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Responses of Glasshouse Grown Cotton to Irrigation with Carbon Dioxide-Saturated Water

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ABSTRACT

Experiments were conducted to test the suitability of using irrigation water as a carrier for CO_2 to enhance growth and productivity of cotton (*Gossypium hirsutum* L.). Potted plants ('DPL 61') were irrigated daily with CO_2-saturated water in a greenhouse in Phoenix, AZ. Growth, productivity, and physiological responses of these plants were compared to those irrigated with deionized water. All plants were fertilized twice weekly with a modified Hoagland's solution. Yield as measured by boll load at maturity was increased 70 and 53% in two sets of experiments. Carbon dioxide exchange rate (CER) was increased 38%. Leaf chlorophyll content and starch content were also increased. None of the carbon in lint samples was derived from the CO_2 in the irrigation water. Leaf Zn and Mn were deficient in the control plants but were sufficient in the plants watered with CO_2-saturated water. The CO_2-saturated irrigation water increased uptake of Zn and Mn, which supported a more robust photosynthetic apparatus in the treated compared to the control plants. The greater photosynthetic activity during boll loading resulted in a significant increase in yield of the treated plants.

BEGINNING WITH THE OBSERVATIONS of Kursanov et al. (1951), there has been sporatic interest in using CO_2-enriched irrigation water as a means of stimulating crop growth. The results have varied from enhancement to inhibition of plant growth. Kursanov et al. (1951), Grinfield (1954), Berquist (1964), and Baron and Gorski (1986) reported that CO_2 was absorbed by root systems and that yields of several species were improved by the treatment. However, Stolwijk and Thimann (1957), Voznesenskii (1958), and Skok et al. (1962) indicated that less that 5% of the CO_2 required by a crop could be expected to be absorbed through the root system and that enhancement of yields by enriching irrigation water with CO_2 was very unlikely.

Several possible rationales have been advanced to explain the responses of those species whose growth and yield have been increased by CO_2-enriched irrigation. In addition to the obvious possibility that CO_2 may be carried in the transpiration stream to the leaves where it could enhance photosynthesis, Misra (1951) suggested that alteration of nutrient uptake from the soil might also be a source of enhanced growth. Increased root elongation has been frequently observed to result from elevated CO_2 concentration in soil or potted experiments (Glinski and Stepniewski, 1985). Increased root organic acid content as well as increased uptake of Ca and Mg were observed by Arteca et al. (1979) when they exposed potato (*Solanum tuberosum* L.) roots in solution culture to high CO_2.

Wittwer (1979) listed CO_2-saturated irrigation water as a possible delivery system for CO_2 fertilization of crops. Nakayama and Bucks (1980) experimentally tested this approach using buried trickle irrigation systems and observed 10%-20% increases in yield of cantaloupe (*Cucumis melo* L.), potato, and wheat (*Triticum aestivum* L.). The amount of CO_2 that can be carried in this manner (1.5 g L^{-1}) has a cost that might make the practice profitable if it resulted in significant yield increases.

Cotton is one of the most responsive crops to CO_2 enrichment (Kimball, 1983). For this reason it is a prime candidate for field enrichment studies. Preliminary to making gaseous or enriched irrigation releases, a greenhouse study to determine whether cotton responds positively or negatively to high CO_2 in the root zone seemed advisable. With this in mind two glasshouse studies were conducted in which cotton was watered daily with CO_2-saturated water. Growth, physiological responses, and final yield were measured.

MATERIALS AND METHODS

Cotton plants (DPL 61) were grown in 12-L black plastic buckets filled with a potting mix (1:1:1, v:v:v, sand:vermiculite:peat). Pots were arranged in rows 0.8 m apart in a well ventilated glasshouse. Each row consisted of 10 pots with two plants per pot. The pot diameter was 25 cm so that the planting density was approximately 8 plants m^{-2}. In Exp. 1, only 4 rows (two replications) were possible; in Exp. 2, six rows (three replications) were used. The carbonated water was applied to alternate rows of this arrangement so that any CO_2 escaping into the atmosphere would enhance growth of both control and treated rows. Sufficient water was applied daily to cause drainage from the perforations in the bottom of the buckets. The daily amount was increased as the plants grew and the evaporative demand increased. When the plants were near maturity the evaporative demand required that the pots be watered twice daily with a total of 3 L of solution, which resulted in about 0.5 L of drainage.

