

# Differing Sensitivity of Photosynthesis to Low Leaf Water Potentials in Corn and Soybean<sup>1</sup>

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## ABSTRACT

Rates of net photosynthesis were studied in soil-grown corn (*Zea mays*) and soybean (*Glycine max*) plants having various leaf water potentials. Soybean was unaffected by desiccation until leaf water potentials were below -11 bars. Rates of photosynthesis in corn were inhibited whenever leaf water potentials dropped below -3.5 bars.

The differences in photosynthetic behavior could be attributed solely to differences in stomatal behavior down to leaf water potentials of -16 bars in soybean and -10 bars in corn. Below these potentials, other factors in addition to stomatal closure caused inhibition, although their effect was relatively small.

Corn, which has the C<sub>4</sub>-dicarboxylic acid pathway for carbon fixation, generally had a higher rate of photosynthesis than soybean during desiccation. Nevertheless, since inhibition of photosynthesis began at higher potentials than in soybean, and since corn was less able to withstand severe desiccation without tissue death, it was concluded that the C<sub>4</sub> pathway confers no particular ability to withstand low leaf water potentials.

This work was undertaken to determine whether there are differences in the photosynthetic behavior of corn and soybean during drought. The problem arises because of a recent suggestion (14) that plant species having the C<sub>4</sub>-dicarboxylic acid pathway for carbon dioxide fixation during photosynthesis may be less sensitive to periods of desiccation than species not possessing the pathway. Evidence was inferred largely from anatomical adaptations of the C<sub>4</sub> species and the frequent exposure of many of them to drought in their natural habitats. There are few data which permit a comparison of the tolerance of photosynthesis to desiccation in different plant species. None are available for C<sub>4</sub> species. Therefore, this study describes a comparison of the photosynthetic behavior of corn, which possesses the pathway, with that of soybean, which does not.

## MATERIALS AND METHODS

Corn (*Zea mays*, var. GSC 50 single cross) and soybean (*Glycine max* (L.) Merr., var. Harosoy) plants were soil-grown

from seed in a constant environment chamber under conditions previously described (5).

After 4 to 5 weeks of growth, water was withheld and rates of net photosynthesis and transpiration were determined daily for the shoots of intact plants with the use of an infrared gas analyzer and assimilation chamber (5, 6). Chamber temperature was 25 ± 0.25 C; relative humidity was 77 ± 2%; wind speed was 1.7 m sec<sup>-1</sup>. Under these conditions, leaf temperatures were within 0.6 C of chamber temperature. Seven 300-w incandescent spotlights filtered through 10 cm of water provided a light intensity of 1.6 cal cm<sup>-2</sup> min<sup>-1</sup> at leaf height, which was saturating for soybean.

After steady rates of gas exchange and transpiration were obtained, the assimilation chamber was opened and a leaf disc was quickly removed from a lower leaf and was placed in a thermocouple psychrometer chamber for isopiestic measurement of leaf water potential (3, 7). The psychrometer chamber was coated with melted and resolidified petrolatum for each determination (4). The air permeability of each leaf was subsequently measured in the light with a modified viscous flow porometer (1) which used a slight vacuum and permitted rapid determinations. The porometer measurements were averaged for each plant and were expressed as the cube root of the time for a standard change in porometer pressure from 3 to 2 cm of H<sub>2</sub>O below ambient. Expressed in this way, the measurements represent a relative and approximate estimate of the diffusive resistance of the stomata (6, 11). The plant was returned to the constant environment chamber after each daily measurement.

The diffusive resistances to the entry of CO<sub>2</sub> into the leaf were calculated by the method of Gaastra (10). The analysis divides the total diffusion pathway into three segments: the boundary layer resistance (*r<sub>a</sub>*), the leaf resistance in the gas phase (*r<sub>l</sub>*), and the so-called mesophyll resistance in the liquid phase (*r<sub>m</sub>*). Changes in leaf resistance are brought about largely by the stomata (10, 12, 21, 26). The mesophyll resistance has been variously interpreted as a resistance to CO<sub>2</sub> diffusion from the cell surface to the site of fixation (10, 12, 21), or as a carboxylation efficiency (9, 13, 16, 24). It seems most likely that it represents elements of both.

