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Effects of High Atmospheric CO_2 and Sink Size on Rates of Photosynthesis of a Soybean Cultivar[1]

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ABSTRACT

The effect of sink strength on photosynthetic rates under conditions of long-term exposure to high CO_2 has been investigated in soybean. Soybean plants (Merr. cv. Fiskeby V) were grown in growth chambers containing 350 microliters CO_2 per liter air until pod set. At that time, plants were trimmed to three trifoliolate leaves and either 21 pods (high sink treatment) or 6 pods (low sink treatment). Trimmed plants were either left in 350 microliters CO_2 per liter of air or placed in 1000 microliters CO_2 per liter of air (high CO_2 treatment) until pod maturity. Whole plant net photosynthetic rates of all plants were measured twice weekly, both at 350 microliters CO_2 per liter of air and 1000 microliters CO_2 per liter of air. Plants were also harvested at this time for dry weight measurements. Photosynthetic rates of high sink plants at both measurement CO_2 concentrations were consistently higher than those of low sink plants, and those of plants given the 350 microliter CO_2 per liter of air treatment were higher at both measurement CO_2 concentrations than those of plants given the 1000 microliters CO_2 per liter of air treatment. When plants were measured under treatment CO_2 levels, however, rates were higher in 1,000 microliter plants than 350 microliter CO_2 plants. Dry weights of all plant parts were higher in the 1,000 microliters CO_2 per liter air treatment than in the 350 microliters CO_2 per liter air treatment, and were higher in the low sink than in the high sink treatments.

Projections of increasing global atmospheric CO_2 concentration with time (13) have stimulated interest in the effects of high CO_2 on photosynthesis and plant growth. Although plant species with the C_3 photosynthetic pathway have been observed (2, 24) to produce more dry matter in high CO_2 than in normal atmospheric CO_2, the basis of this increase in dry matter production is not completely clear. In many studies increased rates of photosynthesis on a leaf area basis during brief exposure of plants to high CO_2 (1, 5, 6, 10) have been extrapolated to explain increased growth in high CO_2 by projecting higher photosynthetic rates over the long term in plants growing in high CO_2 environments (8, 23). In studies (4, 16, 21) where photosynthesis and growth of plants in high CO_2 have been followed for many days or weeks, it has been observed that the initially high rates of growth and photosynthesis per unit of leaf area are maintained for only relatively short periods after exposure to high CO_2, then decline to levels comparable to or lower than those of plants maintained in normal atmospheric CO_2. The length of the enhancement period and the rapidity of decline from the maximum rates apparently depend on the ambient CO_2 concentration, with higher concentrations leading to more rapid loss of enhancement (4). In crop species, it has been reported (11, 16) that exposure to high CO_2 does not result in growth enhancement during all phases of vegetative growth but can increase dry weights significantly during early stages of seedling growth or early fruit development. This suggests that enhanced rates of photosynthesis per unit area may be maintained only during periods of rapid growth when the demand for assimilate is high. During periods of low assimilate demand, rates of photosynthesis per unit leaf area in high-CO_2 grown plants fall off in the manner demonstrated by Aoki and Yabuki (4).

The purpose of the present experiment was to measure the effects of assimilate demand on rate of whole plant net photosynthetic carbon fixation in soybeans during long-term exposure to high CO_2 and to relate these rates to dry matter production under these conditions.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Fiskeby V, an early maturing, strongly determinate cultivar of soybean (*Glycine max.* L. Merrill), was grown in controlled environment chambers at the Duke University Phytotron. Seeds were planted in individual 360-ml plastic pots in a mixture of gravel:vermiculite:Turface (1:1:1; v/v/v) and placed on a 12 h/12 h, light/dark cycle (600 μE m^{-2} s^{-1} at plant height, fluorescent and incandescent lamps) and a 26/20 C temperature cycle. On day 16, seedlings were transplanted into 12.7-cm plastic pots and axillary branches removed. Pots were placed on an automatic watering system and, thereafter, were watered to the drip point with modified half-strength Hoagland solution three times per day.

Treatments were initiated on day 44 when pod growth was rapid but seeds had not yet begun to develop (stage R6 (9)). At this time, vegetative growth was complete with seven to eight mainstem nodes present on all plants. To establish approximately equal leaf area in all plants, trifoliolates at nodes other than 2, 4, and 6 were excised. For low sink treatments the two smallest pods at nodes 2, 4, and 6 were left (total, six pods) and for high sink treatments the three smallest pods at nodes 1 through 7 were left (total, 21 pods). On the second day after pruning plants from each sink treatment were assigned randomly to high CO_2 (1,000 μl CO_2/l air) or "low" CO_2 (350 μl CO_2/l air) chambers. The four resultant treatments were designated as 350 HS (350 μl CO_2-chamber, 21 pods) 350 LS (350 μl CO_2-chamber, six pods), 1,000 HS (1,000 μl CO_2-chamber, 21 pods) and 1,000 LS (1,000 μl CO_2-chamber, six pods).