Twice each week the pots were watered with a modified Hoagland's solution (Guinn, 1974) containing 2 m*M* Ca $(NO_3)_2$, 1 m*M* $MgSO_4$, 1 m*M* KH_2PO_4, 5 m*M* KNO_3, 40 μM H_3BO_4, 10μM Mn SO_4, 1 μM $ZNSO_4$, 0.4μM Na_2MoO_4, 0.2μM $CuSO_4$ and 50 mg L^{-1} Geigy 138 sequestrene Fe [sodium ferric ethylene diamine di(O-hydroxyphenylacetate)]. The diluent for the nutrient solution was carbonated or deionized water as appropriate for the treatment. The carbonated water was produced by commercially available carbonators (Carbo Flow Inc., Phoenix, AZ, Exp. 1; Cornelius Co., Anoka, MN, Exp. 2). The CO_2 was supplied from a high-pressure bottle. The source of the gas was the by-product of petroleum refining.

Experiment I was planted on 12 June 1985 and harvested 19 Aug. 1985, at which time both the controls and treated plants were in cutout, or had ceased to produce blossoms. Experiment II was planted 1 Dec. 1985 and harvested 1 Apr. 1986, again when both treated and control plants had reached cutout. The shorter, cooler days of Exp. 2 reduced the growth rate so that a longer time was needed to reach cutout. At harvest, complete plant maps were made of each plant with the location of each boll identified. In Exp. 2, there were

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two intermediate destructive harvests of six plants (two from each row) from the treated and control groups on 15 Jan. and 15 Feb., 1986. In addition to the destructive harvests in Exp. 2, bolls were counted weekly. For this count all bolls 1 d after anthesis (DAA) or older were included.

Due to the lack of randomization and the *border effect* on growth of the outermost rows, the data of Exp. 2 were analyzed using a paired t test in which the two outer rows (of the six) were paired and the center two rows paired.

Carbon dioxide exchange rate (CER) of the leaves was measured using an ADC[1] model LDC-2 CO_2 analyzer (Analytical Development Co., Ltd., Hoddeson, England) with the Parkinson leaf chamber. This is an open leaf chamber system and the air flow through the chamber (400 mL min^{-1}) is sufficient to keep the leaf from heating. Mid-day solar flux was 1900 μmol m^{-2} sec^{-1}. Carbon dioxide content of the air was 340 μL L^{-1}.

Temperature of the glasshouse for Exp. 1 was controlled by evaporative cooling and daily maximum temperatures ranged between 32 ° and 38 °C. The glasshouse for Exp. 2 had chilled water cooling and the maximum temperature was controlled at a daily average of 25 °C during seedling growth and 30 °C during flowering and maturation.

Carbon dioxide content of the carbonated water was determined by the gravimetic procedure using $Ba(OH)_2$ to precipitate dissolved CO_2 as $BaCO_3$. The pH levels of the irrigation water, the nutrient solution, and the drainage from the pots after irrigation were measured once each week.

Elemental analyses of the leaf tissue was made by a commercial laboratory (IAC, Phoenix, AZ) using the standard procedures of the American Association of Official Chemists. A sample was taken from the composite dried leaf blade tissue from each row.

Chlorophyll was extracted from six leaf punches for each replication. The punches were deposited in cold 80% acetone and stored in the dark in a sealed test tube at −15 °C for 4 wk. At that time the solution was standardized for volume, the color density determined, and the chlorophyll content calculated (Arnon, 1949). Leaf starch was measured enzymatically (Kerr, et al. 1984) from punches taken from the same leaf at dawn and dusk. Results were expressed as the difference between these daily minimal (dawn) and maximal (dusk) values.

Analyses of lint samples from Exp. 1 for C isotope ratios were conducted as suggested by Enoch et al. (1984) to trace the origin of the C in the plant body. Carbon isotope analysis was conducted by the Radiocarbon Laboratory, University of Arizona. The samples were analyzed by mass spectrometry for $^{13}C/^{12}C$ ratio and by shielded counts of Beta emissions for $^{14}C/^{12}C$ ratios.

