Effects of Rhizosphere Carbon Dioxide on the Nutrition and Growth of Peach Trees

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Additional index words. root growth, nitrate, hydroponics, bicarbonate, phosphorus, *Prunus persica*, water use

Abstract. Our objectives in this study were to measure the effects of low levels of root system carbon dioxide on peach tree growth (*Prunus persica* L. Batsch) and nutrient uptake. Using soil and hydroponic systems, we found that increased root CO₂: 1) increased root growth without increasing shoot growth, 2) increased leaf P concentration, 3) decreased leaf N concentration, and 4) reduced water use relative to air injection or no treatment.

Carbon dioxide in the rhizosphere is derived from the respiration of soil microbes and fauna, plant roots, and via diffusion of CO₂ from the atmosphere (Curtis et al., 1995). It indirectly influences microbial populations through increases in available carbon and nutrients (O'Neill, 1994), and directly influences soil pH by altering the formation of carbonic acid (Whitney and Gardner, 1943). These changes, in turn, directly and indirectly influence plant nutrition (Mauney and Hendrix, 1988). Soil-derived CO₂ has accounted for as much as 5% of carbon assimilation by plants (Baron and Gorski, 1986; Yurgalevitch and Janes, 1988). Yields and plant growth can be increased more than 5% by application of CO₂-saturated water or gaseous CO₂ to the root zone (Mauney and Hendrix, 1988; Storlie and Heckman, 1996). Increased aerial CO₂ levels also increase root : shoot ratios and root growth (Stulen and den Hertog, 1993). Some investigators, however, have not observed a growth stimulation from increased CO₂ in the root zone (Hartz and Holt, 1991; Yurgalevitch and Janes, 1988).

Our previous work (Welker and Glenn, 1988) demonstrated that soil management practices, such as the killed sod system, increased soil CO₂ levels and increased tree growth and nutrient uptake. The objectives of these studies were to determine the effects of elevated soil CO₂ on shoot and root growth, plant nutrition, and rhizosphere chemistry.

Materials and Methods

Soil CO₂ experiment. Tennessee Natural peach rootstock was planted in March into four-section root boxes 50 cm deep (Glenn and Welker, 1991), containing a Hagerstown silt loam soil (Typic mesic Hapludalf). Before

planting, perforated 4-mm tubing was placed at 10-, 25-, and 40-cm depths within each of the four 52 × 5-cm sections to distribute the soil gas treatments, and soil gas samplers were installed at a 17-cm depth at the midpoint of each section. The soil gas samplers (four per box) were inverted 10-mL syringes with tubing leading to the soil surface. The soil CO₂ concentration was measured weekly with a Beckman infrared gas analyzer (model 865; Beckman Instruments, Fullerton, Calif.). Carbon dioxide treatments began 3 weeks after planting: control (no gas injection), air (0.03% CO₂, 20% oxygen, balance nitrogen), and CO₂ (5.0% CO₂, 20% oxygen, balance nitrogen). The soil gas treatments of air and 5% CO₂ were supplied by high-pressure gas cylinders connected to each root box. Each treatment received a 1-min pulse of either air or 5% CO₂ every hour, 24 h·d⁻¹ at 0.5 L·min⁻¹. In this manner, each root box was a single treatment with four sections representing independent subsamples, randomly sampled in time for soil and root growth parameters.

Each treatment was replicated four times and the treatments were blocked in the greenhouse. We accounted for CO₂ diffusion from the soil by maintaining constant air circulation within the greenhouse. The CO₂ concentration within the canopy of each tree was measured one to two times each week for a total of 17 times during the study at varying hours to verify uniform mixing. A 1-mL syringe was filled with air from within the canopy of each tree and CO₂ concentration determined with the Beckman infrared gas analyzer. The technician did not breathe while in the greenhouse collecting the sample. Carbon dioxide levels in the tree canopies were not significantly ($P \leq 0.05$) affected by treatment during the experiment (515 and 521 ppm CO₂ for the control and 5% CO₂ treatments, respectively).

