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Stomatal Diffusion Resistance of Snap Beans. II. Effect of Light[1]

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Abstract. The effect of light on the stomatal resistance of abaxial and adaxial leaf surfaces of snap beans (*Phaseolus vulgaris* L.) was studied in the growth chamber and in the field. The adaxial stomata required more light to open than the abaxial stomata; the abaxial stomatal apertures were still about 50% open at 1% full sunlight and light-induced closure was never observed under daytime field conditions. A given value of abaxial stomatal resistance was obtained at a given illumination of the abaxial guard cells whether illumination was adaxial or abaxial.

Photosynthesis and transpiration involve the diffusion of CO_2 and water vapor across the stomatal resistance; an additional resistance is associated with the transport of CO_2 from the mesophyll cell walls to the photosynthesizing sites in the chloroplasts. For many agronomic crops, the stomatal resistance has approximately the same magnitude as the mesophyll resistance (2); hence, crop photosynthesis obtained from models of the light distribution in the canopy must incorporate the effect of light and leaf-water potential on stomatal resistance.

Stomatal resistance is dependent upon the density and aperture of the stomata. On fully expanded leaves, changes in stomatal resistance result primarily from the opening and closing of the stomatal apertures. The stomatal response to light is attributed to the photosynthetic reduction of the internal CO_2 concentration (4); this is consistent with observations that the action spectrum of stomatal opening resembles the absorption spectra of the chlorophylls (14) and the action spectrum of photophosphorylation with maxima in stomatal opening at wavelengths of 432 and 675 nm (9, 12). The nighttime stomatal opening observed in many plant species is also attributed to the internal CO_2 concentration which depends upon respiration and dark fixation of CO_2 (13). However, under daytime field conditions of high soil moisture and normal atmospheric CO_2 concentration, there is a "minimal (light) intensity for opening which varies with species" (7).

Kuiper (8) showed a hyperbolic relationship between the stomatal resistance of bean leaves and the light flux density. Whiteman and Koller (17) found a decrease in stomatal resistance of sunflower leaves (*Helianthus annuus* L.) when light was increased from "500 to 1000 ft-c" but further increase in light resulted in an increase in the stomatal resistance. The increase in resistance at the higher light flux densities may have been due to water stress. Using plants grown in nutrient solution, Ehrler and Van Bavel (1) related the porometer measurements of the abaxial and adaxial leaf surfaces to the incident light at the top of the plants. This assumes that the primary light response of the abaxial and adaxial stomata is to adaxial illumination. They found that for many plant species including snap beans, the adaxial stomata were more sensitive to light than the abaxial stomata.

Although stomata respond to both leaf-water potential and light, we found we could separate the effect of light from the effect of water potential on stomatal resistance. The abaxial stomata of the snap beans are not significantly affected by water deficit at leaf-water potentials higher than −11 bars, as compared to the adaxial stomata which are not significantly affected at leaf-water potentials higher than −8 bars (5). Thus, when the water potential of the leaves does not decrease below −8 bars, differences in stomatal resistance under different light conditions can be attributed to light.

The objective of this paper is to present the effect of light on the adaxial and abaxial stomatal resistance of snap beans (*Phaseolus vulgaris* L., var. Bush Blue Lake).

Methods and Materials

In both growth chamber and field experiments, stomatal resistance, leaf-water potential and light were measured on fully expanded leaflets of 4- to 5-week old bean plants. Stomatal resistance of the abaxial and adaxial surfaces of the leaflets were determined with the stomatal diffusion porometer (6). All resistance values were based on either the adaxial or abaxial surface area of the leaflet and represent an average of 2 to 3 measurements. Variability among these stomatal resistance measurements

[1] Paper II precedes Paper I. See page 1547.

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was usually less than 0.5 sec cm^{-1}. Water potential values were obtained from 1 to 2 measurements on the interveinal areas of the leaflet with the Peltier-type thermocouple psychrometer (5). Light measurements were made with a quanta-response sensor with an output proportional to the number of quanta in the 400- to 700-nm wavelength band (15). The light incident to either surface was measured by placing the sensor parallel to the surface of the leaflet and transmitted light was obtained in a similar manner but holding the sensor near and directed toward the leaf surface.

For the growth chamber experiments, snap beans were planted in 8-liter polyethylene containers, which were filled with silt loam. Under normal lighting of the growth chamber, the light quanta flux density at the top of plants was 45 nE cm^{-2} sec^{-1} (full sunlight $\approx$ 200 nE cm^{-2} sec^{-1}). Other details on the environmental conditions and techniques are given in (5).

