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Effect of Leaf Water Deficit on Stomatal and Nonstomatal Regulation of Net Carbon Dioxide Assimilation[1]

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ABSTRACT

The effect of leaf water deficit on net CO_2 assimilation was studied under two conditions: in one, the stomata were allowed to contribute to the regulation of CO_2 assimilation; in the other, air was forced through the leaf at a constant rate to overcome the effects of change in stomatal resistance accompanying changes in leaf water deficit. When the stomata were allowed to regulate the gaseous diffusive resistance of the leaf, CO_2 assimilation decreased with increasing leaf water deficit. However, when air was forced through the leaf, the rate of assimilation was not inhibited by increasing leaf water deficit. The results indicate that the inhibition of net CO_2 assimilation with increasing leaf water deficit is a consequence of an increase in the diffusive resistance to gas exchange and not of a change in apparent mesophyll resistance.

Two modes of action of leaf water deficits on net CO_2 assimilation are possible: (*a*) a physical effect wherein an increase in leaf water deficit decreases the stomatal aperture, increases the leaf resistance to gas exchange resulting in a decrease in the supply of CO_2 at the surface of the mesophyll cells; (*b*) a biochemical effect associated with partial protoplasmic dehydration and a reduced rate of reaction of CO_2 at the site of assimilation. The available evidence (8, 13) leaves little doubt that the decrease in net CO_2 assimilation accompanying an increase in leaf water deficit can be attributed in part to an increase in leaf resistance to CO_2 transport in the gas phase. Whether, with an increasing water deficit, there is also a change in mesophyll resistance to CO_2 transport within the cells and reaction rates at the site of CO_2 fixation that parallels the change in diffusive resistance is a matter of both controversy and importance. *A priori,* it would not be surprising to find reductions in net CO_2 assimilation brought about by a reduction in the biochemical activity of the chloroplasts as leaf water content approaches very low levels associated with permanent wilting. But our concern is with whether there is an effect of leaf water deficits on the biochemical reactions associated with CO_2 assimilation when these deficits are in the range usually occurring in plants, *i.e.,* between maximum turgor and temporary wilting.

The assumption that leaf water deficits affect CO_2 assimilation independent of changes in leaf resistance to CO_2 diffusing has had rather wide acceptance (8, 12–14). Experiments with *Chlorella* (5) and a liverwort (15), both of which lack stomata, are frequently cited to show reductions in CO_2 assimilation with decreasing cell water potential. In more recent work, Troughton and Slatyer (16) determined the calculated mesophyll resistance of cotton leaves in O_2-free air and normal air over a range of relative leaf water contents with the leaf resistance calculated by the water vapor exchange method. They show that the calculated mesophyll resistance was unaffected by variations in relative water content down to 60%. They conclude that "the constant mesophyll resistance at all levels of water stress indicates that liquid phase diffusion of CO_2 is unaffected, but is not evidence that the photochemical or biochemical reactions associated with CO_2 fixation are unaffected by water stress, unless these components contribute to r_m." [2] Boyer (3), working with sunflower, concluded that at leaf water potentials below −11 bars, photosynthesis under high light was probably limited by reduced photochemical activity of the leaves and not by reduced rates of CO_2 diffusion; however, in an earlier study Boyer (2) found little or no effect of leaf water potential on r_m in corn and soybean, indicating that effect of leaf water potential on r_m may vary among species.

In this report we describe experiments in which air was forced through the leaf at the adaxial surface at a constant flow rate (mass transport). This procedure, a modification of one suggested by Heath (6), eliminates the effect of variations in stomatal resistance on the supply of CO_2 at the mesophyll cell walls and allows an examination of the effect of water deficit on the mesophyll resistance. The r_m includes the diffusion resistance from the surface of the cell wall to the site of reaction and the carboxylation efficiency at the site of reaction.