Two soil samples (436 mL each) were collected from each root box section on three dates (5 June, 23 June, 7 July) near the horizontal midpoint of each section at 15- and 30-cm depths. One sample from each depth was washed to remove roots (Smucker et al., 1982)

and root length determined using a modified line intersect method (Tennant, 1975). The second soil sample was analyzed for soil pH (1:1 soil : 0.01 M CaCl₂) and soil nitrate (nitrate electrode; Orion model 93-07).

Twenty mid-shoot leaves were collected from each tree on three dates (9 June, 23 June, 1 Aug.) and analyzed for macro- and microelements using inductively coupled plasma emission spectrophotometry. Leaf N was determined using a Leco FP 220 nitrogen analyzer. Trees were harvested 1 Aug. and the following parameters were measured: trunk diameter at 15 cm, total shoot length, wood mass above the soil line, leaf mass, and leaf area.

Data were analyzed as a randomized complete-block design with four replications using Ryan-Einot-Gabriel-Welsch mean separation, which controls type I errors when multiple means are different.

Hydroponic root CO₂ experiment. Tennessee Natural seedlings were grown in solution culture for 4 weeks before beginning the rhizosphere gas treatments of 5% CO₂ vs. continuous air. The solution culture was 1 mM KNO₃, 1 mM KH₂PO₄, 2 mM Ca(NO₃)₂·H₂O, 1.1 mM CaCl₂·2H₂O, 1.5 mM MgSO₄·7H₂O, B at 0.6 mg·L⁻¹ as H₃BO₃, Fe at 5.8 mg·L⁻¹ as NaFeEDTA, Cu at 0.25 mg·L⁻¹ as CuSO₄·5H₂O, Mn at 0.5 mg·L⁻¹ as MnSO₄·H₂O, Mo at 0.5 mg·L⁻¹ as H₂MoO₄, and Zn at 0.5 mg·L⁻¹ as ZnSO₄·7H₂O. Plants were grown in 6 L of continuously aerated solution and the solution was changed weekly. Carbon dioxide (5% CO₂, 20% oxygen, 75% N) was injected into the solution for 1 min every 15 min, 24 h·d⁻¹ at a gas flow rate of 0.9 L·min⁻¹. There were six replications of each treatment with plants and two replications without plants. To ensure uniform mixing of air coming from the solution culture containers, fans moved air perpendicular to the paired row of plants, as well as down the central axis of the paired row. On 12 dates, CO₂ concentration within the tree canopy was measured at four locations within the paired row at 1 and 14 min after injection. A 1-mL syringe was filled with air from within the canopy of each tree and CO₂ concentration determined with the Beckman infrared gas analyzer. The technician did not breathe in the greenhouse when the sample was collected. There was no statistical difference in canopy CO₂ concentration due to treatment (452 and 454 ppm CO₂ for the 5% CO₂ and control treatments, respectively).

After 4 weeks of growth in solution culture, trees were randomly assigned treatments in a paired *t* test design. Tree fresh mass and root volume by water displacement were determined. Water use and solution pH were measured weekly when the solution was changed. Trees were grown in treated solution culture for 6 weeks and then harvested to measure: final root volume, root mass, number of laterals per cm of root from 25 root segments, leaf mass, wood mass above the root system, and leaf N, P, Ca, Mg, K, Mn, Fe, Cu, B, Al, and Zn. Leaf N and mineral content were measured as in Expt. 1.

Field study. Soil gas samplers similar to those used in Expt. 1 were installed at 15- and

Received for publication 27 Sept. 1996. Accepted for publication 2 Jan. 1997. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

60-cm depths in a field study of orchard soil management systems. Treatments were killed sod, cultivation, and herbicide. The field plots were 3×10 m with four replications in a randomized complete-block design. The cultivated and herbicide treatments were plowed and tilled for 1 year before soil gas sampling. The killed sod treatment had a 'Kentucky 31' tall fescue sod (*Festuca arundinacea* Schreb.) established 1 year before sampling. In May, the sod was killed with glyphosate [*N*-(phosphonomethyl) glycine] at an a.i. of $2.2 \text{ kg} \cdot \text{ha}^{-1}$ (killed sod). The cultivated treatment was kept weed-free with shallow monthly cultivation, and the herbicide treatment was kept weed-free with a residual herbicide treatment of diuron [*N*-(3,4-dichlorophenyl)-*N,N*-dimethylurea] plus terbacil [5-chloro-3-(1-1-dimethylethyl)-6-methyl-2,4(1*H*,3*H*)-pyrimidinedione], each at an a.i. of $1.12 \text{ kg} \cdot \text{ha}^{-1}$. Soil gas samples were collected monthly from June through September and analyzed for CO_2 using an infrared gas analyzer (Beckman model 865) and for oxygen with an oxygen electrode (YSI model 53).

