Elongation and Branching of Roots on Soybean Plants in a Carbon Dioxide-Enriched Aerial Environment

D. Del Castillo, B. Acock,* V. R. Reddy, and M. C. Acock

ABSTRACT

Plants grown in high CO_2 concentrations ([CO_2]) often have a higher root weight than those grown in low [CO_2]. It is usually assumed that the plants with this extra root weight can explore a greater volume of soil and will, therefore, have more water available to them. To test this assumption, soybean [*Glycine max* (L.) Merr. cv. Forrest] plants were grown in outdoor, sunlit plant-growth chambers in [CO_2] of 330, 450, 600, and 800 $\mu L\ L^{-1}$ throughout the growing season. The soil containers in the growth chambers had a glass side and new root growth appearing at the glass was measured and marked two or three times each week. Root weight at the end of the season (93 d after emergence) was 26 to 31% higher in [CO_2]-enriched chambers compared with the 330 $\mu L\ L^{-1}$ treatment, and cumulative root length was approximately proportional to [CO_2]. However, CO_2 treatment did not affect the rate of elongation of individual root axes. Instead, there was a significant linear increase in the number of actively growing roots with increased [CO_2]. Plants grown in 800 $\mu L\ L^{-1}$ had 65% more actively growing roots than plants grown in 330 $\mu L\ L^{-1}$. Thus, growing a plant in high [CO_2] enabled it to explore a given volume of soil more thoroughly, but did not increase the volume of soil explored.

THERE is currently much interest in how the rising ambient CO_2 concentration and the attendant climate change will affect the growth of crops. Carbon dioxide-enrichment of soybean plants has been shown to increase the dry weight of all organs on the plant (Sionit, 1983; Jones et al., 1984) with, in many cases, proportionately more dry matter going to roots (Hardy and Havelka, 1976; Rogers et al., 1983). Because of this greater root weight, it is often assumed that plants in CO_2-enrichment are less likely to suffer from drought (Wittwer, 1978; Baker et al., 1983). The reasoning is that differences in root weight from CO_2-enrichment would be manifest as differences in root length caused by an increased root elongation rate. This increased root elongation rate would allow a greater volume of soil to be explored (Kimball, 1986).

To test this last assumption, we measured changes in root length appearing on the glass face of a soil container. At intervals during the season, root weights for plants grown in various CO_2 concentrations in growth chambers were also sampled. This study was part of a larger comprehensive experiment to determine the effects of CO_2 on all aspects of soybean growth and development (Acock et al., 1985; Reddy et al., 1989).

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MATERIALS AND METHODS

Seeds of soybean were inoculated with *Rhizobium japonicum* and sown in the growth chambers at Mississippi State University Plant Science Farm on 31 Aug. 1982. The seedlings emerged 6 d later. The seedlings were grown from 8 d after emergence (DAE) until 93 DAE in [CO_2] of 330, 450, 600, or 800 $\mu L\ L^{-1}$.

Controlled Environment Chambers

The sunlit plant growth chambers used in this research have been described in detail by Phene et al. (1978). The rooting medium was contained in a soil bin measuring 1.0 × 2.0 × 0.5 m, consisting mostly of steel, but with one long side made of glass. The soil bin was completely surrounded by a wooden box (with one removable side) to exclude light and prevent rapid changes in soil temperature.

The aerial parts of the plants were in an acrylic plastic box (Plexiglas, Rohm and Haas Co., Burbank, CA) measuring 1.5 × 2.0 × 0.5 m. A chilled solution of antifreeze was supplied to a fan-coil box attached to the north side of each chamber. Each box contained a cooling coil, a heater, and a fan for circulating air through the chamber.

Carbon dioxide concentration, air temperature, and irrigation in the chambers were controlled by a computer, which also monitored solar radiation and humidity. To control [CO_2], each chamber was fitted with its own conductimetric analyzer (Acock et al., 1977). An infrared gas analyzer was used as a secondary calibration standard and the computer adjusted the calibration curves of the conductimetric analyzers when necessary. To maintain [CO_2] concentration in each chamber, pure CO_2 was injected from a gas cylinder through a pressure regulator, solenoid valve, needle valve, and a volume flowmeter. Concentrations were maintained in such a way that for 95% of the time, the values were within ± 20.8 $\mu L\ L^{-1}$ of the set point.