In a second, but converse approach to the problem, the atmosphere surrounding the leaflet was abruptly changed from a relatively high to low humidity (dew-point 15 to −12 C). This abrupt change was a means of effecting gradual stomatal closure and a corresponding decrease in CO_2 and water vapor exchange between leaf and atmosphere without a detectable change in leaf water deficit.

MATERIALS AND METHODS

Experiment 1. Soybean (*Glycine max* [L.] Merr.), mung beans (*Phaseolus aureus,* Berkin), and corn (*Zea mays,* var. Ohio 708) were grown in a greenhouse in clay pots. When the beans were in flower and the corn plant in the early silk stage

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[2] Abbreviations: r_m: mesophyll resistance; RWC: relative water content; *F:* net CO_2 assimilation; *T:* transpiration; *P:* pressure.

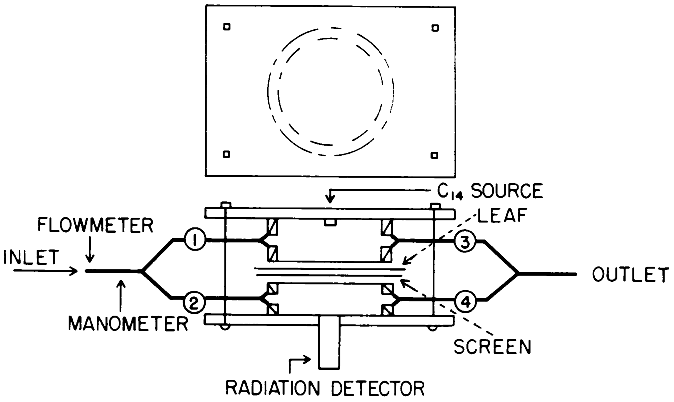

FIG. 1. Schematic diagram of leaf chamber.

of development, they were transferred to a growth chamber where the experiments were conducted. A single, fully expanded bean leaflet or corn leaf was enclosed in a Plexiglas leaf chamber (Fig. 1). The corn and mung bean leaves were attached to the plant during the experiment. The soybean leaf was attached to the plant in the initial portion of the run, but because of an absence of change in assimilation rate, presumably because of a small rate of change in leaf water content, the leaf was severed from the plant to expedite the experiment. The outside diameter of the cylindrical chamber was slightly smaller than the width of the leaf and enclosed a circular portion of the leaf supported on a coarse mesh wire screen. In experiments with corn, the leaf chamber was modified to include a beta gauge with a small ^{14}C source mounted on the ceiling of the upper chamber and a Geiger tube inserted in a hole and sealed in the floor of the lower chamber. The gas inlet tube was connected to a manually adjustable flowmeter and a water-filled manometer. Net CO_2 assimilation was determined by measuring the difference in CO_2 content of the air stream before and after it had passed through the leaf chamber at a constant flow rate. An MSA LIRA M-200 infrared CO_2 analyzer was used for CO_2 measurements. The output of the analyzer was recorded on a strip chart potentiometer. The CO_2 concentration in the input air was constant and about 330 $\mu l\ l^{-1}$. Illumination, provided by cool white fluorescent tubes, was about 0.17 cal cm^{-2} min^{-1} (400–700 nm) at the leaf surface. Growth chamber temperature was maintained at 24 C.

The leaf chamber was designed to permit two modes of operation. In one the air stream was allowed to flow over the upper and lower surface of the leaf (diffusive flow). With this mode of flow, stomatal resistance was operative and allowed to affect CO_2 exchange rates. In the other mode of operation, valves at position 2 and 3 (Fig. 1) were closed and the air was forced through the leaf (mass transport). Assimilation rate with each mode of flow was determined by alternating, in sequence, between the two modes as leaf water content declined. With corn, the relative leaf water content, measured by beta gauging, and assimilation rate were determined simultaneously for each mode of flow throughout the experiment. A constant flow rate was maintained by manual adjustment of a needle valve in the flowmeter. Flow rate was about 85 cm^{3} min^{-1} for beans and about 100 cm^{3} min^{-1} for corn per 18 cm^{2} of leaf surface confined in the circular chamber. Within each experiment the same flow rate was used for both modes of operation. Neither leaf temperature nor transpiration rates were measured in this experiment. Several attempts to measure leaf temperature with a thermocouple placed in contact with the lower surface of the leaf resulted in punctures when the leaf was forced against the screen by air pressure. The punctures invalidated the mass transport mode of operation. Air temperature, measured with a thermocouple suspended in the lower portion of the chamber, was about 27 C. Measurements of transpiration were planned as a part of the experiment, but moisture condensed in the form of droplets on the walls of the lower chamber. The accumulation of condensed water and the possibility of its subsequent release into the air stream voided accurate transpiration measurements. The relatively low flow rate through the chamber and lowered temperature of the bottom half of the chamber caused by shading from the leaf were probably responsible for the condensation.