Data from Exp. 2 were analyzed for statistical significance using a paired t test. Because of pronounced border effects within the treatments, the replications were paired by considering the outermost rows (1 and 6) to be a pair. The remaining rows were paired accordingly (2 and 5; 3 and 4).

RESULTS AND DISCUSSION

The carbonated water was saturated with CO_2 at the time of irrigation and carried 1.5 to 1.8 g L^{-1}. This concentration was approximately 3×10^3 times greater than that in the control water. The half life of this concentration was about 24 h.

[1] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA, and does not imply its approval to the exclusion of other products that may be suitable.

At the time of first flower no differences in leaf area or dry weight were observed between the treated and control plants in either Exp. 1 or 2. However, at the time of full boll load (cutout) when final harvests were made, the plants irrigated with carbonated water had 1.70 and 1.53 times as many bolls as the controls from Exp. 1 and 2, respectively. A list of physiological parameters measured in these plants is shown in Table 1.

The preliminary nature of Exp. 1 and the lack of adequate replications did not allow statistical treatment of these data. However, the yield increase and greener appearance of the CO_2 treated plants was consistent with the observation of Exp. 2. The $^{14}C/^{12}C$ analyses of lint from Exp. 1 indicated that none of the carbon fixed by photosynthesis was derived from the carbonated water. Since CO_2 of fossil origin (which ours was) does not contain ^{14}C, we would expect that had CO_2 been carried by the transpiration stream to the leaves there would be a proportionate reduction in the $^{14}C/^{12}C$ ratio (Enoch, et al., 1984). Since there was none, the yield increase was evidently due to other effects of the carbonated water.

Measurement of CER, diurnal starch differential, and chlorophyll content indicated that the leaves in Exp. 2 were more photosynthetically active during the fruit set and boll filling stages. The boll load present on the plants as measured by the weekly boll counts is shown in Fig. 1. The treated plants did not begin to diverge from the control until 90 d after planting (DAP). At that time the controls began retaining fewer bolls and proceeded into cutout. The treated plants continued to retain bolls and set new flowering sites for a longer time. This resulted in additional plant weight, leaf area, and yield by the treated rows.

An explanation of the more active photosynthetic mechanism may be in the analysis for mineral content of the leaf tissue (Table 2). Though total P was statistically lower in the treated leaves, their contents were

Table 1. Characteristics of cotton plants irrigated with carbonated water (T) and deionized water (C).

	Plant age in days, DAP	T	C	Ratio T/C
	Exp. 1			
Boll no., plant^{-1}	70	7.0	4.1	1.71
Leaf area, m^2 plant^{-1}	70	0.56	0.56	1.00
Boll dry wt., g plant^{-1}	70	22.0	11.0	2.00
Total dry wt., g plant^{-1}	70	87.4	75.8	1.15
$^{14}C/^{12}C$, % modern	70	120	122	0.98
	Exp. 2			
Boll number, plant^{-1}	121	17.3*	11.3	1.53
Leaf area, m^2 plant^{-1}	121	0.86*	0.52	1.65
Boll dry wt., g plant^{-1}	121	59.0*	37.5	1.57
Total dry wt., g plant^{-1}	121	282.9*	125.5	1.61
CER, μmol m^{-2} s^{-1}	50	19	20	0.94
CER, μmol m^{-2} s^{-1}	110	25*	18	1.38
Specific leaf weight, mg cm^{-2}	75	3.2	3.4	0.94
	121	4.6	5.4	0.85
Leaf diurnal starch change, %	120	40.7*	26.8	1.52
Leaf chlorophyll, mg dm^{-2}	120	5.0*	3.4	1.47
pH of Irrigation water		3.6*	4.3	
pH of Nutrient solution		4.4*	6.5	
pH of Pot drainage		7.4	7.0	

* Statistically different from plants watered with deionized water (C) ($P < 0.05$) as determined by a paired t test.

Table 2. Concentration of minerals in leaf blade tissue of cotton at harvest (121 DAP) of Exp. 2. Pots were watered twice weekly with modified Hoagland's solution and on other days with deionized (C) or carbonated water (T).