Plant Culture

The soil bins of the growth chambers were filled with a sand:vermiculite mixture (1:1 by volume). Fertilizer was mixed into the top 0.2 m of soil at rates equivalent to 1000 kg ha^{-1} lime, 110 kg ha^{-1} P, and 200 kg ha^{-1} K.

Within each of the four cabinets, plant spacing was 0.1 m within the row and 0.67 m between rows (15 plants) for plants harvested at 93 DAE. Figure 1 shows how additional rows of plants, harvested at 15 and 25 DAE, were grown in eight removable wooden boxes between these permanent rows. The boxes measured 0.15 × 0.48 × 0.05 m. Until 15 DAE plant spacing was 0.15 m between rows. From 15 to 25 DAE plant spacing was 0.33 m between rows. Root lengths were measured only on plants from the permanent rows. Most of these measurements came from the three plants in each cabinet nearest the glass side.

Throughout the experiment, the air temperature in the growth chambers was 30°C during the day and 22°C at night, with values within ± 1.9°C of the set points for 95% of the time. The plants were irrigated from drip tubes twice daily to keep the whole soil profile moist. The amount of water supplied as irrigation was approximately 1.2 times the amount of water collected off the cooling coils from the previous day. At 12 and 18 d after sowing, the plants in each chamber were given 1.5 L of a full Hoagland nutrient solution (Hewitt, 1952) to keep them growing during the nodule-forming period.

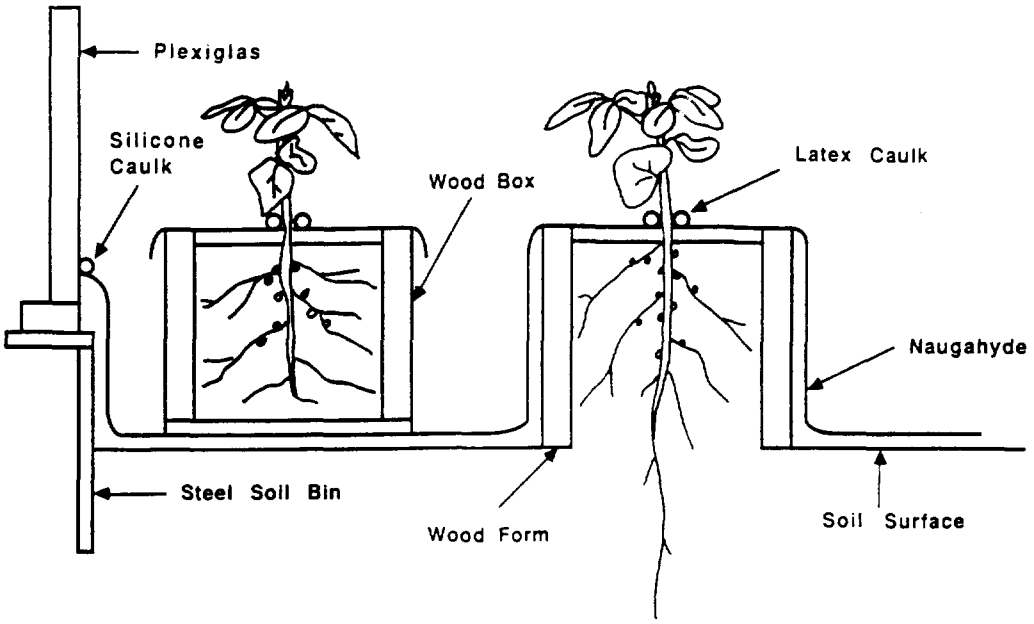

Fig. 1. Diagrammatic cross-section of part of a growth chamber showing the placement of permanent rows of plants, extra rows in wooden boxes, and the sealing of the soil surface with Naugahyde, a vinyl-coated cloth.