The relative water content of the corn leaf was inferred from the β-gauge count rate after establishing the functional relation between RWC and count rate (10) at the end of the run. With corn it was possible to obtain a continuous, simultaneous determination of net CO_2 assimilation and RWC for each mode of flow, over the leaf and through the leaf. With the beans, the initial high level of RWC was determined at the beginning of a run on a leaflet adjacent to the test leaflet and on the test leaflet at the end of the run. In the experiments with corn and mung beans, the leaves remained attached to the plant. In the experiment with the soybean, the change in leaf water content and assimilation rate were occurring very slowly so the leaf was severed from the plant to expedite the experiment. Water was not added to the soil during a run. As soil water deficit increased, RWC of the leaf decreased and CO_2 assimilation rate was determined over a range of RWC by alternate use of the diffusive and mass transport flow modes, *i.e.*, with and without the regulating influence of stomatal resistance.

Experiment 2. In this experiment only soybeans were used. A single attached leaflet was enclosed in a leaf chamber equipped with a β-gauge. The leaf chamber is described in a companion paper (11). Relatively humid air was passed over both sides of the leaf until a constant rate of assimilation was observed. Then the air supply to the leaf chamber was abruptly changed to one with low humidity (10% relative humidity) to induce stomatal closure (9). Net CO_2 assimilation, transpiration, and RWC were continuously and simultaneously monitored for about 80 min following the step change in humidity. At the end of about 80 min, the petiole was cut to interrupt the supply of water to the leaf. Measurements of photosynthesis and transpiration were continued as RWC of the leaf declined. Transpiration was calculated from dew-point determinations of the input and exhaust gas using a dew-point hygrometer (Cambridge System Model 880). Flow rate was approximately 1.7 liter min^{-1} and leaf area 0.6 dm^{2}.

RESULTS AND DISCUSSION

Experiment 1. The results of the experiment with corn (Fig. 2) show that when the air stream flowed over both surfaces of the leaf (diffusive mode), *F* declined as RWC decreased below 92%. When air was forced through the leaf (mass transport mode) to hold constant the effect of increasing stomatal resistance, or other resistances to gas transport, there was no detectable change in F over the range in RWC. The results of experiments with mung beans and soybeans (Figs. 3 and 4) are similar to the one with corn except that when using the mass transport mode wth soybeans there was a decline in assimilation rate when RWC approached the very low value of 50%. The reason for the higher rates of assimilation associated with the mass transport mode are not known. Possibly the boundary layer resistance at the leaf surface, which existed when the diffusive mode was employed, was eliminated or greatly reduced by mass transport. Also, the intercellular resistance to CO_2 transport may have been less with mass transport than with the diffusive mode. The higher assimilation rate associated with mass transport may have been accompanied by a higher water loss rate and correspondingly lower leaf temperature. However, subsequent experiments, conducted for a different purpose, indicate that over an air temperature range of 20 to 30 C, assimilation rates obtained with mass transport are always higher than those obtained with the diffusive mode.