In one growth chamber experiment, the adaxial and abaxial surfaces of a leaflet were exposed to equal incident light by turning the entire plant upside down. A plywood support was designed to hold the soil and root system in the pot during the reversing operation. One leaflet on the plant was enclosed in a cardboard box with an opening at the top which allowed the adjustment of light by placing neutral density filters over the opening. The remainder of the leaves were illuminated with normal growth chamber lights. Stomatal measurements were obtained through an opening in the side of the box. At each level of illumination, stomatal resistance measurements were made at intervals until a steady-state condition was established. The plant was not held continuously upside down for more than 2 hr. All measurements were made during the daytime.

Results and Discussion

Growth Chamber. Prior to the investigation of different light irradiation on the abaxial and adaxial stomata, it was desirable to obtain some knowledge of the time response for the opening and closing of stomata. The plants were subjected to dark and light cycles by switching on and off the normal growth chamber lights and the abaxial stomatal resistance was measured with the diffusion porometer. As seen in Fig. 1, the time for both the opening and closing of abaxial stomata was approximately 20 min. Under normal growth chamber light, the adaxial stomata were closed; therefore, only the abaxial stomatal resistance was measured.

Cyclic oscillations in stomatal aperture, which have been known for a long time (16), can affect the light-stomatal resistance relationship. Shown in Fig. 2 are the cyclic oscillations that occur in abaxial stomata after the lights have been switched on during a normal nighttime period. The oscillations have a period of about 20 to 25 min and an initial amplitude of about 15 sec cm^{-1}. The leaf- and soil-water potentials were approximately -6 and -0.5 bars, respectively, and under non-oscillating conditions, this leaf-water potential would not have affected the resistance. A similar experiment during the daytime did not result in aperture oscillations; the water potential of the leaf was approximately -9 bars which should have enhanced the possibility of oscillations (10); strong endogenous rhythms in snap bean may have prevented the occurrence of oscillations during the daytime.

As shown in Fig. 3, we obtain a single stomatal response curve when the abaxial stomatal resistance is plotted against the light quanta flux density reaching the abaxial guard cells. When illuminated abaxially (plant inverted) the light reaching the abaxial guard cells is approximated by the light incident on the abaxial surface. When illuminated adaxially, the light reaching the abaxial guard cells is that which is transmitted through the leaf plus the portion of reflected and scattered light inside the box that is incident to the abaxial surface. We assume that reflectances of visible light for the abaxial and

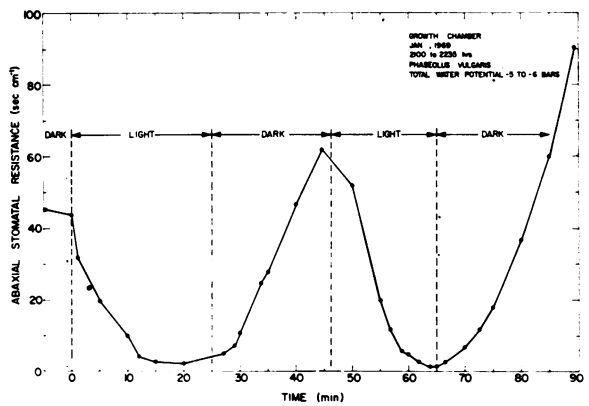

FIG. 1. Stomatal response to light-dark cycles.

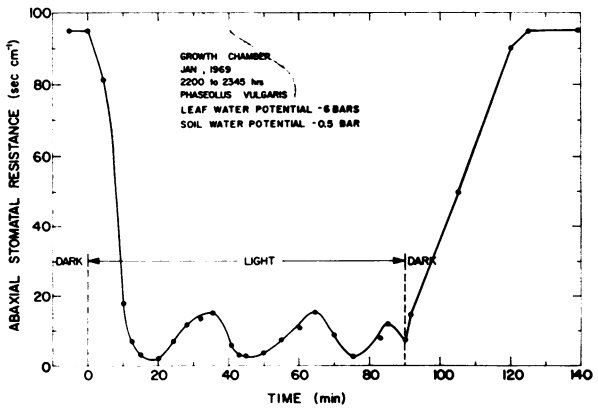

FIG. 2. Cyclic oscillations in abaxial stomata induced by light impulse.

adaxial surfaces are equal and that the spectral differences in the transmitted light can be neglected. It appears that the light which is effective in altering stomatal aperture is the visible light reaching the guard cells; thus, it is probably this light that should be considered when applying a light *versus* stomatal resistance relationship to a light-photosynthesis model.