Physiological Measurements. Starting 4 days after the imposition of CO_2 treatments, rates of photosynthesis per unit leaf area were measured at both 350 and 1,000 μl CO_2/l air on four plants from each of the four treatments. Photosynthetic measurements

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took 2 days. One h after the beginning of the light period on the first day of photosynthetic measurement for a particular sampling date, four 1,000 HS and four 1,000 LS plants were moved to the 350 μl CO_2/l air chamber to acclimate for a minimum of 2 h. Measurements of photosynthesis were then made on the central leaflets of each of the three trifoliolates of the 16 plants, using ambient chamber temperature and light, 350 μl CO_2 air drawn from outside the building, a clamp-on cuvette (18), and a Beckman IR gas analyzer operated in the differential mode. A single measurement could be obtained in 10 to 15 min due to the small volume of the sampling cuvette and all measurements were completed within 4 h. After completion of the measurements, the plants from the 1,000 μl CO_2/l air chamber were returned to their treatment chamber. On the second day of photosynthetic measurements, the eight plants tested the previous day from the 350 μl CO_2/l air chamber were transferred to the 1,000 μl CO_2/l air chamber for acclimation. Measurements were repeated on all 16 plants from the previous day, but using 1,000 μl CO_2/l air bottled gas (20% O_2, remainder N_2, Scott Environmental Technology, Inc.). The plants being given a 350 μl CO_2/l air treatment were then returned to their chamber.

The day following photosynthetic measurements, the 16 plants used for the measurements and 16 additional plants (four from each treatment) were measured photometrically for leaf area (Lambda Instruments LI-3000). Leaves, stems, roots, and pods from each plant were then harvested and dried at 65 C for 2 days, after which time they were weighed.

Rates of photosynthesis measured with the clamp-on cuvette provided data on photosynthetic rates per unit leaf area for each trifoliolate leaf. To obtain whole plant net photosynthetic rates, rates per unit area measured on each leaflet were multiplied by the leaf area of that trifoliolate and the total photosynthesis for the three trifoliolates on the plant summed to provide data on total plant photosynthesis. These total plant rates are based on similar leaf areas, inasmuch as all plants were trimmed to three trifoliolates after leaf expansion was complete. Treatment differences expressed as total plant rates are comparable to differences expressed on a per unit area basis. In previous studies (4, 12, 21) rates have generally been expressed on a unit area basis.

RESULTS

Photosynthesis. When rates of photosynthesis were measured at the treatment CO_2 concentration to estimated *in situ* photosynthetic rates, photosynthetic rates of plants growing and measured in 1,000 μl CO_2/l air were greater than those of plants growing and measured in 350 μl CO_2/l air when plants with equal pod number were compared (Fig. 1.) When rates of plants from both CO_2 treatments were measured at 1,000 μl CO_2/l air (Fig. 2), 350 μl CO_2/l air treated plants had higher rates of photosynthesis than the 1,000 μl CO_2/l air treated plants at all sample dates when plants with equal pod numbers were compared. Sink level also affected photosynthetic rates. Comparing plants from the same CO_2 treatment, high sink plants had greater rates of photosynthesis at 1,000 μl CO_2/l air than the low sink plants. When plants from both CO_2 treatments were measured at 350 μl CO_2/l air (Fig. 3), relationships between the treatments were the same as when measured at 1,000 μl CO_2/l air, *i.e.* photosynthetic rates were higher in high than low sink plants, and 350 μl CO_2/l air treated plants had higher rates of photosynthesis at measurement CO_2 concentrations of 350 μl CO_2/l air than the 1,000 μl CO_2/l air-treated plants. Thus, at both low and high measurement CO_2 concentrations, plants with a high sink capacity had higher rates of photosynthesis than plants with a low sink capacity and plants with a low CO_2 supply during the treatment period had higher photosynthetic rates than plants with a high CO_2 supply.

