Growth of Crabapple Seedlings in Controlled Environments: Effect of CO_2 Level, and Time and Duration of CO_2 Treatment[1]

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Abstract. The growth of cutleaf crabapple [*Malus toringoides* (Rehd.) Hughes] seedlings was greatly accelerated by direct seeding under CO_2-enriched atmospheres (400 or 2000 ppm) in controlled-environment chambers. CO_2 treatment of 2000 ppm for 4 weeks from the time of seeding in the growth chamber produced the most striking results in terms of increase in node number and stem length. By the end of 4 weeks of treatment, stem lengths of seedlings treated for 4 weeks in the growth chamber with 2000 ppm CO_2 were 3 times greater than those of plants grown at ambient CO_2 (ca 350 ppm) in the greenhouse for 4 weeks, and 1.5 times greater than those treated in the growth chamber with 400 ppm CO_2 for 4 weeks. The effect of CO_2 enrichment on stem length was greater than that on node number. The stimulatory effects of CO_2 enrichment persisted for 2-3 months after the plants were moved to the greenhouse at ambient CO_2.

Considerable research has been reported on the effects of temperature, light, moisture, and other environmental factors on the growth and development of woody plants (8), but relatively little has been done on the effects of CO_2 in woody plants. Hellmers and Bonner (5) have indicated that CO_2 may be severely limiting in determining the photosynthetic efficiency of forest trees. Moss (13) has reported that low levels of CO_2 in the natural environment may be much more limiting for photosynthesis in Norway maple than in orchard grass or tobacco.

The stimulatory effects of CO_2-enriched atmospheres on the growth and development of herbaceous plants are well documented (1, 16) but the literature on CO_2 enrichment in woody plants is meager (4, 12, 18, and Tinus, 1970, personal communication).

In a previous study (18) we showed that the growth of crabapple seedlings could be stimulated by growing them in a growth chamber under CO_2-enriched atmospheres for 18 days and that young seedlings (3-7 weeks old) were more responsive to CO_2 enrichment in controlled environments than were older ones (9-14 weeks old).

Recently Krizek (9) and Krizek et al. (10), found that the growth of herbaceous plants could be greatly accelerated if CO_2 enrichment was initiated at the time of seeding the plants. From a practical standpoint this would be the ideal time to begin controlled-environment treatment for woody plants. The feasibility of seeding crabapple plants directly in the growth chamber under CO_2-enriched atmospheres was thereby examined in the present study. The effect of time and duration of CO_2 enrichment and the persistent effects of CO_2 treatment were also investigated.

Materials and Methods

Plant material. Seed of the cutleaf crabapple [*Malus toringoides* (Rehd.) Hughes] were stratified at 1°C in sealed plastic bags for 85 days. Following stratification the seed were placed in petri dishes in an incubator at 25°C on September 10, 1968. After 1 day, the radicles had started to emerge and the germinating seed were planted in 7.5-cm plastic pots containing a peat-vermiculite mix (commercially available as Jiffy Mix[6]). Two seeds were planted in each pot. A week later the seedlings were thinned to one per pot.

Experimental treatment. Experimental treatment began on September 11, 1968 and lasted 4 weeks. The newly seeded pots were divided at random into 7 groups of 9 replicates each. Three groups were treated in an experimental growth chamber with 2000 ppm CO_2, 1 group for the entire 4 weeks, a second for the first 2 weeks only, and a third group for the last 2 weeks only. Three additional groups were treated in a second growth chamber containing 400 ppm CO_2 for the same treatment times described for 2000 ppm CO_2. Those treated in the growth chamber for only 2 weeks spent the other 2 weeks in an air-conditioned greenhouse at ambient CO_2 (ca 350 ppm). An additional group was grown at ambient CO_2 for the entire 4 weeks in an air-conditioned greenhouse. After 4 weeks of treatment, all plants were moved to a standard greenhouse at ambient CO_2 (ca 350 ppm) for an additional 16 weeks to observe the persistence of CO_2 effects.

