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Stomatal Diffusion Resistance of Snap Beans. I. Influence of Leaf-Water Potential[1]

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Abstract. Concurrent measurements of abaxial and adaxial stomatal resistance and leaf-water potentials of snap beans (*Phaseolus vulgaris* L.) in the field and growth chamber show that the stomata on the 2 surfaces of the leaflet react differently to water deficit. The stomata on the abaxial surface, which are about 7 times more numerous than on the adaxial surface, are not significantly affected at leaf-water potentials greater than −11 bars, but with further decrease in leaf-water potential, the resistance rapidly increases. On the other hand, the resistance of the adaxial stomata increases sharply at a leaf-water potential of about −8 bars and is constant at higher water potentials. The average stomatal resistance for both surfaces of the leaf, which is the major diffusive resistance to water vapor, to a first approximation acts as an on-off switch and helps prevent further decline in leaf-water potential. The relation between the leaf-water potential and the stomatal resistance links the soil-water potential to the transpiration stream as needed for soil-plant-atmosphere models.

The relationship between leaf-water potential and stomatal resistance is of intrinsic interest, and also is important in constructing transpiration models for the soil-plant-atmosphere continuum (4). The movement of water in the soil-plant-atmosphere system occurs in response to a potential difference and is in the direction of decreasing energy from the soil, into the plant root and through the xylem to the leaves. As soil-water content decreases, the soil- and leaf-water potentials also decrease (10); consequently, a water deficit develops in the leaves and the guard cells lose turgor which causes the stomata to close. Thus, the resistance for water vapor transport from leaves is increased.

The turgor pressure of the guard cell is affected not only by leaf-water potential but also by temperature, light and CO_2 concentration of the ambient air (14). Under most field conditions, leaf-water potential and light are the primary factors in stomatal movement (20).

Slatyer (20) suggests that water deficit may not affect greatly the stomatal resistance until a critical leaf-water potential is reached and, as the water potential decreases further, there is a progressive increase in stomatal resistance. The data of Ehlig and Gardner (6) for detached leaves also suggest stomatal closure due to water stress occurs within a narrow range of leaf-water potentials.

It is the objective of this paper to present field and growth chamber data on the stomatal resistance *versus* leaf-water potential for snap beans (*Phaseolus vulgaris* L., var. Bush Blue Lake). In a companion paper, the effect of light on the stomatal resistance of snap beans is discussed (12).

Materials and Methods

Concomitant measurements of water potential and stomatal resistance usually were made on the same leaflet. Stomatal resistances were determined for both the adaxial and abaxial surfaces with the diffusion porometer (13) and represent an average of at least 2 porometer readings on each side of the leaflet. Total water and osmotic potentials usually were obtained from single measurements on the leaflet with the Peltier-type thermocouple psychrometer (1). Leaf tissue was placed in the chamber of the psychrometer and equilibrated in a temperature-controlled water bath which was maintained at 25.0°; temperature stability inside the chamber was estimated at ±0.001° during the 2 to 3 hr equilibration period. After the total potential determination, the sample and chamber were removed from the psychrometer and stoppered. The leaf tissue was frozen in the sealed chamber with dry ice and then allowed to attain room temperature over a desiccant to prevent condensation and possible leakage to the inside of the chamber. The chamber was replaced on the psychrometer for the osmotic potential determination.

Field measurements were made at the University of Wisconsin, Hancock Experimental Farm between July 15 and August 20, 1968. During this period the leaf area index (LAI) of the crop increased from about 1.0 to 1.8. The LAI and net assimilation rate were determined from a 1 m² area in the field.

[1] Paper I follows Paper II. See page 1542.

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Field determinations of soil-water potential were made with tensiometers and *in situ* thermocouple psychrometers (18); the potentials also were estimated from the water content of soil samples using a soil-water release curve.