The resistances to diffusion of CO<sub>2</sub> can be related to those for H<sub>2</sub>O according to:

$$r_l + r_a = 1.7 (r_l^{H_2O} + r_a^{H_2O}) \quad (1)$$

where *r<sub>l</sub><sup>H<sub>2</sub>O</sup>* and *r<sub>a</sub><sup>H<sub>2</sub>O</sup>* are the resistances to diffusion of water vapor in the leaf and boundary layer, respectively. Since the assimilation chamber permitted simultaneous measurement of photosynthesis and transpiration, the mesophyll resistance could be calculated from:

$$r_m = r_m + r_l + r_a - 1.7(r_l^{H_2O} + r_a^{H_2O}) \quad (2)$$

$$r_m = \frac{C_a - C_{chl}}{P} + 1.7 \left( \frac{C_l^{H_2O} - C_a^{H_2O}}{T} \right) \quad (3)$$

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where  $C_{chl}$  is the  $CO_2$  concentration, assumed to be the  $CO_2$  compensation point, at the site of  $CO_2$  fixation ( $gm\ cm^{-3}$ );  $C_a$  is the  $CO_2$  concentration in the bulk air;  $P$  is the rate of photosynthesis measured as  $CO_2$  fixation ( $gm\ sec^{-1}\ cm^{-2}$ , leaf area measured from the leaf outline);  $C_s^{H_2O}$  is the saturation concentration of water at leaf temperature ( $gm\ cm^{-3}$ );  $C_a^{H_2O}$  is the water vapor concentration of the bulk air; and  $T$  is the rate of transpiration ( $gm\ sec^{-1}\ cm^{-2}$ ). The resistances ( $r_l + r_a$ ) were calculated from the last term in Equation 3.

RESULTS

Figure 1 shows the response of net photosynthesis in corn and soybean as leaf water potentials decreased. In soybean, photosynthesis was relatively unaffected until leaf water potentials had dropped below  $-11$  bars. Corn showed a decline in rate whenever

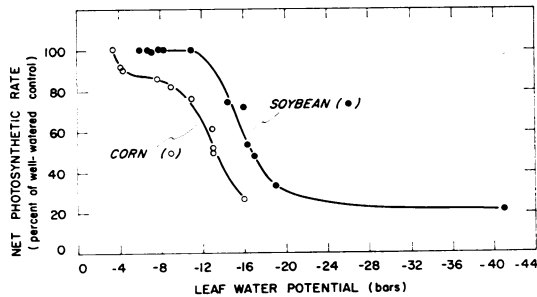


FIG. 1. Rates of net photosynthesis in corn and soybean plants at various leaf water potentials. Rates are expressed as a percentage of the maximum under well watered conditions. The photosynthesis data were collected from two different plants for each species. Maximal rates of photosynthesis were 53 to 55  $mg\ of\ CO_2/hr^{-1}\cdot 100\ cm^{-2}$  of leaf area in corn and 27.5 to 30.6  $mg\ of\ CO_2/hr^{-1}\cdot 100\ cm^{-2}$  in soybean. Plants were 45 to 60 cm tall.

the leaf water potential dropped below  $-3.5$  bars. When leaf water potentials were  $-16$  bars, photosynthesis in soybean was 60%, and in corn 25%, of the rates in well watered plants. On the basis of the leaf water potentials which permitted maximal photosynthetic rates, soybean was less sensitive than corn to low leaf water potentials.

The same idea is suggested by the lowest leaf water potentials at which photosynthesis was recorded in the two species. These potentials approximated the lowest potentials at which death of leaf tissue was negligible upon rewatering. Soybean, which recovered after leaves had been desiccated to as low as  $-41$  bars, appeared to be less sensitive than corn.

