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Limitation of Acetylene Reduction (Nitrogen Fixation) by Photosynthesis in Soybean Having Low Water Potentials[1]

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ABSTRACT

The role of photosynthesis and transpiration in the desiccation-induced inhibition of acetylene reduction (nitrogen fixation) was investigated in soybean (*Glycine max* [L.] Merr. var. Beeson) using an apparatus that permitted simultaneous measurements of acetylene reduction, net photosynthesis, and transpiration. The inhibition of acetylene reduction caused by low water potentials and their aftereffects could be reproduced by depriving shoots of atmospheric CO_2 even though the soil remained at water potentials that should have favored rapid acetylene reduction. The inhibition of acetylene reduction at low water potentials could be partially reversed by exposing the shoots to high CO_2 concentrations. When transpiration was varied independently of photosynthesis and dark respiration in plants having high water potentials, no effects on acetylene reduction could be observed. There was no correlation between transpiration and acetylene reduction in the CO_2 experiments. Therefore, the correlation that was observed between transpiration and acetylene reduction during desiccation was fortuitous. We conclude that the inhibition of shoot photosynthesis accounted for the inhibition of nodule acetylene reduction at low water potentials.

The fixation of N_2 by legumes requires that the soil be able to supply both water and certain gases (2, 9, 18) to the nodules and host plant. Desiccated and flooded soil inhibit N_2 fixation (8, 10, 14-16, 23-26). Acetylene reduction (2, 6, 9, 12) was undetectable in soybean during extremes of soil flooding and desiccation (14). Nodule activity displayed a narrow optimum that was determined by the simultaneous need for the soil to be dry enough to permit gas exchange but wet enough to supply water for the bacteroids and host plant (14).

The rise in nodule activities as the soil desiccated from the flooded condition could be explained by increased gas diffusion through the soil (10, 14, 26). Although inhibitory desiccation (that is, desiccation beyond that required for maximum nodule activity) has been explained in terms of reduced nodule respiration (8, 24, 26), the reduction in respiration was not as severe as that displayed by acetylene reduction (14, 24), and there was a close correlation of photosynthesis and transpiration in the host with acetylene reduction by the nodules (14).

Nodules need a continuous supply of carbohydrate for N_2 fixation (2, 6, 9, 12, 17). Photosynthesis is the source of the carbohydrate (17, 20). Transpiration is also important, since it has been estimated that 80 to 90% of the N_2 fixation by nodules is transported to the host plant via the transpiration stream (20-22) and an accumulation of ammonia can cause repression of nitrogenase synthesis, at least in free-living organisms (7, 19).

In a previous study we showed that acetylene reduction, photosynthesis, and transpiration were inhibited similarly at low water potentials (14). This study was done to determine whether photosynthesis or transpiration controlled rates of acetylene reduction in soybean plants that were subjected to inhibitory desiccation in soil.

MATERIALS AND METHODS

Soybean (*Glycine max* [L.] Merr. var. Beeson) seeds were germinated for 3 days in vermiculite. Single seedlings were inoculated with a commercial preparation of *Rhizobium japonicum* and were transplanted to a mixture of soil, peat, and perlite (2:1:1) in clay pots. The seedlings were grown in a constant environment chamber under the conditions described previously (14). During the first 10 days after transplanting, the seedlings were watered with a Hoagland solution (13) containing nitrate every 3rd day and with water all other days. After 10 days, plants were watered daily either with N_2-free nutrient solution (13) or with water on alternate days. During the growing period, all branches were excised so that only the central stem remained.

After 4 to 5 weeks of growth, rates of net photosynthesis, dark respiration, and transpiration of whole shoots of the intact plants were determined daily with an IR gas analyzer and assimilation chamber (1, 5) under the conditions previously described (14). Light from seven 300-w incandescent spot lamps was filtered through IR-absorbing glass and 10 cm of water and provided an irradiance of 1.6 cal cm^{-2} min^{-1} at leaf height, which was saturating for photosynthesis.

For measuring acetylene reduction, the undisturbed pot-soil-root system of an intact plant was sealed in an acetylene reduction apparatus (14). Acetylene equivalent to 10% of the net air volume of the chamber was injected. Gas samples (0.5 ml) were withdrawn every 30 min, and ethylene production was determined as previously described (14). The apparatus for measuring acetylene reduction was used at the same time the shoot of the plant was enclosed by the assimilation chamber, and shoot and nodule activities were therefore followed simultaneously.