The field measurements were complemented with experiments in a growth chamber (Sherer CEL37-14) which provided daytime and nighttime air temperatures of 24 ± 1° and 20 ± 1°, respectively. The air supply was drawn from both inside and outside the building and over a free-water surface before entering the environmental chamber; therefore, the CO_2 concentration and relative humidity remained relatively constant throughout the study. Two auxiliary fans were installed in the chamber to reduce the boundary layer resistance so that it was a negligible part of the total diffusive resistance to water vapor transport. The light quanta flux density at the top of the plants was maintained at approximately 45 nE cm^{-2} sec^{-1} from 0600 to 1800 hr (nE is nanoEinstein and applies here only to the 400–700 nm waveband—full sunlight ≈ 200 nE cm^{-2} sec^{-1}). Snap beans were planted in a silt loam soil contained in 8-liter polyethylene pots which were 23 cm in diameter and 25-cm deep. Each pot contained an individual plant to give a root distribution which appeared uniform upon visual observation at the conclusion of the experiment. Soil-water potentials were estimated from soil samples and the water-release curve. A thin layer of vermiculite was spread over the soil suface to reduce evaporation. Evapotranspiration rates from 4 stressed plants were obtained from the daily weight losses and the measurements of leaf area per plant. The water loss from the stressed plants was not replaced, while the 4 nonstressed plants were adequately watered. Leaf growth rate was determined by measuring the distance between 2 markings on young, upper leaves. This was a 1-dimensional measure of leaf enlargement normal to the midvein but because the major expansion occurred in this direction, the measure approximated the increase in leaf area. Measurements were made on at least 4 stressed and nonstressed leaves of approximately the same age and position on the plant and their ratio was used as the relative growth rate. The variability among the 4 measurements was usually less than 10%. The leaves from plants used in the evapotranspiration and growth rate analysis were not sampled for the leaf-water potential determinations.

Results and Discussion

Field Experiment. Fig. 1 shows the daily evapotranspiration as determined by lysimeter measurements (3) and the precipitation-irrigation of the snap bean field. There were 2 drying cycles: the first began on July 27 and ended August 3 and the second was from August 10 to August 14. The large decrease in soil-water potential at the 10-cm

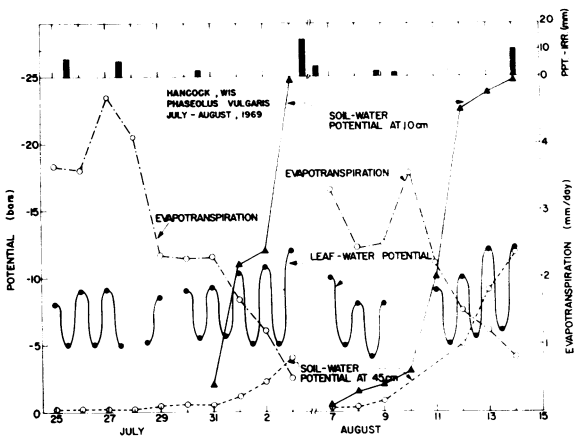

FIG. 1. Trends of soil- and leaf-water potential, evapotranspiration and precipitation-irrigation through 2 drying cycles in snap bean field.

depth and to a lesser extent at the 45-cm depth coincided with a decrease in leaf-water potential below −10 bars (Fig. 1) and the rapidly increasing stomatal resistances (Fig. 2). The leaf-water potential values (Fig. 1) represent an average of 3 to 5 psychrometer measurements. The response of the plant was closely coupled to the soil-water potential in the upper 20 cm of the profile where about 80% (by wt) of the roots were located. This was illustrated by the potential at 10 cm, although the soil-water potential at this depth was affected also by surface evaporation.

