Growth of Crabapple Seedlings in Controlled Environments: Effect of CO₂ Level, and Time and Duration of CO₂ Treatment

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Abstract. The growth of cutleaf crabapple [Malus toringoides (Rehd.) Hughes] seedlings was greatly accelerated by direct seeding under CO₂-enriched atmospheres (400 or 2000 ppm) in controlled-environment chambers. CO₂ 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 CO₂ were 3 times greater than those of plants grown at ambient CO₂ (ca 350 ppm) in the greenhouse for 4 weeks, and 1.5 times greater than those treated in the growth chamber with 400 ppm CO₂ for 4 weeks. The effect of CO₂ enrichment on stem length was greater than that on node number. The stimulatory effects of CO₂ enrichment persisted for 2-3 months after the plants were moved to the greenhouse at ambient CO₂.

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 CO₂ in woody plants. Hellmers and Bonner (5) have indicated that CO₂ may be severely limiting in determining the photosynthetic efficiency of forest trees. Moss (13) has reported that low levels of CO₂ in the natural environment may be much more limiting for photosynthesis in Norway maple than in orchard grass or tobacco.

The stimulatory effects of CO₂-enriched atmospheres on the growth and development of herbaceous plants are well documented (1, 16) but the literature on CO₂ 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 CO₂-enriched atmospheres for 18 days and that young seedlings (3-7 weeks old) were more responsive to CO₂ 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 CO₂ 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 CO₂-enriched atmospheres was thereby examined in the present study. The effect of time and duration of CO₂ enrichment and the persistent effects of CO₂ 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 Mix⁶). 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 CO₂ 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 CO₂ for the same treatment times described for 2000 ppm CO₂. Those treated in the growth chamber for only 2 weeks spent the other 2 weeks in an air-conditioned greenhouse at ambient CO₂ (ca 350 ppm). An additional group was grown at ambient CO₂ 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 CO₂ for an additional 16 weeks to observe the persistence of CO₂ 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 CO₂ 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 (Peters⁹) during the second week, and 1 g/l during the third and fourth weeks. The plexiglass growth chambers are described elsewhere (7, 10).

Environmental 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 CO₂ (ca 350 ppm). The seedlings were watered and fertilized automatically for four hours, three times daily, enough for the excess water or
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 CO₂. 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 CO₂ levels in the growth chambers were monitored and controlled by means of a Beckman model 15A infrared analyzer. The CO₂ system is described elsewhere (1). The CO₂ 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 model 756 light meter. Relative humidities were monitored by means of Hygrodynamics electric hygrometers. Air velocity was measured with an Anemotherm 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 CO₂ enrichment on seedling growth. CO₂ 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 CO₂ 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 CO₂ in the growth chamber or at ambient CO₂ (ca 350 ppm) in the greenhouse (Figs. 1, 5, 6, 7, and Tables 1, 2).

CO₂ enrichment had a greater effect on shoot elongation than on node number. When treatment ended, stem lengths of

Figs. 1, 2. Effect of CO₂ enrichment and controlled-environment treatment on growth of cutleaf crabapple seedlings. Plant on the left grown in an air-conditioned greenhouse at ambient CO₂ (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 CO₂ respectively. Fig. 1. Plants after 4 weeks of treatment. Fig. 2. Plants 4 weeks later after being transferred to a standard greenhouse at ambient CO₂ (ca 350 ppm). Figs. 3, 4. Effect of time and duration of CO₂ enrichment at 2000 ppm CO₂ 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 CO₂ (ca 350 ppm).
crabapple seedlings treated for 4 weeks in the growth chamber at 2000 ppm CO₂ were 3 times those of plants grown at ambient CO₂ in the greenhouse for 4 weeks, and 1.5 times those treated in the growth chamber with 400 ppm CO₂ for 4 weeks (Figs. 1, 5, and Table 1).

Fig. 5. Effect of CO₂ 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 CO₂ (ca 350 ppm). Persistence of CO₂ effects shown for 4-week period after end of differential CO₂ 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 CO₂ level and time and duration of CO₂ treatment on height of cutleaf crabapple seedlings.

<table>
<thead>
<tr>
<th>CO₂ TREATMENT (ppm)</th>
<th>AGE (WEEKS)</th>
<th>Post-treatment</th>
</tr>
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<tbody>
<tr>
<td>GC - 2000</td>
<td>WK 1-2</td>
<td>49a 104a 155a 208a</td>
</tr>
<tr>
<td>GC - 2000</td>
<td>WK 3-4</td>
<td>25b 69b 123b 184abc</td>
</tr>
<tr>
<td>GH - Amb GC - 2000</td>
<td>WK 5-6</td>
<td>35b 88b 141ab 199a</td>
</tr>
<tr>
<td>GC - 400</td>
<td>WK 7-8</td>
<td>35b 76b 116b 162bc</td>
</tr>
<tr>
<td>GC - 400</td>
<td>WK 9-10</td>
<td>28b 74b 127b 188ab</td>
</tr>
<tr>
<td>GH - Amb GC - 400</td>
<td>WK 11-12</td>
<td>25b 73b 126b 184abc</td>
</tr>
<tr>
<td>GH - Amb GH - Amb</td>
<td>WK 13-14</td>
<td>14c 43c 91c 154c</td>
</tr>
</tbody>
</table>

*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 CO₂.

Effect of time and duration of CO₂ 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 CO₂ during weeks 1-2 and those treated during weeks 3-4 (Table 2).

As might be expected, the duration of treatment at 2000 ppm CO₂ 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 CO₂ were significantly greater than those of plants treated at 2000 ppm CO₂ for only 2 weeks (Tables 1, 2). At 400 ppm CO₂, 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 CO₂ effects. The differences in growth established during the 4 weeks of treatment persisted for 2-3 months after CO₂ 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 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 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...
the growth chamber at 2000 ppm CO₂ were still significantly greater than those of seedlings treated in the growth chamber at 400 ppm CO₂ for 4 weeks (Tables 1 and 2).

Significant differences in mean internode length remained between plants given 400 ppm CO₂ and those given 2000 ppm CO₂ 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 CO₂ on seed germination, however, are somewhat conflicting (11) and the role of CO₂ 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 CO₂ enrichment in a controlled environment may persist long after CO₂ 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 CO₂-enriched atmospheres in a controlled environment. There has been little evidence, to date, however, for long-term persistence of the effects of CO₂ 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 CO₂ 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 CO₂ produced a proportionally greater increase in growth over the greenhouse controls than did increasing the CO₂ level in the growth chamber from 400 ppm to 2000 ppm during this time. Increased growth under controlled environments at 400 ppm CO₂ 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 CO₂ level. Enriching the CO₂ level in the growth chamber to 2000 ppm had an additive effect. These studies provide further evidence that elevated CO₂ 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 CO₂-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.

Literature Cited