Environmental conditions. Environmental conditions in the growth chamber consisted of: 2500 ft-c (26.9 klx) of light, provided by 1500 ma cool white fluorescent lamps and 100 watt (130 volt incandescent lamps, the latter providing about 20% of the input wattage); a 16-hour photoperiod; day/night temperature of 30/24°C; 65% relative humidity, day and night; air velocity across the chamber at 10-15 m/min; and CO_2 levels of 400 or 2000 ppm, day and night. The seedlings in the growth chambers were watered or fertilized automatically for one minute, six times daily (ca 25 cc/min). This was sufficient to completely saturate the soil. The plants received distilled water only during the first week, 0.5 g/l of 20-20-20 water soluble fertilizer (Peters[6]) during the second week, and 1 g/l during the third and fourth weeks. The plexiglass growth chambers are described elsewhere (7, 10).

Envirnmental conditions in the air-conditioned greenhouse consisted of a 16-hour photoperiod obtained by extending the natural daylength with 100 ft-c (1.08 klx) of incandescent light from 5:00 to 7:00 AM to 9:00 PM; a day/night temperature of approximately 24/18°C; and ambient CO_2 (ca 350 ppm). The seedlings were watered and fertilized automatically for four minutes, three times daily, enough for the excess water or

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[6]Mention of trademark name or a proprietary product does not imply its approval by the USDA to the exclusion of the other products that may also be available.

fertilizer to drip from the pots. The nutrient regime was comparable to that described for plants grown in the growth chamber.

The plants in the standard greenhouse were grown under natural days supplemented with a night interruption of about 50 ft-c (538 lx) of incandescent light (100 watt, 130 volt) at plant level from 9:00 PM to 3:00 AM. They received a minimum of 18°C night temperature, and ambient CO_2. They were fertilized regularly with a 0.5 g/l solution of 20-20-20. One week after being moved to a standard greenhouse, the seedlings were repotted in 10-cm clay pots containing a mixture of soil, peat, and sand (5:1:1, v/v). When 8 weeks old, they were transferred to 15-cm pots, and at 12 weeks, to 20-cm pots.

Environmental measurements. The CO_2 levels in the growth chambers were monitored and controlled by means of a Beckman[6] model 15A infrared analyzer. The CO_2 system is described elsewhere (1). The CO_2 level in the greenhouse was not controlled. It remained at ambient level. Light levels were set and measured at plant height by means of a Weston[6] model 756 light meter. Relative humidities were monitored by means of Hygrodynamics[6] electric hygrometers. Air velocity was measured with an Anemotherm[6] anemometer. Air temperatures were set in the exhaust air duct. Soil temperatures 1 cm deep in the center of the pot in the middle of the growth chamber were approximately 28-29°C during the day and 20-22°C during the night. Black ball readings, used to simulate leaf temperatures, were approximately 31-33°C during the day, and 21-23°C during the night.

Growth measurements. The days to emergence of the cotyledons were recorded for each seedling. Height of the seedlings above the cotyledonary node and number of nodes were measured at weekly intervals from the time of seeding until week 14, and at biweekly intervals, thereafter.