Stomatal resistances of the abaxial and adaxial surfaces and leaf-water potentials were measured on upper canopy leaves through the first drying period. The time trends are shown on Fig. 2: the log scale facilitates plotting the large range of abaxial and adaxial stomatal resistances. Since nonstomatal control of transpiration as by "incipient drying", is

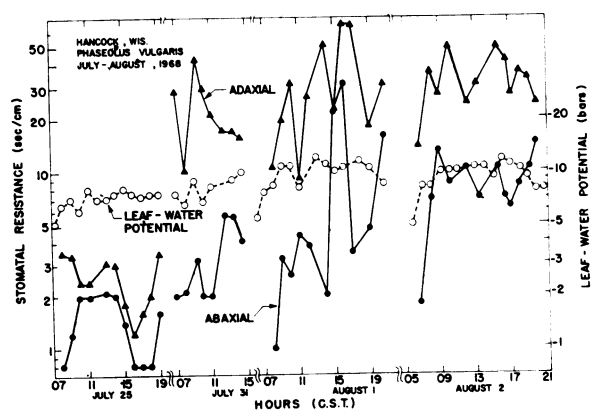

FIG. 2. Hourly trends of adaxial and abaxial stomatal resistances and leaf-water potential during the first drying cycle of upper snap bean leaves in the field.

unlikely to occur at the leaf-water potentials we encountered (19), the porometer measurements reflect almost solely stomatal density and aperture; therefore, the amphistomatic bean leaves, which have about 7 times more stomata on the abaxial surface than on the adaxial surface (16), also have lower resistances on the abaxial surface.

The irregularity in the stomatal resistance curves is due to variability among leaves since each measurement was determined on a different leaflet from the top of the canopy. The sudden increase in stomatal resistance of the abaxial surface at 1300 hrs on July 31 coincided with visible wilting of the upper canopy leaves. The leaves regained turgor about 1500 hr because of decreased solar radiation (clouds) and consequently, the stomatal resistance decreased. The next day, water stress occurred earlier, and the stomatal resistances of the abaxial and adaxial surfaces remained high throughout the day. Similar results were obtained during the second drying cycle.

The stomatal resistances of abaxial and adaxial surfaces *versus* the corresponding leaf-water potentials are given in Fig. 3 for sunny periods of the first drying cycle when the adaxial resistance was not light-limited. The adaxial stomatal resistances progressively increased after the water potential had declined to −8 to −9 bars. The abaxial stomatal resistance remained relatively constant until the leaf-water potential decreased to about −11 to −12 bars, then, rapidly increased and helped prevent a further decline in the water potential of the leaves. Visual observation of the abaxial stomata, using leaf impressions with silicone rubber (22), showed that they are at least 50 % open at approximately −9 bars and appear closed at −11 to −12 bars which is in agreement with the resistances. As indicated in Fig. 2 and as also observed during the second drying cycle, the adaxial stomatal resistance began to increase 1 to 2 days prior to wilting and

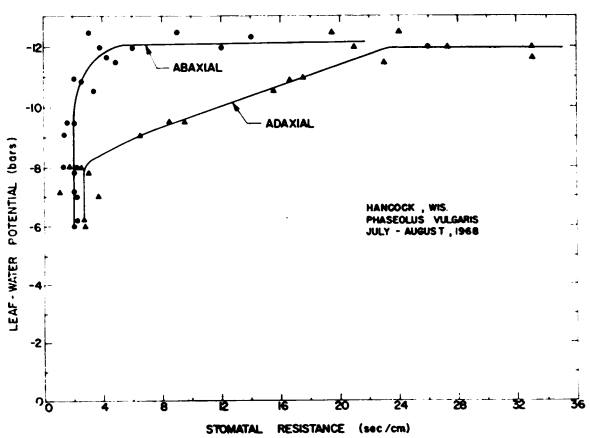

FIG. 3. Relationship between adaxial and abaxial stomatal resistance and leaf-water potential of upper canopy leaves.

the large increases in abaxial resistance; thus, the adaxial resistance may be a useful irrigation indicator. The adaxial stomata of these snap beans were more sensitive to water deficit than the abaxial stomata in contrast to the results found for cotton leaves, in which stomata on both surfaces closed simultaneously (17). Since the adaxial stomata also were more sensitive to light, the adaxial guard cells may have a higher modulus of elasticity; therefore, a given turgor pressure change will cause a larger change in adaxial aperture regardless of the mechanism involved.

