Enoch and Olesen, 1993) as well.

Zinc was the only element to show significant differences between soil treatments. Zinc is usually bound by calcareous soils at high pH and therefore is not as readily available for uptake (Mengel and Kirkby, 1987). The carbonation evidently reduced the pH sufficiently to allow some zinc to become available for uptake. The plants grown in calcareous soil had significantly less Zn uptake than noncalcareous soil (Table 3). This was likely due to lower Zn

levels in the calcareous initially as well as the high pH which limited availability (Table 2). Manganese, iron and copper were available in adequate amounts in both soils, therefore differences in uptake were not noted.

During irrigation with carbonated water, both soils were observed to foam or "puff" up. The calcareous soil appeared to be affected more than the noncalcareous soil. When a test for lime content of soils is preformed an acid is added to the soil to evaluate if bubbles form. The bubbles are CO_2 released when the acid reacts with the $CaCO_3$ (Mc Lean, 1982). It seems likely that the foam or "puffing up" was the CO_2 escaping form the soil.

Nutrient deficiencies became apparent during the sixth week of irrigation treatments. These were exhibited as chlorosis of new leaves and purple markings. This likely indicated nitrogen, iron and phosphorus deficiencies. In a recent study conducted on *Glycine max* L. by Roger et al. (1992), an increase in total N uptake per plant was observed with increased aerial CO_2 (35.5 and 63.0 mg N for 350 and 700 *u*mol mol^{-1} of CO_2, respectively). Although the first deficiencies to become apparent were attributed to iron, the older leaves began to turn a lighter shade of green, indicating a reduction in available nitrogen. This lack of sufficient nutrient may have contributed to the non-significant differences in marketable fruit, fruit fresh and fruit dry weights.

The plants used were a day-neutral cultivar which has the ability to flower and fruit more or less continuously throughout the growing season. There was a marked decrease in flowering as deficiency symptoms became evident. If a slow release fertilizer had been applied, the flowering might have

continued. This may have provided sufficient nitrogen and phosphorus to maximize the potential for photosynthesis. A slow release fertilizer may have also overcome the leaching caused by irrigation and mining of the nutrients by plants as leaves and flowers begin to develop.

The size of the pot used in this study, #1 plastic nursery container, probably had little bearing on the results of this study. The limiting factor was most likely the lack of available nutrients, since this study lasted longer than a few weeks. This is supported by a study conducted by McConnaughay et al., 1993, in which they found that pot size was not a limiting factor but nutrient availability was. *Abutilon theophrasti* and *Setaria faberii* were grown in different pot sizes and different nutrient concentrations. They found that regardless of pot size, there were significant increases in root, stem, leaf and fruit mass for both genera when total N concentration and volume of nutrients were doubled.

The lack of available nutrition may have also contributed to the nonsignificant difference between irrigation treatments and soil types in regard to leaf starch accumulation. The active accumulation and respiration of starch may need a substantial amount of N to form enzymes which facilitate the conversion of glucose to starch and use of starch in the Calvin cycle.

SUMMARY AND CONCLUSIONS

Carbonated water reduced the tap water pH from 6.7 to 4.0. and it also increased the level of CO_2 in both calcareous and noncalcareous soils. Carbonated water generated additional CO_2 from the $CaCO_3$ in the calcareous soil. There was a significant efflux of carbon dioxide during the degassing of carbonated water which resulted in increased levels of aerial CO_2 at 1 cm within the plant canopy.

There was a tendency in the calcareous soil treated with carbonated water to accumulate zinc as compared to the calcareous soil, noncarbonated water treatment although it was not significant. The pH may not have remained low enough for a long enough period of time to facilitate adequate uptake. Also, this shows that N may have been limiting before Zn.

Carbonated water irrigation of the calcareous soil appeared to convey earliness with regard to buds and open flowers. The lack of available nutrients as indicated by chlorosis while the plants were fruiting could have contributed to the non-significant marketable fruit yields, fresh fruit and dry fruit weight. There was a trend towards increased marketable fruit, which with better nutrition could have lead to increased numbers of marketable fruit with the carbonated water treatment. The higher number of buds, open flowers and the

crown dry weight for the carbonated water irrigation could be a factor in higher fruit yield.

Subsequent experiments should address nutrients as a factor limiting response in pot studies when irrigating with carbonated water. A slow release or liquid fertilizer could be used in a long term study using day-neutral strawberry plants in the greenhouse. Also, the acidified water pH should be adjusted to a level closer to the pH of the carbonated water.