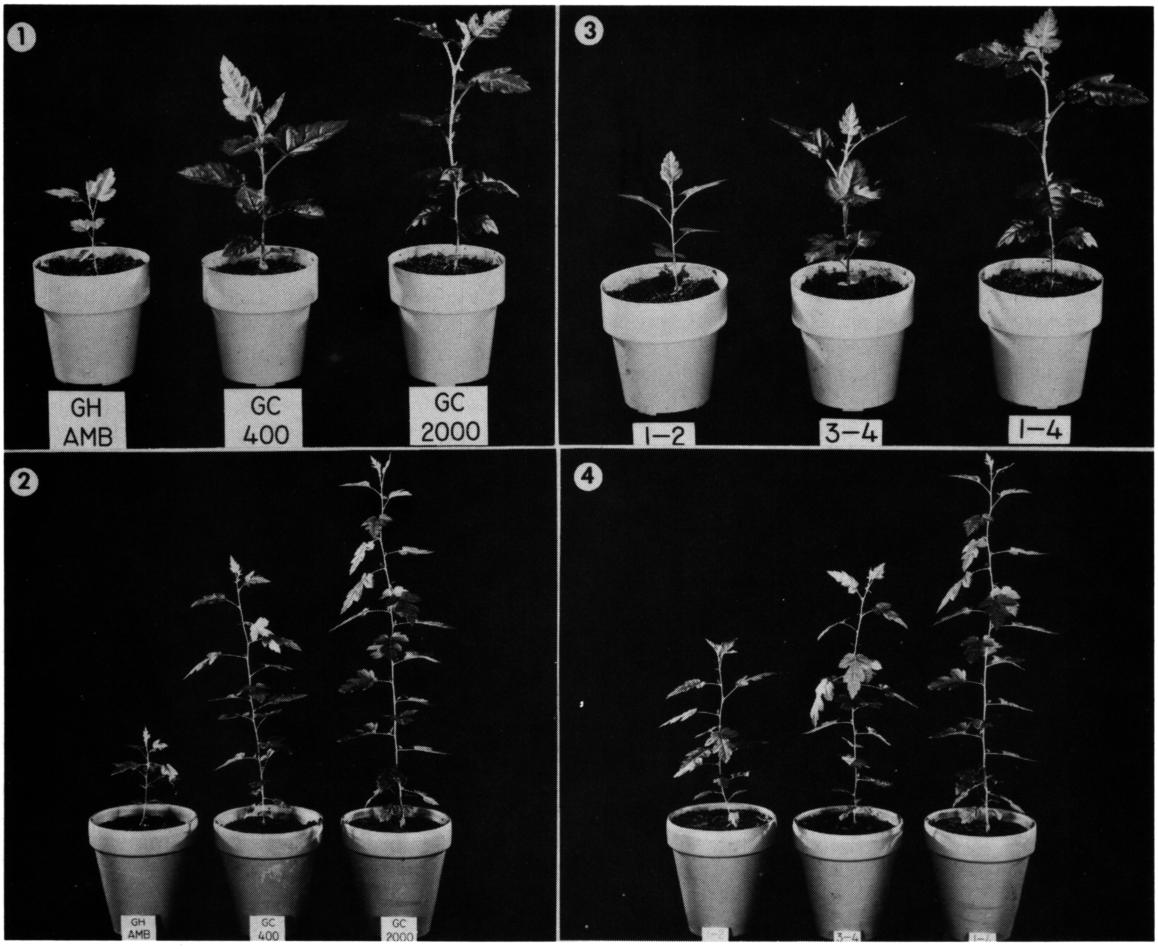
Analysis of data. Data were analyzed by analysis of variance and by the Duncan Multiple Range Test (2) for all mean separations at the 5% level of probability.

Results

Effect of CO_2 enrichment on seedling growth. CO_2 treatment had no significant effect on the time of seedling emergence. Each lot took approximately 3-4 days to emerge from the soil mix.

Enriching the CO_2 content of the atmosphere to 2000 ppm for 4 weeks from the time of seeding produced significant increases in height, number of nodes, and mean internode length over those grown at 400 ppm CO_2 in the growth chamber or at ambient CO_2 (ca 350 ppm) in the greenhouse (Figs. 1, 5, 6, 7, and Tables 1, 2).

CO_2 enrichment had a greater effect on shoot elongation than on node number. When treatment ended, stem lengths of



Figs. 1, 2. Effect of CO_2 enrichment and controlled-environment treatment on growth of cutleaf crabapple seedlings. Plant on the left grown in an air-conditioned greenhouse at ambient CO_2 (ca 350 ppm) for 4 weeks from seed; plant in the center and on the right, grown in controlled-environment chambers for 4 weeks from seed at 400 and 2000 ppm CO_2 respectively. *Fig. 1.* Plants after 4 weeks of treatment. *Fig. 2.* Plants 4 weeks later after being transferred to a standard greenhouse at ambient CO_2 (ca 350 ppm). *Figs. 3, 4.* Effect of time and duration of CO_2 enrichment at 2000 ppm CO_2 on the growth of cutleaf crabapple seedlings. Plant on the left treated for the first 2 weeks; plant in the center treated during the next 2 weeks; and plant on the right treated during the entire 4-week period. *Fig. 3.* Plants after 4 weeks of treatment. *Fig. 4.* Plants 4 weeks later, after being transferred to a standard greenhouse at ambient CO_2 (ca 350 ppm).

crabapple seedlings treated for 4 weeks in the growth chamber at 2000 ppm CO_2 were 3 times those of plants grown at ambient CO_2 in the greenhouse for 4 weeks, and 1.5 times those treated in the growth chamber with 400 ppm CO_2 for 4 weeks (Figs. 1, 5, and Table 1).