The average stomatal resistance based on the total surface area of the leaflet (sum of adaxial and abaxial) is given by

$$r_s = 2r_t r_b/(r_t + r_b) \qquad \text{I}$$

where r_t and r_b are adaxial and abaxial stomatal resistances, respectively. It is noted that r_s of equation I should not be simply added to the air boundary layer resistance to obtain the total diffusive resistance to water vapor from the leaflet since a boundary layer resistance is in series with both r_t and r_b; however, the error is slight if the boundary layer resistance is an order of magnitude smaller than the stomatal resistance, as has been shown for our field conditions (13). As seen from equation I, when r_t is much greater than r_b (—10 to −12 bars leaf-water potential), the average stomatal resistance is about twice the abaxial resistance. When the leaf-water potential is quite high, r_t is approximately twice r_b, and r_s is about 1.3 r_b. Thus, the transpirational losses from the bean canopy are largely controlled by the abaxial stomata, particularly at low leaf-water potentials.

Variations in stomatal resistances and water potentials of leaves on the west side, east side and top of the N-S row on August 12 are shown in Fig. 4. The low leaf-water potentials appear relatively uniform throughout the canopy. This is attributed to the orientation of the upper leaves to a near-vertical position with the leaf tip upward; thus, solar radiation penetrated deeply to give a more uniform transpiration rate throughout the canopy. During a period of low water deficit, the lower, shaded leaves have a substantially greater water potential than the upper, rapidly transpiring leaves (12). The August 12 data and some data on August 11 and 13 show that the upper leaves in the canopy have a lower stomatal resistance than the bottom leaves. This may be a psysiological adaptation which permits the young, upper, meristematic portions of the plant to continue to photosynthesize at the expense of the older leaves.

The increase in stomatal resistance during a drying cycle increases the diffusive resistance of both water vapor and CO_2; therefore, if the CO_2 supply to the photosynthetic sites is limiting, we would expect a decrease in photosynthesis as well as transpiration. In order to indicate the effect of leaf-water potential and stomatal closure on photosyn-

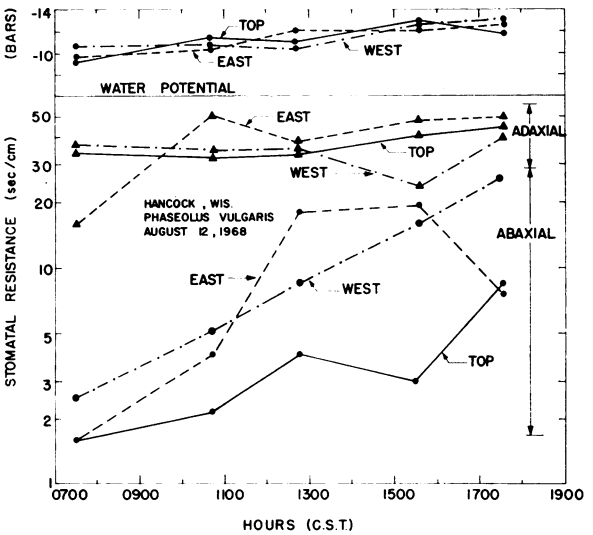

FIG. 4. Hourly variations in adaxial and abaxial stomatal resistance and leaf-water potential at various positions in the N-S row.