Evidence reported here indicates the benifit of optimizing soil pH through the use of carbonated water. The question of direct enhancement of photosynthesis using carbonated water remains unanswered.

LITERATURE CITED

Arienzo, M., G. Basile, R. d'Andria, V. Magliulo and A. Maggio. 1993. Fertilization via carbonated water and mineral concentration in tomato crop. Communications in Soil Sci. & Plant Anal. 24:2281-2291.

Arteca, R.N. and B.W. Poovaiah. 1982. Absorption of $H_2{}^{-14}CO_2$ by potato roots and its subsequent translocation. J. Amer. Soc. Hort. Sci. 107:398-401.

Arteca, R.N., B.W. Poovaiah, and O.E. Smith. 1979. Changes in carbon fixation, tuberization and growth induced by CO_2 application to the root zones of potato plants. Science 205:1278-1280.

Baron, J.J., and Gorski, S.F., 1986. Response of eggplant to a root environment enriched with CO2. HortSci. 21:495-498.

Berdi, A.K., Walare, A., and Rhoads W.A. 1960. Assimilation of bicarbonate by different plant species. Soil Sci. 89:257-263.

Boodley, J.W. 1981. The Commercial Greenhouse. Delmar Publishing Inc., Albany, N.Y.

Burinsma, J. 1963. The quantitative analysis of chlorophylls a and b in plant extracts. Photochem. and Photobiol. (Chlor. Methabol. Sym.). Vol 2 Pergamon Press Ltd., Great Britian. p. 241-249.

Cahn, H. K. 1989. Carbon Dioxide Profiles in the Field and Response to Enriched Atmospheres Using Strawberry. M.S. Thesis. Colorado State University, Fort Collins. 179 p.

Clegg, M. D., C. Y. Sullivan, and J. D. Eastin. 1978. A sensitive technique for the rapid measurement of carbon dioxide concentration. Plant Phys. 62:924-926.

Cummings, M. B. and C. H. Jones. 1918. The aerial fertilization of plants with carbon dioxide. Vermont Agric. Exp. Stn. Bull. 211. 56 p.

Darrow, G. M. 1966. The Strawberry, History, Breeding and Physiology. Holt Publ. Co., New York

Enoch, H. Z. and B. A. Kimball, (ed.). 1986. Carbon Dioxide Enrichment of Greenhouse Crops. Vol. 1. CRC Press, Inc., Boca Raton, Florida, p.25-27.

Enoch H. Z. and J. M. Olesen. 1993 Tansely Review No. 54. Plant Response to irrigation with water enriched with carbon dioxide. New Phyto. 125:249-258.

Enoch, H. Z., I. Rylski and M. Spiegelma. 1976. CO_2 enrichment of strawberry and cucumber plants grown in unheated greenhouses in Israel. Sci Hort. 5:33-41.

Enoch, H. Z. and N. Zieslin. 1988. Growth and development of plants in response to carbon dioxide concentrations. Applied Agricultural Research 3:248-256.

Fletcher, S. W. 1917. The Strawberry in North America History, Origin, Botany and Breeding. MacMillian Publ. Co., New York.

Geisler, G., 1963. Morphogenic influence of ($CO_2 + HCO^{3-}$) on roots. Plant Phys. 38:77-80.

Hanan, J. J., W. D. Holly and K. L. Goldsberry. 1978. Greenhouse Management. Springer-Verlag, New York. p. 326.

Hartz, T. K. and D. B. Holt. 1991. Root-zone carbon dioxide enrichment in field does not improve tomato or cucumber yield. HortSci. 26:1423.

International Standardization of Fruit and Vegetables. Strawberries. 1979. Organization for Economic Co-operation and Development. Paris

Jones, J.B. and V. W. Case. 1990. Sampling and handling and analyzing plant tissue samples. p. 389-427. *In* R. L. Westermen (ed.). Soil Test and Plant Analysis. 3rd ed. SSSA Book Series 3. SSSA Madison, WI.

Kumakura, H. and Shishido, Y. 1994. Effects of diurnal temperatures and leaf position on the translocation and distribution of C-14-photosyntates in fruiting strawberry cv Morioka-16. J of Jap. Soc. for Hort. Sci. 62:833-838.

Lindsay, W. L. 1979. Chemical Equilibrium in Soils. John Wiley. Inc., New York.

Livingston, B.E. and Ruth Beal. 1934. The soil as a direct source of carbon dioxide. Plant Phys. 9:237-259.

Mauney, J. R. and D. L. Hendrix. 1988. Responses of glasshouse grown cotton to irrigation with carbon dioxide saturated water. Crop Sci. 28:835-838.