These data suggest that some portion of the photosynthetic mechanism is more sensitive to desiccation in corn than in soybean. Because resistances to  $CO_2$  diffusion (10, 12, 21) and the activity of the enzymes for dark fixation (9, 13, 16, 24) limit rates of photosynthesis at the high light intensities used in the present experiments, it was necessary to distinguish between these two factors to explain the behavior of the two species. Two methods were used to distinguish between resistive and enzymatic effects.

In the first, transpiration, the relative diffusive resistance of the stomata, and rates of net photosynthesis were measured simultaneously. If the differing sensitivity of photosynthesis in corn and soybeans was due solely to the diffusive resistance of the stomata, stomatal closure would have occurred in corn well before it did in soybean, and this would be reflected by transpiration and the relative diffusive resistance of the leaves. On the other hand, if photosynthesis was limited by changes in resistance within the mesophyll cells or by enzyme activity, stomatal behavior would bear little relationship to the inhibition of photosynthesis. Figure 2 shows that as leaf water potentials dropped, inhibition of photosynthesis did not occur unless stomatal closure and reduction in transpiration also occurred in the two species. Therefore, the initial inhibition of photosynthesis appeared to be well correlated with stomatal behavior.

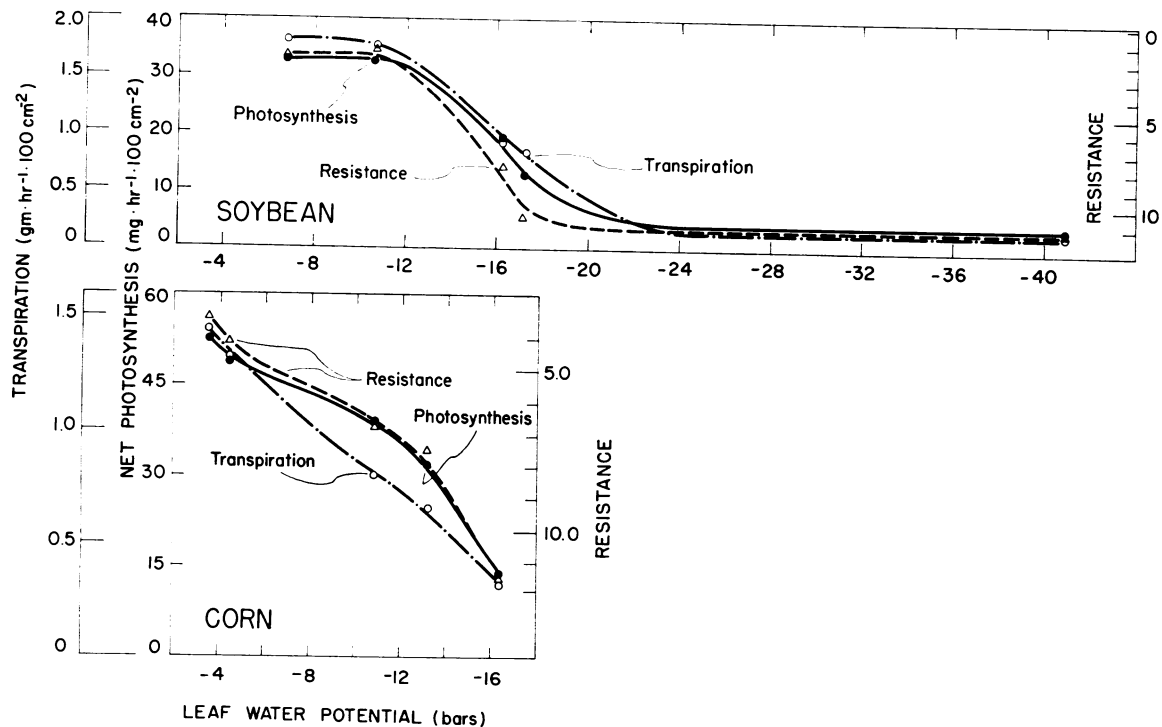


FIG. 2. Net photosynthesis, transpiration, and relative diffusive resistances of stomata in corn and soybean at various leaf water potentials. Relative diffusive resistances were determined as the cube root of the time required for the viscous flow of a standard quantity of air through the leaf under a standard pressure difference.