Results

Soil CO_2 experiment. The 5% CO_2 treatment significantly ($P \leq 0.05$) elevated, and the air treatment significantly depressed, soil CO_2 levels relative to the control during the experiment (1.98%, 0.64%, and 0.80%, for 5% CO_2 , air, and control treatments, respectively, $N = 17$). The air treatment probably reduced soil CO_2 by displacing soil gas with air at 0.03% CO_2 .

Trunk diameter, total shoot length, leaf mass, wood mass, leaf area, total aboveground mass, and soil pH were not significantly affected by the treatments (data not presented). Root length density was greatest for the 5% CO_2 treatment and least for the control and air treatments (Table 1). Leaf mineral content at harvest was unaffected by the treatments except for P, which was higher in the 5% CO_2 and control treatments than in the air treatment (0.27%, 0.27%, and 0.25% P, respectively, $P \leq 0.05$). Leaf N concentration in the 5% CO_2 treatment was significantly reduced compared to the air and control treatments at all three sampling dates (4.0%, 4.3%, and 4.3%, respectively, $P \leq 0.10$). Date $\times$ treatment interaction was nonsignificant for leaf N concentration.

Hydroponic root CO_2 experiment. The gas treatments did not affect seedling fresh mass and root volume (85 g and 42 mL vs. 81 g and 39 mL, for 5% CO_2 and control treatments, respectively, $P \leq 0.05$). Over all sampling dates, the pH of the nutrient solution was not significantly increased by the CO_2 treatments when plants were present (6.8 vs. 6.7, respectively, for the CO_2 and control treatments, $P \leq 0.05$). However, the CO_2 treatment did significantly increase the pH of the nutrient solution without plants (5.4 vs. 4.9, for CO_2 vs. control treatments, respectively, $P \leq 0.05$). Elevated rhizosphere CO_2 increased root dry mass and number of laterals per cm root length but not leaf, wood, or total seedling mass (Table 2).

Weekly water use was significantly lower in the CO_2 treatment compared with the control. Leaf N concentration was reduced, but leaf P was increased, by the CO_2 treatment. The other mineral elements were not significantly affected by the treatments.

Field experiment. Carbon dioxide levels in the soil were 2-fold higher in June and July in the killed sod treatment compared with the other treatments (Fig. 1). The herbicide and cultivated treatments did not differ significantly ($P \leq 0.05$).

Discussion

In the field, a killed sod maintained higher levels of soil CO_2 in June and July, presumably due to the sod roots decomposing in the soil. The levels of soil CO_2 were below 5% in all treatments, a level shown to be detrimental to root growth (Yurgalevitch and Janes, 1988). Soil management systems that influence soil gas composition by increasing soil organic matter will increase levels of soil CO_2 , as we (Welker and Glenn, 1988) and others (Stolzy, 1974) have shown. Plastic mulch also increases soil CO_2 levels (Baron and Gorski, 1981) by reducing diffusion from the soil.

In the greenhouse studies, we manipulated soil CO_2 level to reflect that found in the field. Four patterns emerged in the studies. 1) Root growth was stimulated by the CO_2 treatments. Previous studies have generally demonstrated an increase in root biomass and root length with increased aerial and soil CO_2 levels (Idso and Kimball, 1992; Stulen and den Hertog, 1993). 2) Leaf N concentration was reduced. Other studies have shown no effect or reduced N in tissues associated with elevated CO_2 levels in the aerial and soil environment (den Hertog and Stulen, 1990; Norby et al., 1986b); however, Cramer and Lips (1995) did show N level increases under salinity stress plus CO_2 enrichment of the rhizosphere. Because HCO_3^- is a counter ion for NO_3^- uptake (Barber, 1974), the increased availability of HCO_3^- in the rhizosphere from CO_2 injection may inhibit NO_3^- uptake. 3) Leaf P concentrations were increased, as shown by Norby et al. (1986a); however, Mauney and Hendrix (1988) found a reduced level of plant P due to an increase in solution pH from CO_2 -saturated

water. 4) Water use was reduced by CO_2 -saturated water. Chang and Loomis (1945) first reported a reduction in water use and N and P uptake in hydroponic culture when the solution was enriched with CO_2 .