In the mass transport mode of operation the leaf chamber and manometer function as a viscous flow porometer with the manometer pressure change providing an indirect indication of a change in the combined stomatal and intercellular resistance to gas transport. As RWC declined with time, the pressure required to maintain a constant flow rate increased indicating an increase in viscous flow resistance. The increase in viscous flow resistance is taken as an indication of an increase in the diffusive or stomatal resistance on the basis of the relation between viscous flow and diffusive resistance (7). Although air was forced through the leaf at all data points in the mass transport mode (Figs. 3 and 4), measurements of pressure in Figure 4 were discontinued after 70 min because pressure was increasing too rapidly to determine with reasonable accuracy.

The decrease in CO_2 assimilation rate (diffusive mode) associated with declining RWC reported here and in a companion paper (11) has been observed by others. This decrease in CO_2 assimilation has been attributed to either an increase in gas-phase resistance in the stomatal and intercellular pathway, or to an increase in mesophyll resistance, or to both. The mesophyll resistance contains components of CO_2 absorption at the liquid-air interface in the surface of the mesophyll cell walls, CO_2 transfer components within the cell, and the chemical reaction of CO_2 at the site of fixation. Because the effect of a change in gas-phase resistance was held constant in the mass-

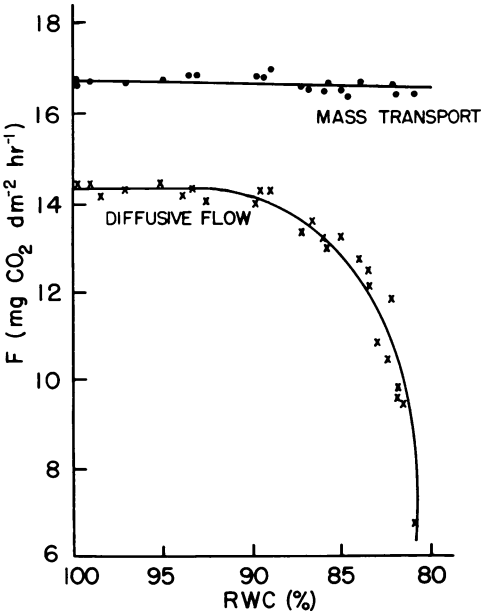

FIG. 2. Net CO_2 assimilation (*F*) by corn at various relative leaf water contents (RWC) for diffusive and mass transport modes of air flux.

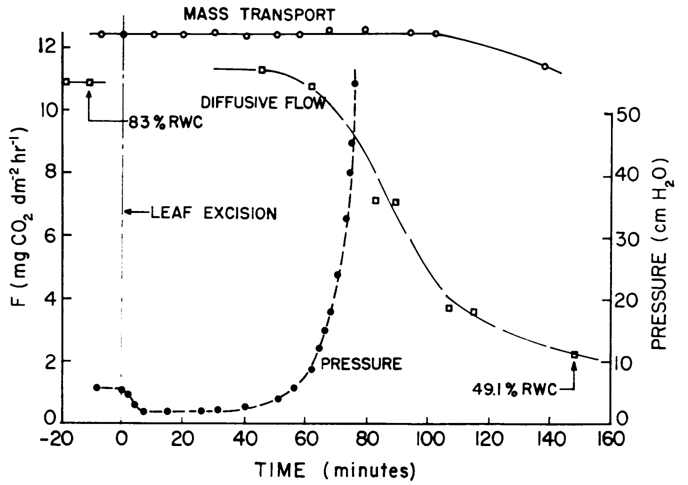

FIG. 4. Time course of net CO_2 assimilation by soybeans for diffusional and mass transport modes of air flux as relative water content declined from 83% to 49.1%.