Dry Weights. In the present study, dry weight gain was mainly an indication of photosynthate storage. At the time treatments were imposed, vegetative growth had ended and reproductive growth had just started. After pruning, all plants were similar in terms of leaf areas and dry weights except for experimentally imposed differences in the number of pods per plant. As a result, vegetative dry weight gain represented storage of photosynthate rather than growth of new plant parts. Reproductive dry weight

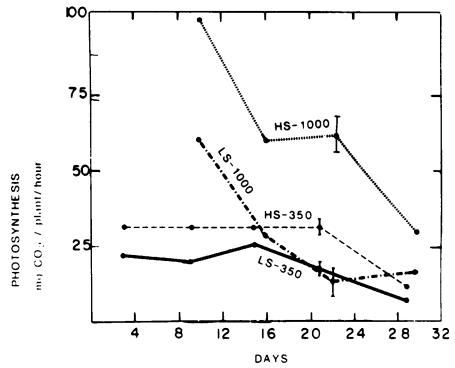

FIG. 1. Whole plant rates of net photosynthesis for plants grown at 350 μl CO_2/l air until early pod set, then trimmed to 6 or 21 pods per plant and exposed to 350 μl CO_2/l air or 1,000 μl CO_2/l air on day 0. Plants with 21 pods were treated and measured at 1,000 μl CO_2/l air (HS - 1000) or 350 μl CO_2/l air (HS - 350). Plants with six pods were treated and measured at 1,000 μl CO_2/l air (LS - 1000) or 350 μl CO_2/l air (LS - 350). N = 4 at each sample point. Error bars indicate ±1 SE for sample date indicated, which was representative of sampling variability. No data were obtained for the first sampling data in 1,000 μl CO_2/l air-treated plants because of technical problems with the system.

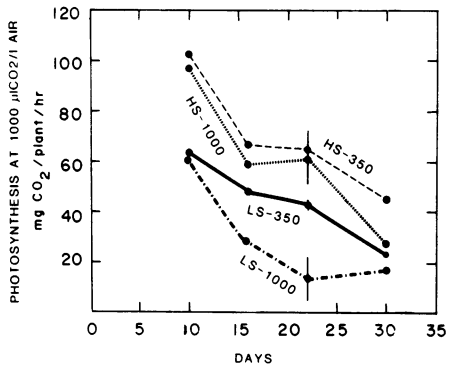

FIG. 2. Treatments as in Figure 1, except whole-plant net photosynthesis was measured in all plants at 1,000 μl CO_2/l air. Plants treated previously at 350 μl CO_2/l air were exposed to 1,000 μl CO_2/l air for at least 2 h prior to actual measurement.

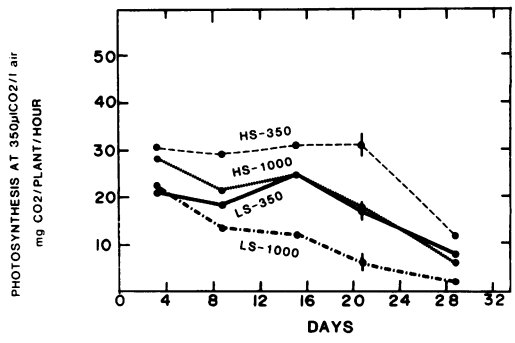

FIG. 3. As in Figure 1 except whole-plant net photosynthesis was measured only at 350 μl CO_2/l air. Plants treated previously at 1,000 μl CO_2 were exposed to measurement CO_2 concentrations for at least 2 h prior to actual measurement.

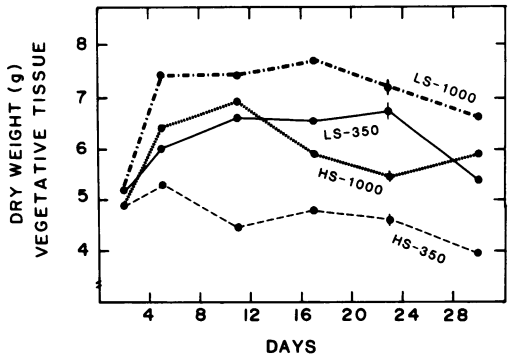

FIG. 4. Dry weights of vegetative tissues for treatments described in Figure 1; N = 8 at each sample point.

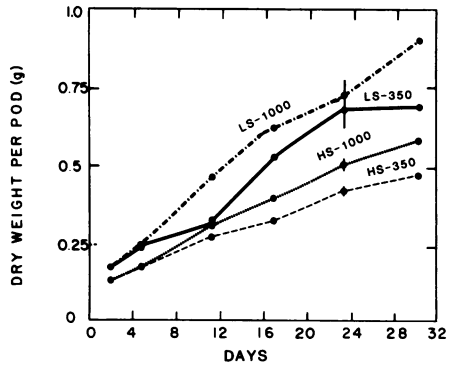

FIG. 5. Per pod dry weights for treatments described in Figure 1; N = 8 at each sample point.

gain represented both new growth and storage.