McConnaughay, K. D. M., G. M. Berntson and F. A. Bazzaz. 1993. Limitations of CO_2-induced growth enhancement in pot studies. Oecologia 94:550-557.

McLean, E. O. 1982. Soil pH and lime requirement. *in* Methods of Soil Analysis. A. L. Page (ed.). Part 2. Chemical and Microbial Properties 2nd edition. SSSA Madison, WI.

Mengel K. and E. A. Kirkby. 1987. Principals of Plant Nutrition. International Potash Institute, Publ. Switzerland.

Moe, R. 1986. CO2 Enrichment In Norway. *In* H. Z. Enoch and B. A. Kimball (ed.) Carbon Dioxide Enrichment of Greenhouse Crops. Vol. 1. CRC Press, Inc., Boca Raton, Florida, p. 67.

Moore, F. D. 1990. Potential for irrigation with carbon dioxide. p. 171-178. *In* A. Alvino (ed.) Symposium on Scheduling of Irrigation for Vegetable Crops under Field Conditions. Acta Hortic. Vol. 1. No. 278.

Murphy, L. S. 1982. Plant-Soil-Fertility Relationships. *in* The Fertilizer Handbook. White, W. C. and D. N. Collins (eds.) The Fertilizer Institute, Washington D. C., p. 111-136.

Nakayama, F.S., and Bucks D.A. 1980. Using subsurface trickle system for carbon dioxide enrichment. p.13-18. *In* N. T. Oebker (ed.) Proc. 15th Nat. Agri. Plastics Conf. Tucson, AZ. 13-17 April. Nat. Agri. Plastics Assoc., Manchester, MO.

Nelson, P. V. 1978. Greenhouse Operation and Management. Reston Publishing Co. Inc., Reston Virginia. p. 286.

Novero, R. 1991. Yield Response of Field Grown Tomatoes Irrigated with Carbonated Water. M.S. Thesis. Colorado State University, Fort Collins. 80 p.

Novero, R., D. H. Smith, F. D. Moore, J. R. Shanahan, and R. d'Andria. 1991. Field-grown tomato response to carbonated water application. Agron J. 83:911-916.

Porter, M. A. and B. Grodzinski. 1984. Acclimation to high CO_2 in bean. Carbonic anhydrase and ribulose bisphoshate carboxylase. Plant Phys. 74:413-416.

Porter, M. A. and B. Grodzinski. 1985. CO_2 enrichment of protected crops. *in* Horticultural Reviews, Vol. 7. J. Janick (ed.). AVI Publ. Co., Inc. Westport, Conn.

Rogers, H. H., C. M. Peterson, J. N. McCrimmom and J. D. Cure. 1992. Response of plant roots to elevated atmospheric carbon dioxide. Plant, Cell and Enc. 15:749-752.

Salisbury, F. B. and C. W. Ross. 1985. Plant Physiology. Wadsworth Publ. Co. Belmont, California.

Schafer, W. 1988. Pflanzenwachstum durch CO_2/HCO^{3-} Enting uber die Wurzel. (English abstract) J. Agron. Crop Sci. 160:228-234.

Van Berkel, N. 1986. CO2 Enrichment in the Netherlands. *In* H. Z. Enoch and B. A. Kimball (ed.) Carbon Dioxide Enrichment of Greenhouse Crops.. Vol. 1. CRC Press, Inc., Boca Raton, Florida, p. 25-27.

Wilhelm, S. and J. Sagen. 1974. A History of the Strawberry. University of Calif. Berkley, California.

Zornbach, VONW Schickedanz F. 1993. *Der Einfluss von CO_2 impregniertem Giesswasser auf verschiedene Mikosen an Zierpflanzen.In* Enoch H. Z. and J. M. Olesen. Tansely Review No. 54. Plant response to irrigation with water enriched with carbon dioxide. New Phyto., 125:249-258.

APPENDIX

Table 9. Total number of buds per plant over nine weekly observations.

Source	d.f.	EMS
Rep	19	20.52
H2O pH (A)	1	340.31*
Soil (B)	1	154.01*
AxB	1	52.81
Error	57	25.48

Table 10. Total number of open flowers per plant over nine weekly observations.

Source	d.f.	EMS
Rep	19	10.17
H2O pH (A)	1	125.00*
Soil (B)	1	151.25*
AxB	1	42.05
Error	57	10.78

Table 11. Total marketable fruit over nine weeks.