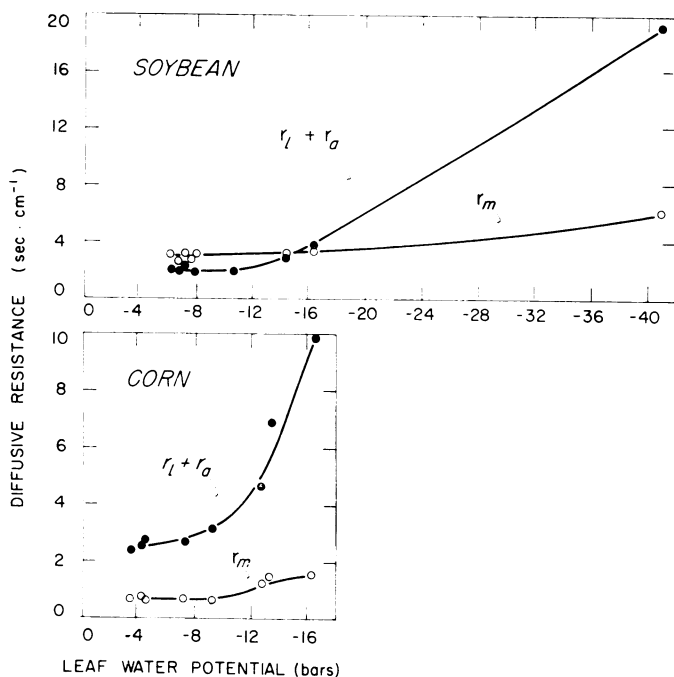


FIG. 3. Diffusive resistances to  $\text{CO}_2$  transfer in corn and soybean leaves having various water potentials. Resistances were measured in the intact shoots. Resistances ( $r_l + r_a$ ) represent the combined resistances of the gas phase in the leaf ( $r_l$ ) and the boundary layer around the leaf ( $r_a$ ). Boundary layer resistance was kept constant.

The second means of testing whether photosynthetic reduction was due to resistive or enzymatic changes involved calculating the diffusive resistances to the entry of  $\text{CO}_2$  into the leaf. The  $\text{CO}_2$  compensation points were  $0 \mu\text{l/liter}$  in corn and  $38 \mu\text{l/liter}$  in soybean (W. R. Ogren, University of Illinois, private communication). The  $\text{CO}_2$  compensation point does not appear to change as plant tissue becomes desiccated (24). Measurements with sunflower (Boyer, unpublished data) also confirm this in plants with leaf water potentials as low as  $-14$  bars.

Figure 3 shows  $r_m$  and ( $r_l + r_a$ ) for shoots of intact plants in the assimilation chamber. Boundary layer resistances were held constant during desiccation by similar positioning of the plants and stirring of the air during each measurement. As a result, changes in ( $r_l + r_a$ ) were primarily due to changes in  $r_l$ . In soybean, ( $r_l + r_a$ ) remained constant until leaf water potentials dropped to  $-11$  bars. In corn, ( $r_l + r_a$ ) rose whenever leaf water potential dropped. Thus, changes in ( $r_l + r_a$ ) matched those of photosynthesis.

If inhibition of photosynthetic rates results only from stomatal closure,  $r_m$  should remain constant as leaf water potentials decrease. This was true in soybean until leaf water potentials dropped to  $-16$  bars and in corn, to  $-10$  bars. Below these water potentials,  $r_m$  increased and ultimately doubled during severe desiccation.

## DISCUSSION

The photosynthetic behavior of corn and soybean differed considerably during desiccation. Net photosynthesis in corn decreased whenever leaf water potentials dropped; in soybean it was unaffected until leaf water potentials became lower than  $-11$  bars. Soybean was also less subject to death of tissue during severe drought than was corn. On the basis of these criteria, soybean appeared more able to withstand drought than corn. However, the photosynthetic rate of corn was higher than that of soybean over most of the desiccation range (Figs. 1 and 2).