Vegetative dry weights increased during the first 5 days of treatment in response to pod removal and CO_2 treatment (Fig. 4). Dry weights of 1,000 μl CO_2/l air-treated plants were greater than the dry weights of 350 μl CO_2/l air-treated plants with an equal pod number. Dry weights of low sink plants were greater than the dry weights of the high sink plants from the same CO_2 treatment. Thus, the ranking of the treatments for vegetative dry weight is the reverse of the ranking for rates of photosynthesis at either high or low measurement CO_2 concentrations (Figs. 2 and 3).

After day 5, the pods became the most important component of dry weight gain in the plants. Individual pods of 1,000 μl CO_2/l air treated plants weighed more than individual pods of 350 μl CO_2/l air-treated plants with an equal pod number (Fig. 5). Individual pods from low sink plants weighed more than the individual pods of the high sink plants in the same CO_2 treatment (Fig. 5). As with the vegetative dry weights, these rankings among the treatments with respect to individual pod weights are the reverse of the rankings for the photosynthetic data obtained at either high or low measurement concentrations (Figs. 2 and 3).

DISCUSSION

Plants of equal pod number treated and measured at 1,000 μl CO_2/l air had higher whole plant net photosynthetic rates than plants treated and measured at 350 μl CO_2/l air for virtually the entire treatment period (Fig. 1). This resulted in greater dry weights of vegetative and reproductive tissues in plants from the high CO_2 treatments even though rates of photosynthesis were lower in 1,000 μl CO_2/l air-treated plants when plants from the two CO_2 treatments were compared at the same photosynthetic measurement conditions (Figs. 2 and 3).

Vegetative dry weights and per pod dry weights were higher in low sink compared to high sink plants, but total reproductive weights were lower because high per pod weights did not compensate for fewer pods. Total plant weights were also lower in low sink plants because increased vegetative weights did not compensate completely for greater pod number in high sink plants. Thus, in the low sink treatments, lower photosynthetic rates were associated with lower total weights, but with higher vegetative weights and per pod weight because when pod number was restricted assimilates accumulated both in vegetative tissue and in tissues of the remaining pods.

Accumulation also occurred in vegetative tissues of high CO_2-treated plants as evidenced by their higher weights. In high CO_2-treated plants this accumulation was associated with lower photosynthetic rates at any one measurement CO_2 concentration. This accumulation in vegetative tissue may be a partial cause of decreased photosynthesis in 1,000 compared to 350 μl CO_2/l air treated plants and in low compared to high sink plants. In *Vitis vinifera* (14) high CO_2-treated plants also had greater dry matter production, but lower photosynthetic rates at 350 μl CO_2/l air compared to 350-treated plants. In *Vitis*, however, photosynthetic rates at 1,000 μl CO_2/l air were higher in high CO_2-grown plants, possibly because of continued rapid growth in high-CO_2 treatments.

The hypothesis of assimilate control of photosynthesis reviewed by Neales and Incoll (17) has been supported by evidence linking high source:sink ratios with low rates of photosynthesis (7, 20, 22) but the actual mechanism of control has not been demonstrated.

Our data are compatible with the hypothesis of assimilate control of photosynthesis. There is a correlation across all treatments between accumulation of dry weight in vegetative tissues and depression of rates of photosynthesis. Inasmuch as no significant new vegetative tissue was added, the dry weight increase we observed in roots, leaves, and stems probably represented starch accumulation. Starch accumulation has been observed in high CO_2-grown plants (12, 15) and in plants with low demand for assimilate (7). While starch accumulation may not directly affect rates of photosynthesis, in plants where considerable starch buildup occurs, it is likely that other metabolic pools are also filling. Computer stimulation of metabolic pathways suggests that enzymes controlling starch synthesis are a likely control point in the reductive pentose phosphate pathway (3); thus, starch buildup in plants with low sink demand may affect rates of photosynthesis indirectly.

Our observations point out the complexity of predicting how plants will respond to increased atmospheric CO_2. As previously reported (4, 14, 16), we found that plants grown in high CO_2 can have reduced rates of photosynthesis relative to controls when tested at a single CO_2 concentration, but we also found that availability of sink capacity for photosynthate storage strongly influenced the amount of reduction of photosynthesis which occurred. Our data suggest that the more rapidly storage tissues are filled, the more rapidly rates of photosynthesis decline. As a result, we believe that a more accurate understanding of the relationship between photosynthetic changes and plant growth under CO_2 enrichment requires consideration of whether active meristematic and storage tissues can utilize photosynthate at a sufficient rate to overcome the type of apparent photosynthetic inhibition observed in the current study. Such a source:sink interaction would offer a plausible explanation for the variation in response to high CO_2 between species (4, 14) and within species at different stages of development (11, 19). If we are to predict how world vegetation patterns may change in response to increasing atmospheric CO_2, we must first have a better understanding of the processes linking production, distribution, and use of photosynthate in the plant.

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