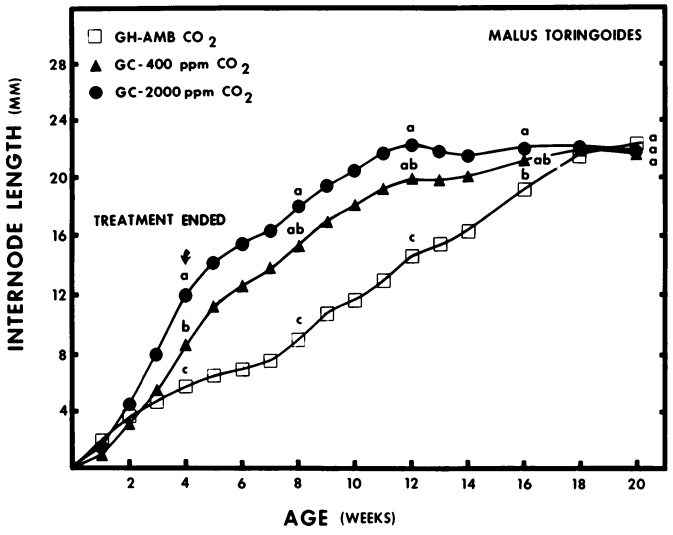


Fig. 5. Effect of CO_2 enrichment on increase in height of cutleaf crabapple seedlings. Plants treated for 4 weeks from seed and then transferred to a standard greenhouse at ambient CO_2 (ca 350 ppm). Persistence of CO_2 effects shown for 4-week period after end of differential CO_2 treatment. Means not followed by the same letter are significantly different at the 5% level by Duncan's Multiple Range Test.

Table 1. Effect of CO_2 level and time and duration of CO_2 treatment on height of cutleaf crabapple seedlings.

CO_2 TREATMENT (ppm) Treatment			HEIGHT (cm)* Post-treatment			
WK - 1-2	WK - 3-4	4 WK	8 WK	12 WK	16 WK	20 WK
GC - 2000	GC - 2000	13.5a	49a	104a	155a	208a
GC - 2000	GH - Amb	6.6bc	25b	69b	123b	184abc
GH - Amb	GC - 2000	8.7b	35b	88b	141ab	199a
GC - 400	GC - 400	8.6b	35b	76b	116b	162bc
GC - 400	GH - Amb	7.8b	28b	74b	127b	188ab
GH - Amb	GC - 400	5.6bc	25b	73b	126b	184abc
GH - Amb	GH - Amb	4.0c	14c	43c	91c	154c

*Means within each column not followed by same letter are significantly different by Duncan's Multiple Range Test.
Abbreviations: GC - Growth Chamber; GH - Greenhouse; Amb - ca 350 ppm CO_2.

Effect of time and duration of CO_2 enrichment. Data taken after 4 weeks of treatment are shown in Figs. 3 and 4 and Tables 1 and 2. By the end of 4 weeks of treatment, there were no significant differences in stem length or node number between plants enriched with 2000 ppm CO_2 during weeks 1-2 and those treated during weeks 3-4 (Table 2).

As might be expected, the duration of treatment at 2000 ppm CO_2 was of greater consequence than the time of treatment. When treatment ended, stem lengths and node numbers of crabapple seedlings treated for 4 weeks in the growth chamber at 2000 ppm CO_2 were significantly greater than those of plants treated at 2000 ppm CO_2 for only 2 weeks (Tables 1,2). At 400 ppm CO_2, however, increasing the time of treatment from 2 weeks to 4 weeks had no significant effect on either height or number of nodes.