The plants were sprayed to control insects and fungi as necessary, but any treatment necessary in one chamber was always given to the other chambers. All soil surfaces were covered with Naugahyde[1] (Uniroyal, Inc., Mishawaka, IN), a vinyl impregnated cloth, so that CO_2 respired by roots, nodules, and soil microorganisms could not enter the shoot enclosure. The root chambers were vented separately from the shoot enclosure, but no attempt was made to flush out respired CO_2.

At least twice each week new root length appearing at the glass face of the soil bin was measured and then marked with a wax pencil. The top 0.1 m of root medium was obscured by boxes and metal framing for the glass side. At 15 and 25 DAE, 10 plants were harvested from the wooden boxes. Sand and vermiculite were washed off roots placed on a screen and root dry weight was measured. After the final destructive harvest at 93 DAE, the rooting medium in the soil bins was removed layer by layer, the roots washed out, dried, and weighed. Further details of the experiment are given in Acock et al. (1985).

Statistical Analysis

Trend analysis was used to determine the influence of $[CO_2]$ on number of actively growing root tips, root elongation rate, root length, and root weight at various soil depths at 93 DAE. Root weight at 93 DAE by soil depth was also analyzed by analysis of variance (ANOVA). The $[CO_2] \times$ soil depth interaction was used as the estimate of error variance. Root weights at 15, 25, and 93 DAE were analyzed by ANOVA, testing the hypothesis that CO_2-enrichment increases root weight. The $[CO_2] \times$ harvest date interaction was used as the estimate of error variance.

RESULTS AND DISCUSSION

Root weight and distribution.

Table 1 shows the effect of CO_2 concentration on root weight for the three occasions when organ dry weights were measured. All $[CO_2]$ treatments above 330 $\mu L\ L^{-1}$ produced more root weight. The distribution of this root dry matter with depth at final harvest (93 DAE) is shown in Fig. 2. In all $[CO_2]$ treatments, the greatest root weight was in the top 0.2 m and there was negligible weight of root below 0.8 m depth. This is similar to the root distribution found by Arya et al. (1975), except that they found a lower proportion of total root dry weight in the 0 to 0.2 m soil layer. Increased root dry weight in the top 0.2 m was associated with increased $[CO_2]$ up to 450 $\mu L\ L^{-1}$ CO_2, after which root dry weight did not increase appreciably for increases in $[CO_2]$. Root dry weight located at a depth of 0.2 to 0.4 m increased linearly with increased $[CO_2]$, but this response contributed very little to the overall effect of $[CO_2]$ on root dry weight. Below 0.4 m, there was no consistent pattern of response to increased $[CO_2]$. Evidently, the extra dry weight available to plants in high $[CO_2]$ was not being used to explore deeper in the total soil volume.

Root length.

Accumulated root length measured against the glass side of the soil bins is shown in Fig. 3. Early in the season there was no significant relationship between root length and $[CO_2]$ treatment. However, at 63 DAE,

Table 1. Root dry weights of plants from various CO_2 concentrations on several occasions.

Days after emergence	CO_2 concentrations ($\mu L\ L^{-1}$) 330	450	600	800
	dry weight (g plant^{-1})			
15	0.32	0.34	0.40	0.34
25	0.81	1.12	0.96	0.96
93	2.30	2.89	2.97	3.01
Mean	1.14	1.45*	1.44*	1.46*

LSD (0.05) using one-sided t = ±0.27

* Means significantly different from control at the 0.05 probability level.

[1] Mention of proprietary products is for the convenience of the reader only, and does not constitute endorsement or preferential treatment of these products by Mississippi State University or the USDA.

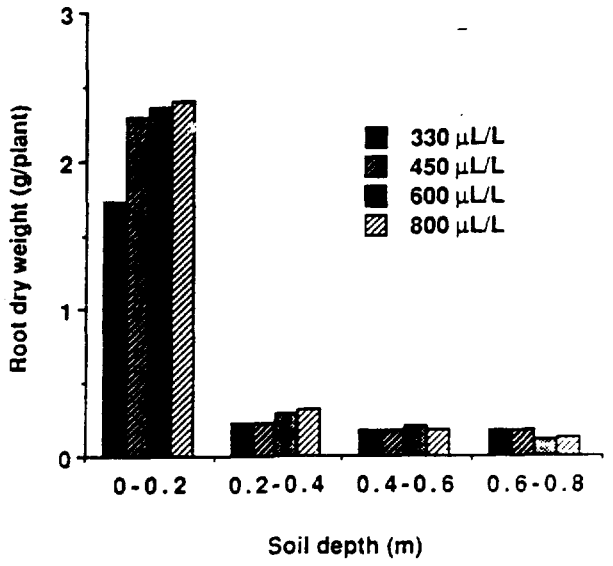

Fig. 2. Variation in root dry weight with depth at final harvest (93 DAE) for soybean grown in various CO_2 concentrations. SE between means = ± 0.094.