These responses illustrate the difficulties in assigning levels of drought tolerance to plants. Species such as corn, which have the  $\text{C}_4$ -dicarboxylic acid cycle for fixing  $\text{CO}_2$ , often exhibit higher rates of photosynthesis than other species if well hydrated. If this pattern is characteristic of most species having the  $\text{C}_4$  pathway, it is probable that they are capable of greater carbon fixation during drought than are species that lack the pathway. In this sense, the data support the idea that these species are more tolerant of drought than those not having the pathway (14). The inability of corn to survive completely leaf water potentials below  $-20$  to  $-25$  bars may not be characteristic of the group as a whole. The *Atriplex* species known to have the pathway (22) are frequently exposed to desiccation and may be able to remain viable under more extreme conditions than corn. Also, the recovery of photosynthesis after a period of desiccation may be more rapid in  $\text{C}_4$  species than in other species. However, on the basis of the available data for corn, it appears that plants having the  $\text{C}_4$  pathway could be considered more tolerant to drought than other species only in the sense that they often have larger rates of  $\text{CO}_2$  fixation rather than any particular ability to withstand low leaf water potentials without reduction in photosynthetic rates and without tissue death.

The high sensitivity of photosynthesis to desiccation in corn is also suggested by comparison with species other than soybean. In sunflower, inhibition of photosynthesis begins at about  $-8$  bars (6), and consequently, its response is intermediate between corn and soybean. Photosynthesis in tomato and pine (8) also is less sensitive than in corn at most leaf water potentials. However, the complete inhibition that occurs in tomato and pine at leaf water potentials of  $-12$  to  $-15$  bars does not take place in sunflower or corn at these potentials.

The mechanism which determines the degree of desiccation bringing a reduction in photosynthetic rate is undoubtedly complex. As leaf water potentials drop, the levels of metabolic intermediates change (17), photosynthetic electron transport is inhibited (6, 15, 18), stomatal closure occurs (2, 8, 10, 11, 20, 23–25), and rates of respiration may increase or decrease (8, 19). Changes in rates of dark respiration of corn and soybean are too small to have an appreciable effect on rates of net photosynthesis (5). Also, the high light intensities used in the present experiments should have obscured any but the most extreme inhibition of photosynthetic electron transport (6). Alterations in the availability of metabolic intermediates or in enzyme levels should have been reflected by a change in the  $\text{CO}_2$  compensation point or in  $r_m$ , inasmuch as both of these factors are influenced by enzymatic activity. However, no change in  $r_m$  was observed in corn or soybean at potentials as low as  $-10$  and  $-16$  bars, respectively. Furthermore, when  $r_m$  did increase, the increase was small relative to the total magnitude of the diffusive resistance. For example, resistances ( $r_l + r_a$ ) accounted for 19 units of resistance in soybean at  $-41$  bars, but  $r_m$  accounted for 6 units and represented an increase of only 3.5 units. Consequently, the increase in  $r_m$  had little effect on photosynthetic response to desiccation because the rate-limiting portion of the diffusion pathway was located in the gas phase of the leaf, presumably as a result of stomatal closure.

In both species, therefore, the diffusive resistance of the stomata to  $\text{CO}_2$  entry appeared to be the primary factor limiting net photosynthesis in high light. The following observations also support this conclusion. (a) Inhibition of photosynthesis was always accompanied by inhibition of transpiration, regardless of whether it occurred at high potentials as in corn, or at moderately low potentials as in soybean. (b) Relative estimates of stomatal diffusive resistance indicate that resistance increased whenever rates of photosynthesis decreased in the two species. A similar behavior of photosynthesis, transpiration, and relative stomatal resistance has been found in sunflower (6). These findings are

consistent with those obtained with cotton (2, 23, 24) and pepper (2). However, it has been suggested (20) that inhibition of photosynthesis and transpiration is attributable to more than stomatal closure in corn. In the present paper, no evidence was found for these additional effects at moderate potentials. At potentials below  $-10$  bars, photosynthesis in corn declined slightly more than could be attributed to stomatal closure.

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