Evidence exists that CO_2 in the root system acts in an anapleurotic manner to increase plant carbon and make more carbon available for root growth. The mechanisms include assimilation of inorganic carbon into sugar (Baron and Gorski, 1986; Cramer et al., 1993) and, specifically, the dark fixation of bicarbonate ion and, assimilation via phosphoenolpyruvate carboxylase activity (Vuorinen et al., 1992). Additionally, CO_2 interferes with ethylene action and increases root elongation where ethylene levels are supraoptimal (Abeles, 1972; Otani and Ae, 1993). Respiration rates are reduced by CO_2 -enriched aerial (Bunce, 1995) and soil (Qi et al., 1994) environments and may provide a mechanism for increased carbon partitioning to the root; however, reduced respiration in aerial tissues associated with enriched CO_2 is often a short-term phenomenon. Wullschlegel et al. (1994) and Reuveni et al. (1995) have shown that high ambient CO_2 does not affect respiration by suppressing the alternative, cyanide-resistant pathway. Increasing atmospheric CO_2 decreases water use and increases water use efficiency (Kimball and Idso, 1983) and we found similar results when CO_2 levels were increased in the root zone.

The killed sod system increases root growth and P levels in peach trees (Glenn and Welker, 1991) similar to the elevated soil CO_2 responses demonstrated in this study and those cited. However, the killed sod system also increases plant N levels whereas our studies suggest a suppression of N uptake. Our previous studies (Glenn and Welker, 1991) have shown that the killed sod system increases the levels of soil solution nitrate and this is probably the reason for increased N levels in field studies. The increased N availability in the killed sod system probably overcomes the nitrate uptake inhibition from elevated soil CO_2 . Stimulation of root growth by elevated soil CO_2 in the rhizosphere may have increased P uptake. Increasing organic matter turnover in the soil not only increases nutrient availability and improves soil tilth, but the

Table 1. The effect of soil CO_2 treatments on root length density and aboveground mass of peach trees.

Treatment	Root length density ($\text{cm} \cdot \text{cm}^{-3}$)			Leaf plus wood mass (g)
	5 June	23 June	6 July	
5% CO_2	1.30 A ^a	1.83 A	3.11 A	416 ^{ns}
Air	0.95 B	1.40 B	2.12 B	373
Control	0.85 B	1.48 B	1.78 B	320

^aMean separation within columns by Ryan-Einot-Gabriel-Welsch mean separation, $P \leq 0.05$.

^{ns}Nonsignificant at $P \leq 0.05$.

Table 2. Effect of root applied CO_2 treatments on peach trees in solution culture.

Treatment	Dry mass (g)		Root laterals (no./cm)	Water use (mL/week)	Leaf N (%)	Leaf P (%)
	Leaf shoot	Root				
5% CO_2	16.6 ^{a, ns}	11.4 A	0.10 A	3085 B	3.98 B	0.64 A
Control	15.3	10.3 B	0.09 B	3274 A	4.69 A	0.59 B

^aMean separation within columns by paired *t* test, $P \leq 0.05$.

^{ns}Nonsignificant.

Fig. 1. Effect of soil management system on soil CO₂ concentration. Data points are the means of 15- and 60-cm depth samplings (N = 8).

organic matter stimulates microbial growth. Subsequent respiration has beneficial effects on root growth due to the physiological action of CO₂ on the root system. Increased soil CO₂ in the rhizosphere is a mechanism in part responsible for the growth increase in peach trees planted in a killed sod system (Glenn and Welker, 1989; Welker and Glenn, 1988); however, soil management effects on plant physiology are complex and the elevation of soil CO₂ levels alone does not explain the growth response found in field studies.

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