	Mineral element										
	N	P	K	S	Ca	Mg	Zn	Mn	Fe	Cu	B
	mg g^{-1}						$\mu g\ g^{-1}$				
Control, C	33	7.8	45	11	35	7.3	17	13	62	7.6	216
Treated, T	32	5.5*	40	11	36	7.2	28*	54*	62	8.4	221

* Statistically different from the control plants ($P < 0.05$) as determined by the paired t test.

within the acceptable guidelines for adequate nutrition of this crop (Joham, 1986). Only Zn and Mn in the control leaves were in the deficient range. These data indicate that the carbonated water irrigation made Zn and Mn more easily available for uptake, possibly due to the shift of pH in the carbonated water (Table 1). The well established involvement of Mn as a cofactor in the photosynthetic splitting of water and its involvement with the biosynthesis of chlorophyll suggest that the difference in Mn content enabled the leaves to retain a more active CER, and thereby delay cutout until a greater boll load was set.

Microscopic examination of cross sections of leaf tissue from Exp. 2 showed that there was no change in number of cells or chloroplasts in the treated plants. Staining for starch showed that the starch content of the chloroplasts at dusk was greater in the treated than untreated plants (Table 1).

These data demonstrate once again the positive correlation between CER and productivity of the cotton plant. Mauney et al. (1978) showed that a 17% increase in CER over the life of the crop would increase leaf area and more than double the harvested yield. Kimball (1983) lists cotton as one of the most responsive species to CO_2 enrichment of the atmosphere.

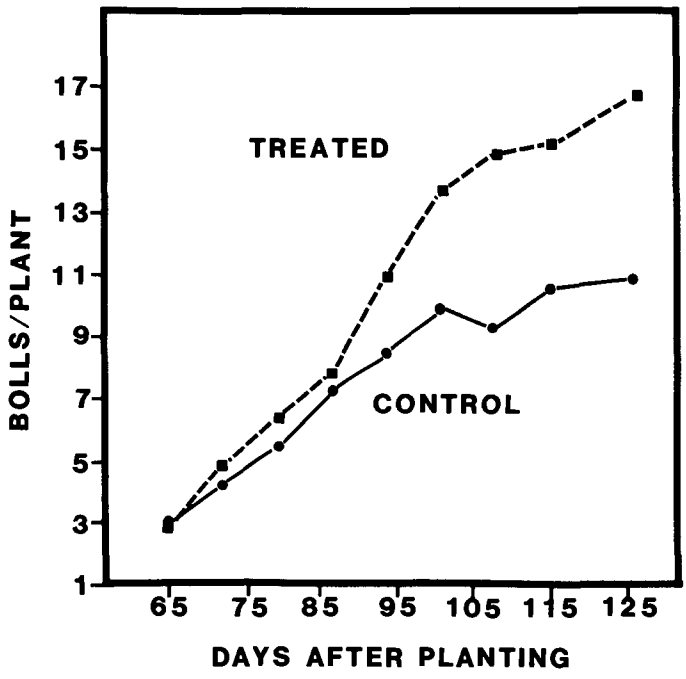

Fig. 1. Boll load on cotton plants from Exp. 2 irrigated with deionized water (control) or carbonated water (treated). Data points are average of 48 plants. Statistical analysis by paired t test, which paired the outermost replications (rows 1 and 6) and paired the other replications accordingly (2 and 5; 3 and 4) showed that the divergence of the treatments became significant ($P < 0.05$) on day 107. Error bars are not included on the graph because this analysis gives a standard error for the difference.

These data are consistent with those of Misra (1951) who observed a significant increase in leaf area and plant height of greenhouse-grown, potted cotton irrigated with CO_2-saturated water. Misra (1951) did not report the boll load or yield of his experiments. Leonard and Pinckard (1946) observed inhibition of growth of cotton grown in solution culture when CO_2 content was greater than 100 mg L^{-1}. The data from solution culture apparently are not reliable for prediction of responses of cotton to soil concentrations of CO_2.

The data presented here show a consistent pattern of increase in photosynthetic activity during fruiting based upon greater chlorophyll content of the leaves due, apparently, to a more nearly adequate Mn supply. The Mn content of the leaves was not monitored frequently during this experiment, but we speculate that competition for minerals such as Mn and Zn between bolls and leaves during boll maturation may create a deficiency of one of these elements in the leaves that hastens the natural tendency of the plant to cease flowering under boll load.

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