After the measurements of CO_2 exchange and transpiration by the shoot, the assimilation chamber was opened and a leaf toward the bottom of the plant was sampled for a determination of leaf

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water potential. For the determination, a disc was removed from interveinal tissue, which had been washed with distilled H_2O and permitted to dry before the measurements of gas exchange, and the disc was placed in a thermocouple psychrometer chamber for an isopiestic measurement (3) of leaf water potential as previously described (14). Immediately after the leaf had been sampled, the plant was removed from the gas exchange and acetylene reduction apparatus and a soil sample was loaded into a psychrometer chamber for an isopiestic measurement of the soil water potential, as previously described (14).

RESULTS

Photosynthesis. Photosynthate might limit nodule activity at low water potentials, but only if the roots and nodules did not have a large reserve that could be used by the bacteroids. To test whether reserves for acetylene reduction existed in the soybean roots, the stem of a plant was girdled to remove the phloem just above the soil level, and acetylene reduction, photosynthesis, transpiration, dark respiration by the shoot, and leaf, nodule, and soil water potentials were measured for several days. Figure 1 shows that in the absence of translocation to the roots, acetylene reduction was inhibited 24 hr after girdling. Thus, root reserves were not adequate to support rapid acetylene reduction for more than a few hours. The inhibition could not be attributed to changes in transpiration, dark respiration, or water potentials which remained constant (Fig. 1). The slight decrease in ne photosynthesis probably did not affect nodule activity because of the girdled stem.

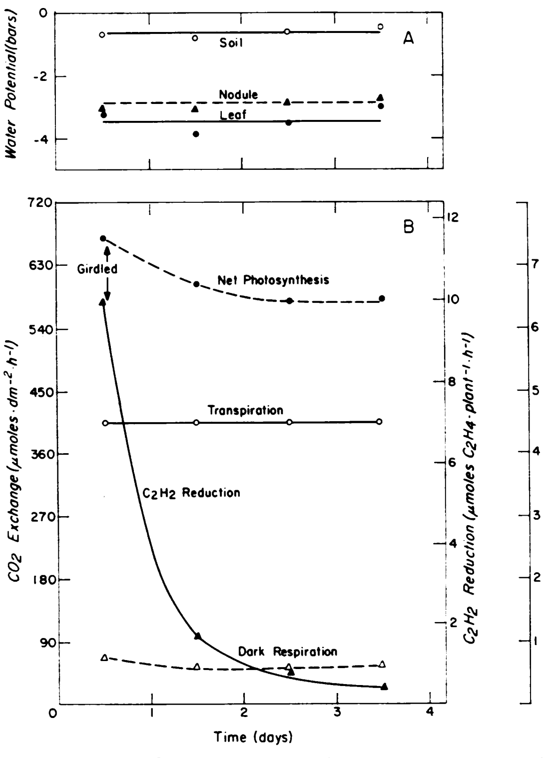

FIG. 1. Response of acetylene reduction, net photosynthesis, dark respiration of the shoot, and transpiration of soybean to girdling of the stem. A length of 0.5 cm of the tissues external to the xylem was carefully removed 3 cm above the base of the stem. The girdled section was coated with a thin layer of petrolatum to prevent water loss directly from the exposed xylem. Water was added to the soil in the usual way to maintain high leaf water potentials throughout the experiment. A: Soil, nodule, and leaf water potentials. The nodule water potentials were determined from a relationship measured previously with a separate group of plants (14). B: Acetylene reduction, net photosynthesis, dark respiration by the shoot, and transpiration.