Persistence of CO_2 effects. The differences in growth established during the 4 weeks of treatment persisted for 2-3 months after CO_2 treatment was terminated and growth chamber-grown plants were moved to the greenhouse (Figs. 2, 5, 6, and Tables 1, 2). For purposes of illustration, comparative

Table 2. Effect of CO_2 level and time and duration of CO_2 treatment on number of nodes formed on cutleaf crabapple seedlings.

CO_2 TREATMENT (ppm) Treatment			NUMBER OF NODES* Post-treatment			
WK 1-2	WK 3-4	4 WK	8WK	12WK	16WK	20WK
GC - 2000	GC - 2000	11.6a	28.1a	47.3a	71.0a	97.3a
GC - 2000	GH - Amb	8.8c	20.4c	36.7b	58.0b	83.1bc
GH - Amb	GC - 2000	9.9bc	23.6b	52.0b	62.6b	90.4ab
GC - 400	GC - 400	10.4b	23.2b	38.4b	54.8bc	74.6cd
GC - 400	GH - Amb	9.9bc	23.6bc	38.0b	59.6b	86.6abc
GH - Amb	GC - 400	9.3bc	20.4c	37.7b	58.6b	85.0abc
GH - Amb	GH - Amb	7.7d	16.3d	28.9c	47.3c	70.1d

*Means within each column not followed by same letter are significantly different at the 5% level by Duncan's Multiple Range Test.
Abbreviations: GC - Growth Chamber; GH - Greenhouse; Amb - ca 350 ppm CO_2.

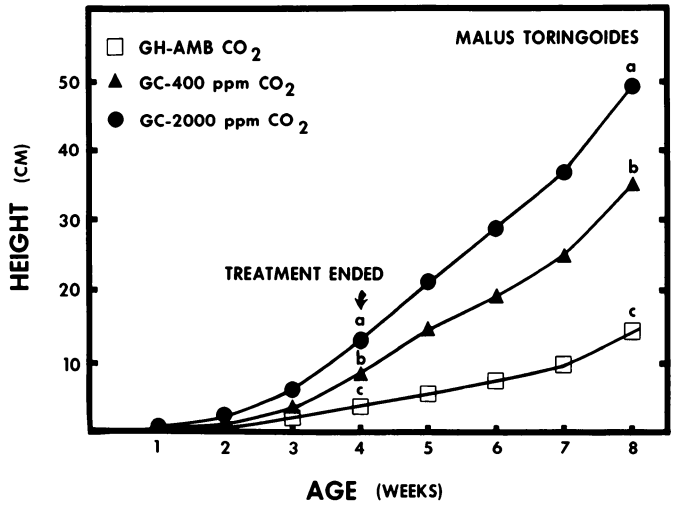


Fig. 6. Effect of CO_2 enrichment on increase in number of nodes formed on cutleaf crabapple seedlings. Plants treated for 4 weeks from seed and then transferred to a standard greenhouse at ambient CO_2 (ca 350 ppm). Persistence of CO_2 effects shown for 4-week period after end of differential CO_2 treatment. Means not followed by the same letter are significantly different at the 5% level by Duncan's Multiple Range Test.

growth rates based on measurements of height and node number are shown in Figs. 5 and 6 for the treatment period plus the first 4 weeks after treatment ended. After 20 weeks, stem lengths and node numbers of crabapple seedlings treated for 4 weeks in