The adaxial stomata were closed ($\approx$ 100 sec cm^{-1}) at an adaxial illumination of 45 nE cm^{-2} sec^{-1} and their resistance decreased to about 33 sec cm^{-1} at 66 nE cm^{-2} sec^{-1}; on the other hand the abaxial stomata were almost fully open at 2 nE cm^{-2} sec^{-1} (Fig. 3). Thus, the abaxial and adaxial stomata behave quite differently and indepedently of each other with respect to light, as well as water deficit (5).

When a plant was exposed to a constant light level (45 nE cm^{-2} sec^{-1}) for 24 hr, we found that the abaxial stomatal resistance measured during the daytime hours was lower than that measured during the nighttime hours as shown in Fig. 4; this presumably is due to endogenous rhythms. There was a maximum stomatal closure at about midnight and a minimum near noon. Maskell (11) and Gregory and Pearse (3) have reported similar endogeneous rhythms in the stomatal aperture of cherry and *Pelargonium*. Also shown in Fig. 4 are the total leaf-water and osmotic potentials obtained by sampling leaves corresponding to approximately the same position and age as the upper leaflet. The total water and osmotic potentials increased during the normal nighttime period while the turgor pressure (total minus osmotic potential) remained fairly constant throughout the 24-hr period. The turgor pressure was not uniquely related to stomatal resistance, presumably because it is an average of the entire

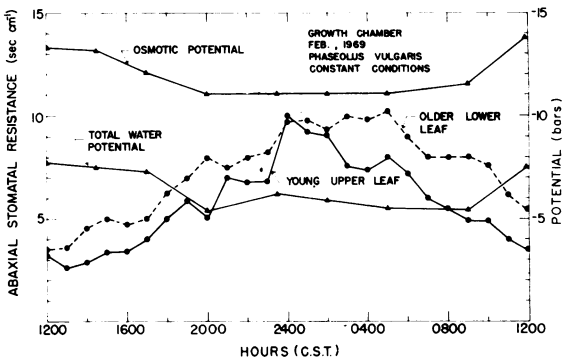

FIG. 4. Hourly trends in abaxial stomatal resistance (young and older leaves), leaf-water potential and osmotic potential under continuous illumination.

leaf tissue, and not the turgor pressure of the guard cells and subsidiary cells.

Field Experiment. Field measurements were made at the University of Wisconsin, Hancock Experimental Farm in the central sand plains, on 5-week old plants with a leaf area index of about 1.3.

On a clear day, stomatal resistances and leaf-water potentials were measured on the upper, sunlit and lower, naturally-shaded leaves of the bean canopy (Fig. 5 and 6). Each experimental point was determined on a different leaflet. Light measurements were not available for this period of the field measurements; however, later measurements indicated that the incident light quanta flux densities on the adaxial surfaces of sunlit and shaded leaves were approximately 110 and 20 nE cm^{-2} sec^{-1}, respectively. The abaxial stomatal resistances of the sunlit and shaded leaves were almost identical, but resistances of the adaxial surfaces of the shaded leaves were much greater than that of the sunlit leaves. Since the water potential of the shaded leaf did not de-

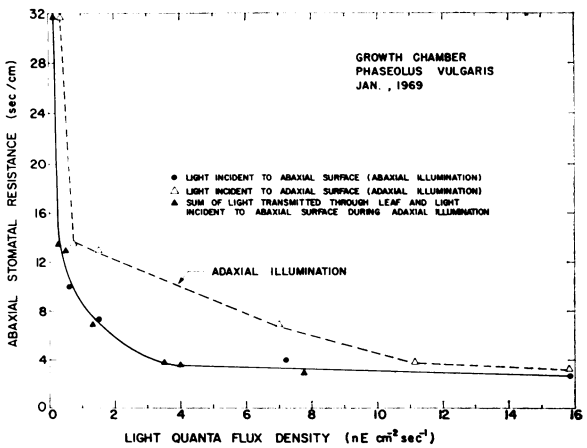

FIG. 3. Abaxial stomatal resistance *versus* the estimated light to the abaxial guard cells (solid curve). The dashed curve represents light incident on the adaxial surface when illuminated adaxially.