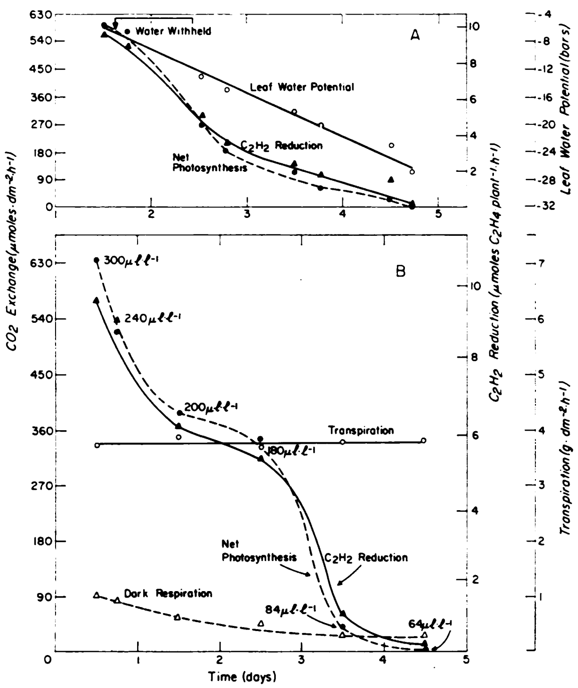

FIG. 2. Effect of low external CO_2 concentrations on net photosynthesis, dark respiration of the shoot, transpiration, and acetylene reduction of a soybean plant. The plant exposed to low CO_2 (B) was well watered and had soil, nodule, and leaf water potentials of -2, -4, and -5 bars, respectively, throughout the experiment. The CO_2 concentrations were maintained for the entire photoperiod of each day and are shown beside the photosynthesis data. During the dark period, the plant was returned to the controlled environment chamber in which the plant had been grown. For comparison, a plant was subjected to low water potentials (A) but was exposed to 300 $\mu l \cdot l^{-1}$ of external CO_2 throughout the measurements. Water was withheld as in a typical desiccation experiment, and net photosynthesis and acetyllene reduction were measured.

Since there were few root reserves for acetylene reduction, nodule activity should have been closely coupled to the photosynthetic activity of the shoot. If photosynthesis limited nodule activity at low leaf water potentials, it should have been possible to reproduce the effects of inhibitory desiccation by depriving the shoot of CO_2, even though soil water was available and should have favored acetylene reduction. Figure 2B shows that lowered CO_2 concentrations could give rates of photosynthesis and acetylene reduction that were similar to those occurring during desiccation (Fig. 2A). Leaf, nodule, and soil water potentials remained high and constant throughout the experiment. These data support the hypothesis that photosynthesis limited acetylene reduction at low water potentials. Dark respiration continued at significant rates while acetylene reduction was reduced virtually to zero (Fig. 2B). Thus, respiration was unlikely to cause the decreases in acetylene reduction. It is also noteworthy that transpiration remained constant. If the effects of desiccation were caused by decreased transpiration rather than decreased photosynthesis, acetylene reduction should have been unaffected during the CO_2 deprivation experiment.

To support the conclusions of the CO_2 deprivation experiment, an experiment was done to determine whether the effects of desiccation could be reversed if photosynthesis increased. A soybean plant was desiccated in the usual way, but measurements were made early each day at a CO_2 concentration of 300 $\mu l \cdot l^{-1}$ and

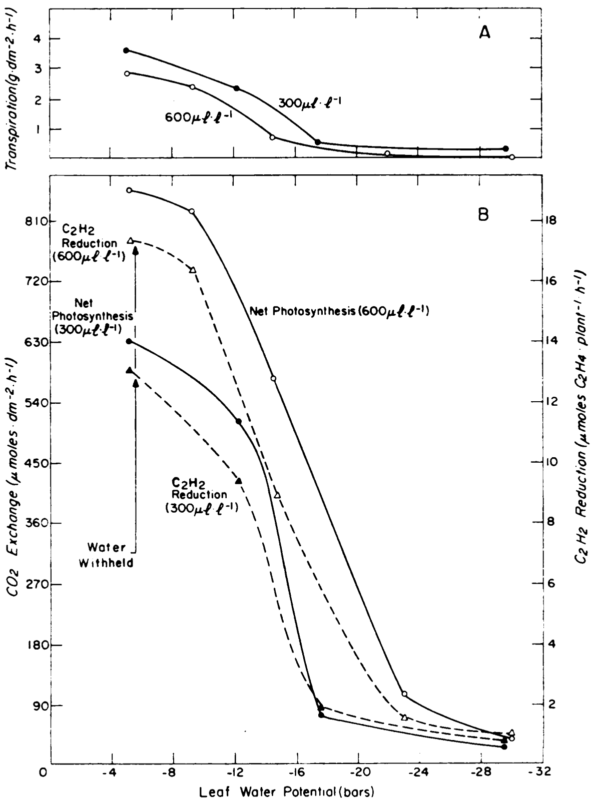

FIG. 3. Transpiration (A), acetylene reduction and net photosynthesis (B) at various leaf water potentials in a soybean plant exposed to air containing 300 and 600 $\mu l \cdot l^{-1}$ of CO_2. Exposure to 300 $\mu l \cdot l^{-1}$ occurred from 0700 until 1230 hr and exposure to 600 $\mu l \cdot l^{-1}$ occurred from 1230 until 1730 hr. The last 2 hr of each exposure period were used for measurements. Leaf water potentials were determined immediately at the end of each treatment.