Source	d.f.	EMS
Rep	19	9.62
H2O pH (A)	1	31.25
Soil (B)	1	16.20
AxB	1	0.20
Error	57	9.66

*Significant difference at the $P<0.05$ level.

Table 12. Fruit fresh weight (g) totaled over nine weeks.

Source	d.f.	EMS
Rep	19	479.81
H2O pH (A)	1	909.36
Soil (B)	1	114.53
AxB	1	101.43
Error	57	368.60

Table 13. Fruit dry weight (g) totaled over nine weeks.

Source	d.f.	EMS
Rep	19	5.190
H2O pH (A)	1	0.092
Soil (B)	1	0.002
AxB	1	1.970
Error	57	3.740

Table 14. Leaf area measured in cm^2.

Source	d.f.	EMS
Rep	3	3429.57
H2O pH (A)	1	392.24
Soil (B)	1	7964.67*
AxB	1	2.32
Error	9	837.06

*Significant difference at the $P<0.05$ level.

Table 15. Leaf dry weight measured in grams.

Source	d.f.	EMS
Rep	3	0.10
H2O pH (A)	1	0.09
Soil (B)	1	0.50*
AxB	1	0.02
Error	9	0.05

Table 16. Leaf per plant over nine weeks.

Source	d.f.	EMS
Rep	19	3.17
H2O pH (A)	1	3.20
Soil (B)	1	6.05
AxB	1	8.45
Error	57	6.49

Table 17. Leaf chlorophyll in mg/g.

Source	d.f.	EMS
Rep	3	0.06
H2O pH (A)	1	0.69*
Soil (B)	1	0.04
AxB	2	0.16
Error	9	0.04

*Significant difference at the $P<0.05$ level.

Table 18. Crown dry weight in g.

Source	d.f.	EMS
Rep	19	0.27
H2O	1	1.56*
Soil	1	0.64
AxB	1	0.11
Error	57	0.23

Table 19. Plant dry weight in g.

Source	d.f	EMS
Rep	19	122.68
H2O pH (A)	1	3.99
soil (B)	1	9.48
AxB	1	0.23
Error	57	114.09

Table 20. Specific leaf area in cm^2/g.

Source	d.f.	EMS
Rep	3	242.65
H2O pH (A)	1	285.69
Soil (B)	1	74.43
AxB	1	130.82
Error	9	195.28

*Significant difference at the $P<0.05$ level.

Table 21. Specific leaf weight in 10^{-2} g/cm^2.

Source	d.f.	EMS
Rep	3	2.95
H2O pH (A)	1	3.67
Soil (B)	1	3.23
AxB	1	3.69
Error	9	3.14

Table 22. Zinc concentration of leaf tissue in ppm.

Source	d.f.	EMS
Rep	2	0.065
H2O pH (A)	1	0.027
Soil (B)	1	0.214*
AxB	1	0.009
Time (C)	1	0.000
AC	1	0.003
BC	1	0.000
ABC	1	0.000
Error	14	0.022

Table 23. Iron concentration of leaf tissue in ppm.

Source	d.f.	EMS
Rep	2	0.69
H2O pH (A)	1	4.36
Soil (B)	1	7.55
AxB	1	0.03
Time (C)	1	7.15
AC	1	0.23
BC	1	0.47
ABC	1	3.81
Error	14	2.71

*Significant difference at the $P<0.05$ level.

Table 24. Manganese concentration of leaf tissue in ppm.

Source	d.f.	EMS
Rep	2	9.65
H2O pH (A)	1	8.56
Soil (B)	1	34.83
AxB	1	5.99
Time (C)	1	1.11
AC	1	13.04
BC	1	7.49
ABC	1	2.58
Error	14	9.12

Table 25. Copper concentration of leaf tissue in ppm.

Source	d.f.	EMS
Rep	2	0.002
H2O pH (A)	1	0.001
Soil (B)	1	0.004
AxB	1	0.006
Time (C)	1	0.000
AC	1	0.002
BC	1	0.003
ABC	1	0.001
Error	14	0.002

Table 26. Soil pH in response to irrigation treatments.

Source	d.f.	EMS
Rep	2	0.41
H2O pH (A)	1	10.81*
Soil (B)	1	14.85*
AxB	1	0.31
Time (C)	5	0.53*
AC	5	0.23
BC	5	0.06*
ABC	5	0.16*
Error	46	0.12

Table 27 Morning starch accumulation in 10^{-3} mg/g for May 21, 1993.

Source	d.f.	EMS
Rep	2	0.009
H2O pH (A)	1	0.001
Soil (B)	1	0.004
AxB	1	0.001
Error	11	0.006

Table 28. Evening starch accumulation in 10^{-3} mg/g for May 21, 1993.