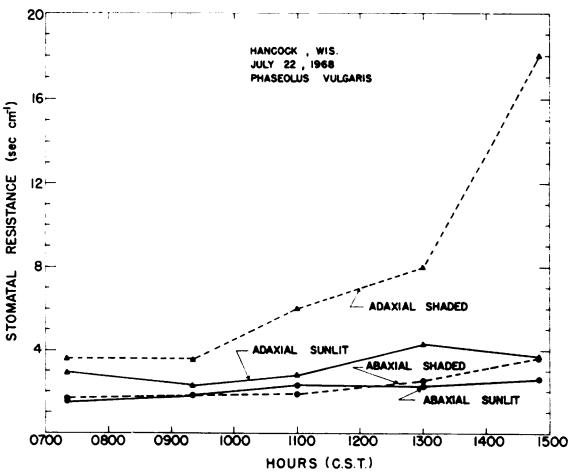

FIG. 5. Trends in stomatal resistances of sunlit and naturally shaded leaves in the field.

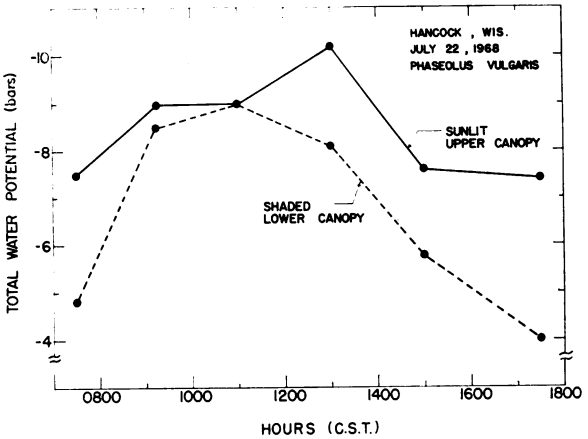

FIG. 6. Trends in leaf-water potential of sunlit and naturally shaded leaves in the field.

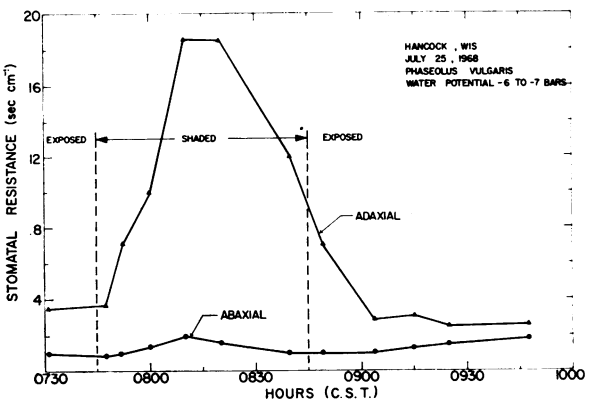

FIG. 7. Trends in stomatal resistance of the adaxial and abaxial surfaces of an artificially shaded leaf in the field.

crease below −9 bars, water deficit did not limit the opening of the abaxial stomata, but at times it would have limited the opening of the adaxial stomata. The progressive increase in adaxial stomatal resistance of the shaded leaves throughout the day may be due to our sampling leaves deeper in the canopy, or to an increase in water deficits. As shown in Fig. 6, there was a significant water-potential difference between the bottom and top of the plant, which was attributed to a greater transpiration at the top of the canopy.

A piece of aluminum foil about 3 times the adaxial area of the leaflet was positioned about 6 cm above the leaf to shade the adaxial surface of the leaflet from the incident light without significantly altering the incident light to the abaxial surface. The incident light quanta flux density on the abaxial surface was approximately 20 nE cm^{-2} sec^{-1}. The water potentials of leaves in approximately the same position in the canopy were −6 to −7 bars; thus, stomatal resistance would not be affected appreciably by the water stress. The adaxial stomatal resistance increased with the decrease in light (Fig. 7). The slight decrease in resistance toward the end of the shading period may be due to an increase in the water potential under the decreased evaporation, thus causing the decrease in stomatal resistance.

The field measurements were consistent with the growth chamber observations in that the abaxial and adaxial stomata responded differently to light. A substantially higher light level is required to open the adaxial stomata, while according to Kuiper's (8) stomatal resistance *versus* aperture curve for snap beans, our abaxial stomata are 50 % open at 1 % full sunlight. Closure of the abaxial stomata was never observed in the field, as a consequence of a low light intensity during daytime.

The abaxial and adaxial stomata of snap beans react differently to light and water deficit; therefore, it is essential that light-photosynthesis, and transpiration models incorporate these physiological characteristics of the stomata.

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