later in the day at 600 $\mu l \cdot l^{-1}$. For the 3 hours prior to the measurements, the plants were pretreated under measurement conditions. The experiment was designed in this way because acetylene reduction responds to changes in shoot activities within 30 to 60 min under our conditions (14). New, reasonably stable rates of acetylene reduction occur within 2 to 3 hr (14). The 3-hr pretreatment permitted any changes in photosynthesis to be expressed as changes in acetylene reduction. By carrying out the entire experiment with a single plant, any observed differences were significant.

Both nodule activity and photosynthesis increased after exposure of the plant to 600 $\mu l \cdot l^{-1}$ of CO_2 (Fig. 3B) at all leaf water potentials. CO_2 effects on transpiration were not related to effects on acetylene reduction (Fig. 3, A and B).

Transpiration. In the above experiments, photosynthesis and transpiration changed simultaneously. Hence, an experiment was done in which transpiration was varied in a way that was independent of net photosynthesis and dark respiration.

In plants having high leaf water potentials, a decrease in transpiration to 0.43 of the original rate (experiment 1, Table I) had no effect on acetylene reduction, net photosynthesis, or dark respiration of the shoot. An increase in the transpiration from 0.45 to 1 × (experiment 2, Table I) also was without effect. If a similar change in transpiration had been caused by desiccation, nodule activity would have been expected to change by about the same amounts as transpiration (14).

Direct Effects of Low Nodule Water Potentials. An attempt was made to estimate the direct effects of desiccation on nodule activity by excising the nodules and 1 cm of root, desiccating the nodules in air in the dark for various times, and measuring their activities for acetylene reduction. Nodule water potentials were then measured after the activities for acetylene reduction had been determined.

Regardless of nodule water potential, detached nodules had lower activity than those that remained attached and undisturbed in the soil (Fig. 4). Furthermore, nodule activity was inhibited to a greater extent by desiccation in air than by desiccation *in situ*. Since this experiment did not duplicate the effect of desiccation *in situ*, it is likely that artifacts had been caused by detachment of the nodules and this line of investigation was discontinued.

Recovery from Low Leaf Water Potentials. When soybean plants were desiccated for 4 days and then rewatered, acetylene reduction, net photosynthesis, and transpiration remained below the initial rates even though leaf, nodule, and soil water potentials rapidly returned to the initial levels (Fig. 5, A and B). Clearly, exposure of the plants to low water potentials caused aftereffects. The aftereffects on photosynthesis and transpiration have been attributed largely to incomplete stomatal opening (4).

If the lack of photosynthate at low water potentials accounts for the decrease in acetylene reduction, it might also account for the aftereffects of low water potentials on acetylene reduction. To determine whether this was true, we deprived a plant of external CO_2 without desiccating the soil. Figure 6 shows that nodule activity remained inhibited when CO_2 was resupplied after the plant was exposed to low CO_2 concentrations for 3 days. Thus, the decrease in photosynthate caused by CO_2 deprivation also resulted in an inhibition of nodule activity well after the return of photosynthesis to reasonably high levels.

DISCUSSION

The girdling experiment demonstrated that root reserves supporting acetylene reduction were low in soybean. Consequently, the activity of the nodules must have depended largely on current photosynthesis. This activity agrees with previous work that showed a rapid response of nodule activity to excision of the whole shoot (14). The sustained but low activity observed (Fig. 1B) after the initial large decrease suggests that there are reserves capable of supporting low nodule activity for a considerable time. It has been suggested that nodules contain compounds, notably

Table I. *Effect of Different Rates of Transpiration on Photosynthesis, Respiration of Shoot, and Acetylene Reduction of Well Watered Soybean Plants*

Leaf, nodule, and soil water potentials were −6, −4, and −2 bars, respectively, throughout the experiment. The low wind treatment involved a relative humidity of 61.5% and average wind speed of 0.4 m·sec⁻¹, and the high wind treatment involved a relative humidity of 58.5% and average wind speed of 1.5 m·sec⁻¹. Otherwise, all measurement conditions were as described.