thesis, we approximate, as did Denmead and Shaw (5), the net assimilation rate by

$$NAR = \Delta W/\overline{(LAI)}\Delta t \qquad \text{II}$$

where LAI is the average leaf area index for the field, and ΔW is the change in dry weight per plant during the time interval, Δt. Water stress affects NAR through its effect on photosynthesis and on respiration. In general, as water stress develops, respiration first increases but as water deficit increases the respiration rate decreases (15). The differences in respiration responses of different species to water deficit may have been due to the rate at which stress was applied (20). The reduction in NAR (Fig. 5), which occurs when stomatal resistances and leaf-water potentials are rapidly changing, could possibly be attributed to: (a) increased respiration rate due to higher leaf and root temperatures brought about by a decrease in transpiration and a higher heat flux to the soil, and (b) decreased photosynthesis due to a decrease in CO_2 supply and hydration effects on biochemical processes. The average stomatal resistances (Fig. 5) are obtained from hourly r_s values.

Growth Chamber. A controlled-environment experiment was conducted to substantiate the field data on the effect of water deficit on stomatal resistance and growth rates. The plants were grown in silt loam, instead of sand, to allow a more gradual increase in water stress. During this period, evapotranspiration, relative leaf-growth rate, stomatal resistance, soil- and leaf-water potential and leaf-osmotic potential were determined on 4- to 6-week old plants.

Leaf-water deficit affects growth through the closely integrated processes of cell division, cell enlargement and photosynthesis. If we assume that leaf expansion is primarily controlled by photosynthesis, then a concurrent decrease in transpiration rate and relative growth rate will result unless the mesophyll resistance constitutes a major proportion of the total diffusive resistance for CO_2 (8, 20). There are conflicting data in the literature on the relative magnitude of the mesophyll resistance (2, 7, 9). Since there appeared to be a concomitant decrease in the relative transpiration and growth rates during the first week of the drying period (Fig. 6), the mesophyll resistance must not have been large compared to the stomatal resistance. The large decrease in transpiration and growth rates at modest soil water potentials may then be attributed to the increased stomatal resistance. The transpiration and growth curves diverge during the end of the drying cycle, which corresponds to the period of near-zero turgor pressure and wilting of the leaves. During a wilting period, rate of cell enlargement and division are markedly decreased and there is a decrease in osmotic potential of the leaves which, in addition to

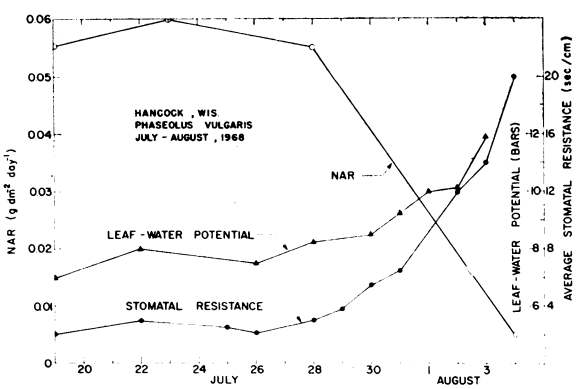

FIG. 5. Trends in NAR (net assimilation rate), average stomatal resistance and leaf-water potential during the first drying cycle.

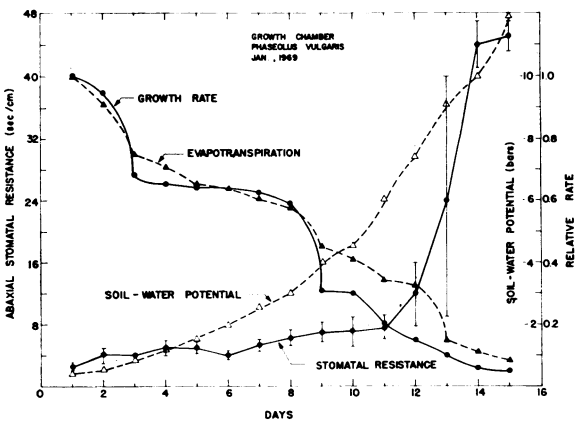

FIG. 6. Daily trend in relative-growth and evapotranspiration rates, soil-water potential and stomatal resistance in growth chamber.

the impeded CO_2 transport, causes a decline in photosynthesis (20, 21). As the water stress increases, respiration becomes more dominant in determining the net assimilation rate since photosynthesis is reduced.