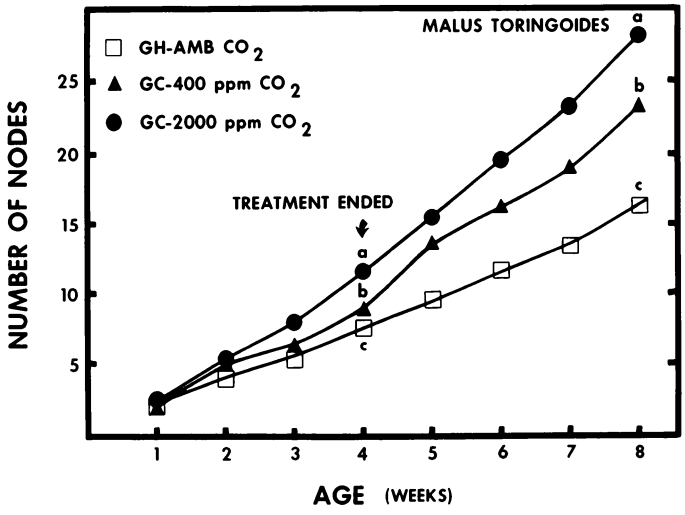


Fig. 7. Effect of CO_2 enrichment on average internode length of cutleaf crabapple seedlings. Plants treated for 4 weeks from seed and then transferred to a standard greenhouse at ambient CO_2 (ca 350 ppm). Means not followed by the same letter are significantly different at the 5% level applying Duncan's Multiple Range Test.

the growth chamber at 2000 ppm CO_2 were still significantly greater than those of seedlings treated in the growth chamber at 400 ppm CO_2 for 4 weeks (Tables 1 and 2).

Significant differences in mean internode length remained between plants given 400 ppm CO_2 and those given 2000 ppm CO_2 until week 8. By week 18, there were no significant differences in mean internode length among any of the treatments (Fig. 7).

Discussion

The importance of early environmental conditions in determining the subsequent fate of the plant has long been known (6). The effects of CO_2 on seed germination, however, are somewhat conflicting (11) and the role of CO_2 during early seedling development has not been investigated to any extent.

The present study demonstrates that the pattern of growth established during early seedling development in a woody species as a consequence of CO_2 enrichment in a controlled environment may persist long after CO_2 treatment is ended. French and Humphries (3) have reported persistent effects of early controlled-environment treatment in sugar beet, and Krizek (9) and Krizek et al. (10) have reported similar persistent effects with petunia and other herbaceous plants grown under CO_2-enriched atmospheres in a controlled environment. There has been little evidence, to date, however, for long-term persistence of the effects of CO_2 enrichment and controlled-environment treatment in woody plants.

Greenhouse control plants eventually caught up to plants grown for either 2 or 4 weeks in the growth chamber and then moved to the greenhouse. This was presumably caused by limitations imposed by restricted root growth in 20-cm pots. Since growth chamber-treated plants showed the greatest initial growth rate, they would be expected to be the first affected by limited pot size. By 20 weeks, greenhouse plants appeared to be equally limited by pot size as evidenced by the plateau reached in their average internode length (Fig. 7).

The primary effect of CO_2 enrichment on shoot growth of cutleaf crabapple seedlings was to stimulate internodal elongation. Similar results were obtained in a previous study with tea crabapple, *Malus hupehensis* Rehd. (18).

The choice of environmental conditions in this experiment was based on results obtained with petunia, and other herbaceous plants (9, 10). The fact that crabapple seedlings grew rapidly in the growth chamber under the same conditions as those used to accelerate the growth of herbaceous plants indicates the generality of these conditions in the culture of higher plants.

During the 4-week treatment, controlled-environment treatment at 400 ppm CO_2 produced a proportionally greater increase in growth over the greenhouse controls than did increasing the CO_2 level in the growth chamber from 400 ppm to 2000 ppm during this time. Increased growth under controlled environments at 400 ppm CO_2 during the four weeks of treatment was probably caused by a combination of factors such as controlled humidity, higher temperatures than those normally experienced in the autumn greenhouse, high light levels and a slight increase in CO_2 level. Enriching the CO_2 level in the growth chamber to 2000 ppm had an additive effect. These studies provide further evidence that elevated CO_2 levels and controlled-environment treatment are required for optimum seedling growth of woody plants.

Our results show that it is feasible to grow woody plants from seed in a CO_2-enriched atmosphere under controlled-environment conditions. Since the attainment of minimum size is important for flowering in woody plants (14, 15, 17), the acceleration in growth provided by this technique may be of considerable value in studies on juvenility, flowering, and other developmental problems. This technique may also be important in the commercial production of woody plants.

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