	Activities			
	Expt. 1		Expt. II	
	Low wind	High wind	Low wind	High wind
Transpiration (g dm^{-2} hr^{-1})	1.60	3.68	1.59	3.53
C_2H_2 reduction (μmoles C_2H_2 $plant^{-1}$ hr^{-1})	8.00	8.20	8.40	8.60
Photosynthesis (μmoles dm^{-2} hr^{-1})	583	589	568	576
Dark respiration (μmoles dm^{-2} hr^{-1})	75	73	71	73

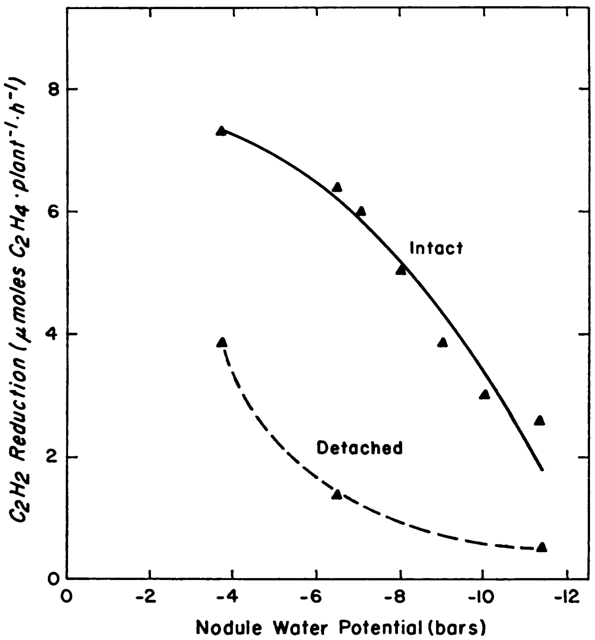

FIG. 4. Acetylene reduction at various nodule water potentials for nodules *in situ* (intact) and nodules excised with a 1 cm segment of root (detached). The detached nodules were desiccated in air in the dark in the controlled environment room. After 0, 20, and 40 min of desiccation, they were sealed in a bottle of 122 ml containing an atmosphere saturated with water vapor. Acetylene reduction was measured inside the bottle. The water potentials of the detached nodules were measured immediately after the acetylene reduction assays were completed. For intact nodules, acetylene reduction was measured on an undisturbed pot-soil-root system of an intact plant that had been desiccated by withholding water from the soil. Leaf and soil water potentials were then measured. Nodule water potentials were determined from the relationship between nodule water potentials and soil and leaf water potentials observed with a separate group of plants (14).

poly-β-hydroxybutyrate, that might support nodule activity (28). However, during the 3 to 4 days of the present experiments, even these reserves appeared to be almost depleted (Fig. 1B).

As a result, it was possible to simulate the effects of desiccation on nodule activity by controlling the rate of photosynthesis (Fig. 2B). The rate of photosynthesis at low leaf water potentials was also increased by increasing the CO_2 concentration around the shoot, and a concomitant increase in the activity of the nodules took place (Fig. 3B). This experiment provides strong evidence that acetylene reduction was not only correlated with photosynthesis but was indeed limited by the inhibition of photosynthesis at low leaf water potentials. If some other factor had limited acetylene reduction, an increase in rates of photosynthesis should have had little effect on nodule activity.

Others have shown that N_2 fixation can be increased by increasing the CO_2 available for photosynthesis (11, 27). They concluded that the availability of photosynthates limited the rate of N_2 fixation. The present work indicates that this conclusion extends to all levels of water availability except those in moist soil, where gas diffusion through the soil can become more limiting than photosynthate (14).

The acetylene reduction assay should have responded to the activity of bacteroid nitrogenase and the availability of reductant and ATP for N_2 fixation. It would not have reflected the availability of the carbon chains with which ammonia combines, however, since the product of acetylene reduction, ethylene, diffuses out of the nodules as a gas. Consequently, these experiments would not have detected a limitation of N_2 fixation due to carbon chain availability. Since the carbon chains are derived from photosynthate, however, our conclusions would be unaltered.

In addition to the involvement of photosynthesis in nodule activity during desiccation, photosynthesis accounts for the aftereffects of desiccation. The aftereffects of desiccation on nodule activity could be reproduced by depriving the nodules of photosynthate when the plants were kept well watered (Fig. 6). We do not know why nodule activity did not readily recover when the plants returned to high water potentials, but perhaps regrowth of bacteroid tissue is required after a period of photosynthate deprivation.