Source	d.f.	EMS
Rep	2	9.67
H2O pH (A)	1	0.09
Soil (B)	1	2.24
AxB	1	4.76
Error	11	1.69

*Significant difference at the $P<0.05$ level.

Table 29. Starch accumulation in 10^{-3} mg/g for May 21, morning and evening.

Source	d.f.	EMS
Rep	2	5.01
H2O pH (A)	1	1.21
Soil (B)	1	0.06
AB	1	2.33
Time (C) am & pm	1	177.79*
AC	1	1.03
BC	1	0.04
ABC	1	2.43
Error	14	2.35

Table 30. Morning starch accumulation in 10^{-3} mg/g for June 4, 1993.

Source	d.f.	EMS
Rep	5	2.07
H2O pH (A)	1	1.24
Soil (B)	1	0.98
AxB	1	3.49
Error	15	2.35

Table 31. Evening starch accumulation in 10^{-3} mg/g for June 4, 1993.

Source	d.f.	EMS
Rep	5	12.53
H2O pH (A)	1	0.00
Soil (B)	1	2.68
AxB	1	9.75
Error	15	7.35

*Significant difference at the $P<0.05$ level.

Table 32. Starch accumulation in 10^{-3} mg/g for June 4, morning and evening.

Source	d.f.	EMS
Rep	5	4.75
H2O pH (A)	1	0.65
Soil (B)	1	0.21
AB	1	12.46
Time (C) am & pm	1	792.55*
AC	1	0.60
BC	1	3.45
ABC	1	0.79
Error	35	5.57

Table 33. Soil air CO_2 (ppm) 7 hours after irrigation treatments.

Source	d.f.	EMS
Rep	5	32630.60
H2O pH (A)	2	11905151.31*
Soil (B)	1	16365087.77*
AB	2	3910914.13*
Time (C)	5	256062.12*
AC	10	66315.78
BC	5	87694.11
ABC	10	145204.67*
Error	175	55860.85

*Significant difference at the $P<0.05$ level.

Table 34. Soil air CO_2 (ppm) 48 hours after irrigation treatments.

Source	d.f.	EMS
Rep	5	7489.80
H2O pH (A)	2	479150.61*
Soil (B)	1	1487957.64*
AxB	2	443463.74*
Time (C)	5	2492859.61*
AC	10	346160.86*
BC	5	452035.25*
ABC	10	105993.41*
Error	175	14617.33

Table 35. Aerial CO_2 (ppm) 1/2 hour after irrigation treatments.

Source	d.f.	EMS
Rep	5	10805.99
H2O pH (A)	2	3985692.28*
Soil (B)	1	701714.13*
AB	2	389273.61*
Time (C)	5	339480.38*
AC	10	261101.21*
BC	5	71682.77*
ABC	10	90987.95*
Position (D)	1	7175437.82*
AD	2	3809648.01*
BD	1	581653.18*
ABD	2	434943.78*
CD	5	192324.34*
ACD	10	246763.08*
BCD	5	96562.71*
ABCD	10	82609.38*
Error	355	11623.47

*Significant difference at the $P<0.05$ level.

Table 36. Aerial CO_2 (ppm) 24 hours after irrigation treatments.

Source	d.f.	EMS
Rep	5	1897.99
H2O pH (A)	2	26860.73*
Soil (B)	1	22072.90*
AB	2	87.64*
Time (C)	5	43413.76*
AC	10	4193.93*
BC	5	714.53
ABC	10	1642.30
Position (D)	1	59360.87*
AD	2	32723.87*
BD	1	29153.72*
ABD	2	5087.97*
CD	5	8121.50*
ACD	10	2922.66*
BCD	5	3400.12*
ABCD	10	758.32
Error	355	1421.16

*Significant difference at the $P<0.05$ level.

Table 37. Aerial CO_2 (ppm) 48 hours after irrigation treatments

Source	d.f.	EMS
Rep	5	558.35
H2OpH (A)	2	14895.09*
Soil (B)	1	15101.71*
AB	2	3159.24
Time (C)	5	10483.91*
AC	10	2437.15*
BC	5	4164.07*
ABC	10	1824.68
Position (D)	1	29812.29*
AD	2	11551.98*
BD	1	7306.28*
ABD	2	1878.72
CD	5	6295.72*
ACD	10	2350.29*
BCD	5	2150.46
ABCD	10	2250.12
Error	355	1144.71

*Significant difference at the $P<0.05$ level.