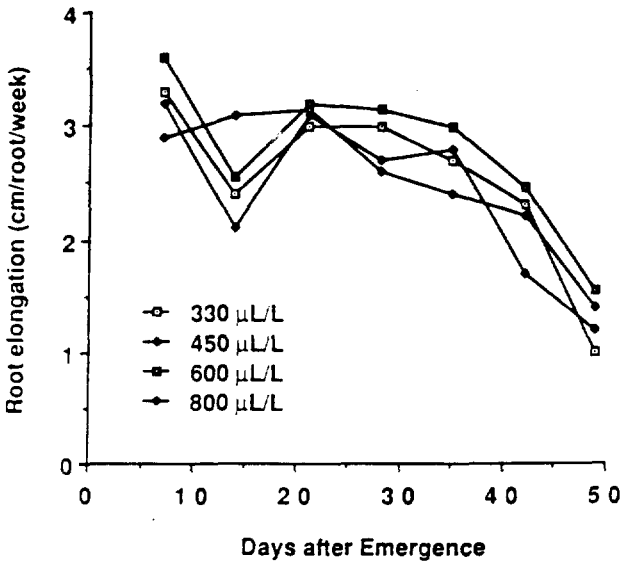

Fig. 4. Mean elongation rate of individual root axes during vegetative growth for soybean in various CO_2 concentrations.

cumulative root length was a linear function of $[CO_2]$ (R^2 = 0.95); the plants in 800 μL L^{-1} accumulated almost 50% more root length than the plants in 330 μL L^{-1}.

The differences in root length found between $[CO_2]$ treatments were not caused by differences in the elongation rate of individual root axes (Fig. 4). There was no significant relationship between root elongation rate per axis and $[CO_2]$ although there was a gradual decline in root elongation rate in all $[CO_2]$ treatments towards the end of the vegetative growth phase. Instead, the differences in root length were caused by differences in the number of actively growing root tips (Fig. 5). There was a significant linear increase in actively growing root tips with increase in $[CO_2]$ (R^2 = 0.94). The elongation of root axes was erratic. Individual root tips would elongate actively at rates of up to 0.025 m d^{-1} for several days and then stop abruptly. Rates from 0.025 to 0.050 m d^{-1} have been observed by Mitchell and Russell (1971) under optimum field conditions.

Differences in the number of actively growing roots in various $[CO_2]$ could have been caused by (i) individual root axes staying active for longer or (ii) the formation of more root axes through branching. We believe that the difference in number of actively growing roots was caused by branching because (i) all root axes observed were growing away from the base of the plant, yet most of the roots were in the top layer of soil (Fig. 2), and (ii) mean root elongation rate per axis did not differ between treatments (Fig. 4).

If individual root axes had stayed active for longer in the higher $[CO_2]$ treatments, the roots would have been distributed over more of the profile since all roots were observed to be growing away from the base of the plant. Increased branching, on the other hand, would tend to increase the population of roots in those parts of the soil profile already occupied by roots, rather