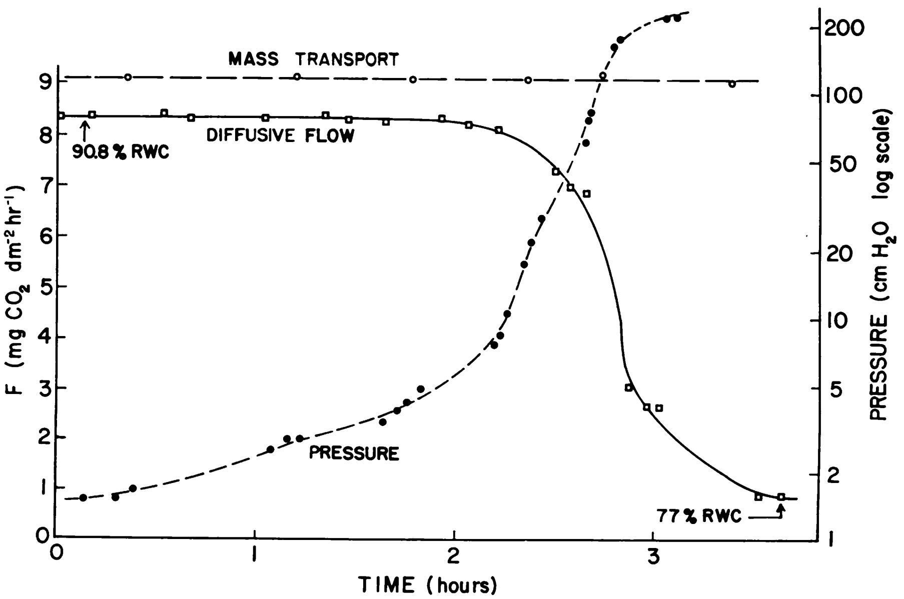

FIG. 3. Time course of net CO_2 assimilation (*F*) by mung beans for diffusive and mass transport modes of air flux as relative water content (RWC) declined from 90.8% to 77%. Manometer pressure curve (●) indicates increase in pressure required to maintain constant air flux through the leaf and corresponding increase in gas phase resistance.

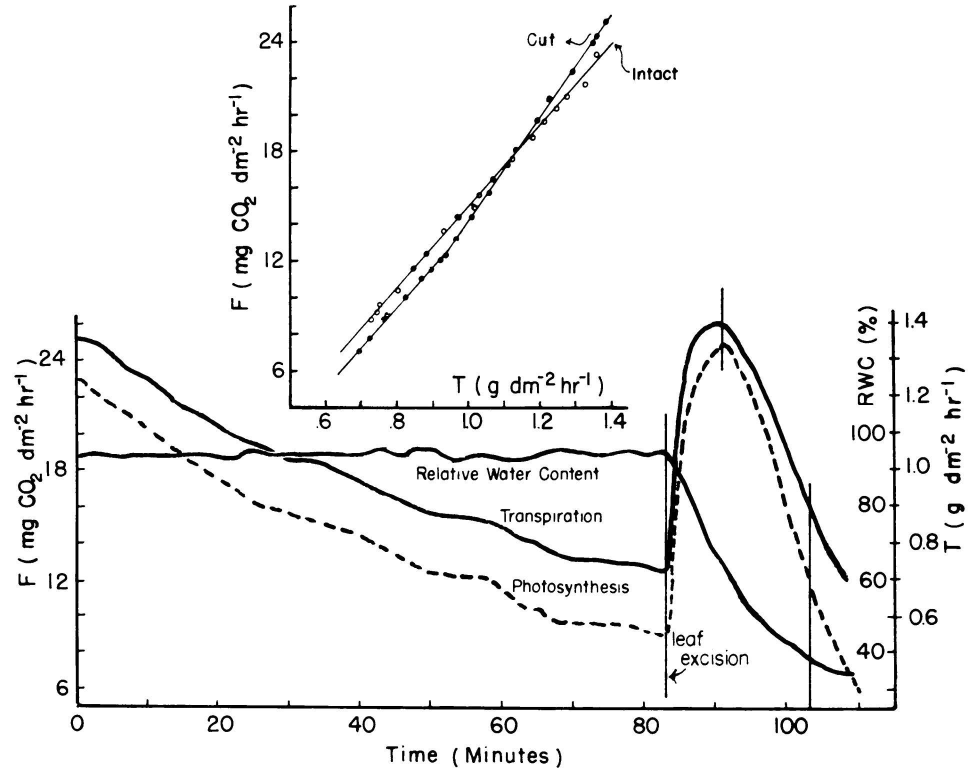

FIG. 5. Time course of net CO_2 assimilation, transpiration, and relative water content of an attached soybean leaf immediately following the abrupt change in the air stream from high to low humidity and after leaf excision. The inset graph indicates the relation between net CO_2 assimilation and transpiration during constant and declining relative leaf water content.