Only the abaxial stomatal resistance is shown in Fig. 6 since the adaxial stomata remained closed throughout the experiment. Stomatal resistance measurements were taken 3 to 5 times each day. The vertical lines represent the daytime variations in stomatal resistance on the same leaflets. The large variations in stomatal resistance on the tenth through the thirteenth day correspond to the period in which wilting occurred. During this period the upper leaves had a smaller stomatal resistance than the lower leaves and were oriented in a near-vertical position with their leaf tips pointed upward. This behavior occurred in the field and in the growth chamber at a leaf-water potential of —12 to —13 bars. Orientation in a vertical position reduced the radiation load on the leaf and, consequently, also transpiration.

Shown in Fig. 7 are the relationships between the abaxial stomatal resistance and the leaf-water and pressure potentials. The pressure potential, usually referred to as the turgor pressure, is obtained by subtracting the osmotic from the leaf water potential; thus, the errors in both potential determinations are accumulated in the turgor pressure calculation. The turgor pressure of the guard cells and subsidiary cells should be uniquely related to the stomatal resistance; however, our measurements represent average potentials of the mesophyll and epidermal cells which may be different from those of the guard cells.

At high turgor pressures, the stomatal resistance increases due to the mechanical pressure applied by the subsidiary cells. There is an abrupt change in elasticity of the cell wall of several plant species at a turgor pressure of about 2 bars (11); this value of the turgor pressure also corresponds to visual wilting of the bean leaves.

The relationship of leaf-water potential to abaxial resistance found in the growth chamber is similar to that of the field data (Fig. 3); however at high water potentials, the stomatal resistances from the growth chamber are slightly higher whereas at high resistances, the water potential is lower. The slightly higher resistance at high potentials obtained in the growth chamber may be light-induced since abaxial stomatal resistance increases about 30 % at higher light levels (12). The lower water potentials at the high stomatal resistances found in the growth chamber experiment may be due to the differences between the osmotic potentials of the soil solutions in the field and growth chamber studies.

We do not expect the curves in Fig. 7 to be unique for all plant species, but their shapes indicate to a first approximation an on-off relationship between the leaf-water potential and stomatal resistance. The on-off stomatal mechanism should not cause an on-off daily transpiration flux which is high one-day and virtually nil the next because (a) diurnal trends in leaf-water potential indicate a gradual increase in stomatal resistance before the critical water potential is reached, then a rapid increase in resistance, and (b) as the drying cycle progresses, the stomata close earlier during the day; hence, the interval of stomatal closure is increasing from day to day. The shape of the curves shown in Fig. 3 and 7 provide useful information in constructing soil-plant-atmosphere models, since such a relationship links the plant environment as reflected in the leaf-water potential to the stomatal-controlled, transpiration flux.

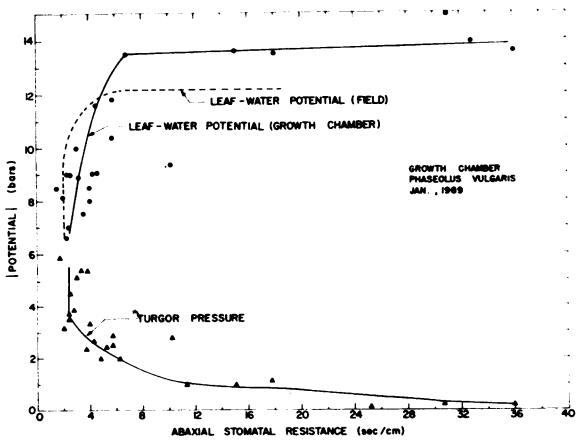

FIG. 7. Relationship between abaxial stomatal resistance and leaf-water potential and turgor pressure of growth chamber plants. The dashed curve taken from Fig. 3.

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