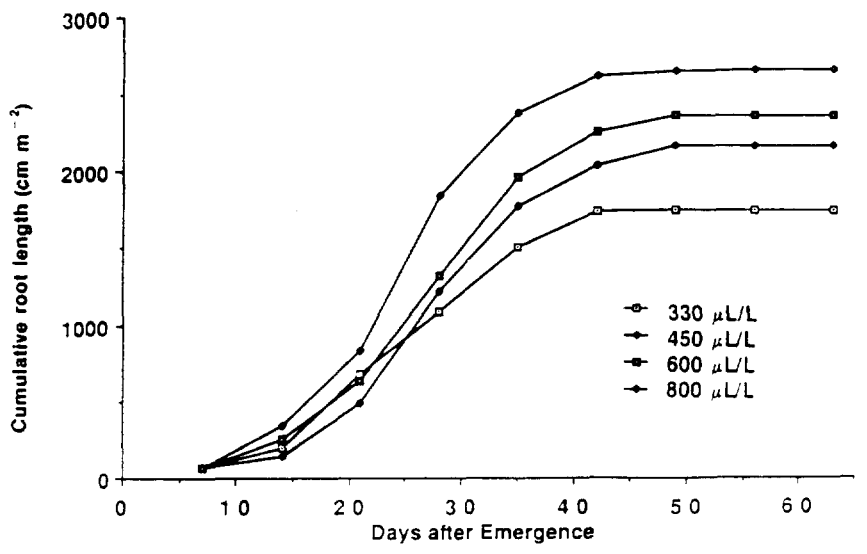

Fig. 3. New root length visible at a vertical soil/glass interface accumulated over the season for soybean in various CO_2 concentrations.

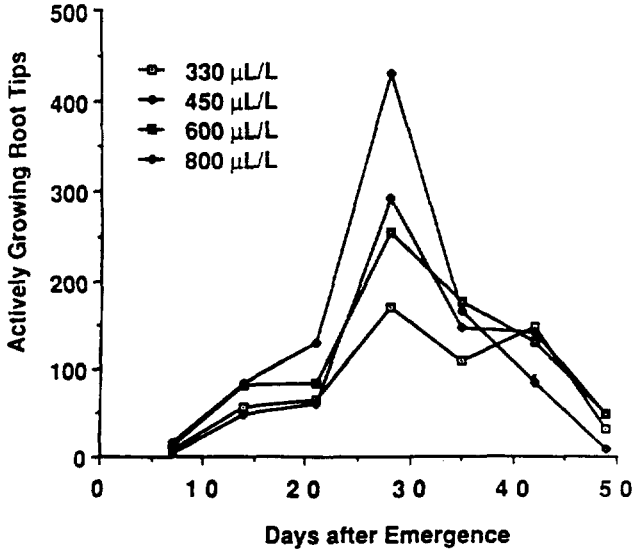

Fig. 5. Number of actively growing roots visible at a vertical soil/glass interface during vegetative growth for soybean in various CO_2 concentrations.

than increasing the rooted volume of the soil. Our observations are best explained by increased root branching in high [CO_2] conditions. Although increased [CO_2] may not increase the total volume of soil explored, it must be remembered that soil exploration can be affected by irrigation treatment (Mayaki et al., 1976). What is clear from this work is that high [CO_2] did not increase the root dry weight by increasing root elongation rate, but by increasing the number of active root axes (branching). Under well-watered conditions this would not influence the amount of water available to the plants. Under drought conditions the more thorough exploration of the rooted soil volume might enable the plant to extract slightly more water from that rooted volume. However, the additional amount of water available would be greater if the new roots penetrated deeper into the soil, rather than re-exploring soil partially dried by older roots. Further work is needed to determine how root growth is affected by water stress in CO_2-enrichment.

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ERRATA

In the article "Water Use Efficiency and Dry Matter Distribution in Nitrogen- and Water-Stressed Winter Wheat" by J.J. Heitholt, *Agron. J.* 81:464–469, the first two lines are missing from the first sentence in the final paragraph of the left-hand column on page 465. The sentence should read:

"After photosynthesis and transpiration measurements, leaf area and dry matter of the measured leaf were determined, the number of tillers on each plant counted (main stem included as a tiller), and the entire plant harvested."

Also, in the first paragraph of the right-hand column on page 467, the "IN treatments" referred to in the last sentence should read "LN treatments."

In the article "Ergopeptine Alkaloids in Grazed Tall Fescue" by D.P. Belesky, J.A. Stuedemann, R.D. Plattner, and S.R. Wilkinson, *Agron. J.* 80:209–212, the y-axis units in Fig. 1 should read ($\mu g\ kg^{-1}$).