flow mode, the absence of a change in rate of CO_2 assimilation as RWC declined indicates that there was no change in mesophyll resistance associated with a decline in RWC. From these results we infer that the decrease in assimilation associated with declining RWC is caused by an increase in gas-phase resistance and not by changes in mesophyll resistance. The duration of leaf water deficits in these experiments was relatively short, (2 to 3 hr with beans and about 8 hr with corn). Extending the duration of the water deficit over several days may produce an effect on mesophyll resistance that was not detectable in the experiments reported here.

Experiment 2. The lower portion of Figure 5 shows the time course of *F*, *T*, and RWC, immediately following the abrupt step change from high to low humidity in the bulk air passing over the leaf. Immediately following the change *F* and *T* decreased with time, but surprisingly there was no significant change in RWC over the same interval of time. Lange *et al.* (9) indicate that when the water vapor pressure gradient between the guard cells and the atmosphere surrounding the leaf is abruptly increased, there is a direct loss of water from the guard cells, a decrease in their turgor pressure, and partial to complete closure of the stomata. The parallel decrease in *T* and *F* is evidence that stomatal resistance was increasing with time after the step change. That this stomatal resistance increase occurred in the absence of any measurable change in RWC indicates that although the water content of the leaf remained relatively high and unchanged, the flow of water to the guard cells was not sufficient to maintain their turgor.

At 82 min the petiole of the leaflet was cut to interrupt the supply of water to the leaf. Immediately there was a parallel rise in both *F* and *T* to a peak followed by a decline while RWC declined rapidly in the same interval. The rapid rise in *F* and *T* following removal of the leaf from the plant is a well known phenomenon. It is attributed to the transient opening of the stomata brought about by a decrease in the turgor pressure of the epidermal cells surrounding the guard cells and a relaxation of the force of the epidermal cells on the guard cells allowing the stomata to open (11, 12). With a continuing drop in RWC the turgor pressure in the guard cells declines and the stomata gradually close and reduce the rate of gas exchange between the leaf and surrounding atmosphere.

The upper portion of Figure 5 is a plot of the association between F and T before severing the leaf from the plant (0- to 80-min interval) and after cutting the leaf from the plant. The data for the cut leaf were taken in the interval between the two verticle lines of the lower graph (points b and c) when *F* and *T* were decreasing. The ratio *F*/*T* when RWC was constant and *F* was declining (0–80 min) was not sensibly different from what it was after the leaf was cut and RWC declined along with the decline in *F* and *T*. These data indicate that net CO_2 assimilation is regulated by the stomata without a detectable effect of leaf water deficit on the mesophyll resistance (1). *F* and *T* are independent processes but both are regulated proportionally by stomatal resistance. Had the mesophyll resistance been affected by water stress, the slope or intercepts of the *F*/*T* lines, or both, would have been different because of the independent effect of RWC on *F*. The coincidence of the two ratios of *F*/*T*, one under a condition of constant RWC and the other under declining RWC also indicates that stomatal resistance is the primary factor regulating *F* and that declining RWC or leaf water potential has little or no direct effect of *F*. Apparently the change in RWC did not have an appreciable effect on the water vapor concentration in the substomatal cavity and did not change the difference in the water vapor concentration between leaf and atmosphere.

The results of these experiments are consistent with those of

Boyer (2), who concluded that by using water vapor exchange techniques for determining r_m in both soybeans and corn, diffusive resistance of the stomata to CO_2 entry appeared to be the primary factor limiting CO_2 assimilation.

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