A relationship could not be demonstrated for transpiration and nodule activity under dry conditions (Figs. 2B, 3B, 6; Table I). Of the experiments that were conducted, the most straightforward is that in which transpiration was varied independently of other shoot activities (Table I). Thus, although the transpiration stream is responsible for transporting the majority of the products of N_2 fixation (20–22), the inhibition of transpiration apparently did not inhibit nodule activity at low water potentials.

SpRent (24, 25) explains the effects of desiccation in terms of withdrawal of bacteroid cytoplasm from cell walls. She suggests that disruption of the plasmodesmata might cause a barrier to the exchange of metabolites between the bacteroids

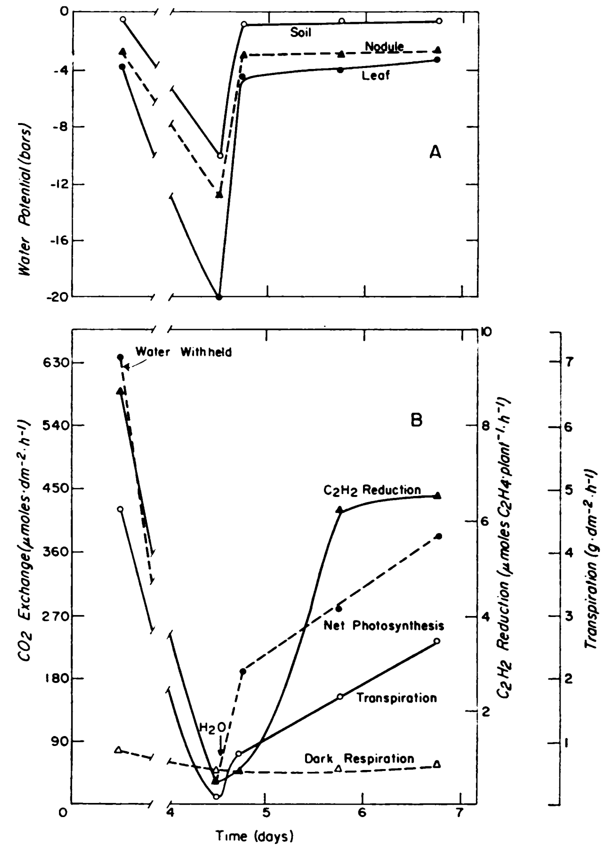

FIG. 5. Recovery of rates of photosynthesis, transpiration, dark respiration of the shoots, and acetylene reduction in a soybean plant after desiccation to a leaf water potential of −20.1 bars. A: Soil, nodule, and leaf water potentials; B: net photosynthesis, transpiration, dark respiration of the shoot, and acetylene reduction. The plant was returned to the constant environment chamber after each daily measurement had been completed. The nodule water potentials were obtained from the relationship between nodule water potentials and soil and leaf water potentials determined with a separate group of plants (14).

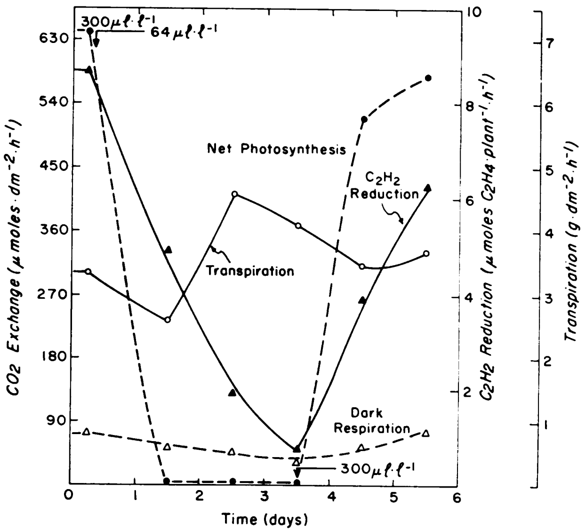

FIG. 6. Acetylene reduction, net photosynthesis, dark respiration of the shoot, and transpiration following the exposure of a soybean plant to a low CO_2 concentration for 3 days. The plant was exposed to 64 $\mu l \cdot l^{-1}$ for the entire photoperiod each day and then to 300 $\mu l \cdot l^{-1}$ for 2 days. During the dark period each day the plant was returned to the controlled environment chamber in which it had been grown. The plant had soil, nodule, and leaf water potentials of −2, −4, and −5 bars, respectively, throughout the experiment.

and the host tissues. Since her work was done with excised nodules, this interpretation should be approached with caution in view of the large differences in the response of excised and intact nodules (Fig. 4).

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