Stomatal Behavior and Water Relations in Sugar Beet Leaves Infected by *Erysiphe polygoni*

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Accepted for publication 25 September 1981.

ABSTRACT

Gordon, T. R., and Duniway, J. M. 1982. Stomatal behavior and water relations in sugar beet leaves infected by *Erysiphe polygoni*. Phytopathology 72:723-726.

Sugar beet plants (*Beta vulgaris* L.) infected by *Erysiphe polygoni* (cause of powdery mildew) on their adaxial leaf surfaces showed altered stomatal behavior. In the dark, both diffusive conductance to water vapor loss and viscous flow conductance of mildew-infected leaves were increasingly higher with time after inoculation, as compared to healthy leaves. This indicates that stomata on infected leaves failed to close completely in the dark. Water stress was induced in healthy and mildew-infected leaves by withholding water from the soil in which plants were growing. Illuminated healthy leaves showed a substantial decrease in leaf conductance to water vapor loss as leaf water potential dropped, whereas for mildewed leaves conductance to water vapor loss in the light was not significantly affected by leaf water potential values as low as −39 bars. As a result, mildewed leaves transpired more than healthy leaves at low leaf water potentials. At soil water potentials less than −6 bars the water potential values of leaves on heavily mildewed plants were much lower than leaves on uninfected plants. The relationship between turgor pressure and leaf water potential was not altered by the disease.

Results presented previously (7,8) showed a decrease in stomatal aperture of mildew-infected sugar beet leaves in the light. This decrease in stomatal aperture did not have an important effect on photosynthesis because it was accompanied by more drastic effects of the disease on the photosynthetic capacity of the mesophyll. It did, however, cause a significant reduction in the rate of transpiration from diseased leaves in the light. Because of the central role of stomata in the control of leaf water loss, their ability to respond to certain environmental cues is critical to maintenance of a favorable water balance in the plant (17). For this reason, we have examined the response of stomata on mildew-infected leaves to darkness and to water stress, both of which cause nearly complete stomatal closure in healthy leaves. We also evaluated water potential-turgor relationships in bulk leaf tissue from mildew-infected sugar beet leaves.

MATERIALS AND METHODS

Sugar beet plants (*Beta vulgaris* L. USH10) were grown in 4-L pots of sterilized soil in a controlled environment chamber. Growing conditions and inoculations of single leaves were as described previously (7). For one set of water stress experiments, entire plants were placed in the inoculation chamber, rather than single leaves. Three plants were inoculated simultaneously with a uniform inoculum density of 40–60 viable conidia per square centimeter of leaf area. Seven or eight leaves, representing all unfolded, non-senescent leaves, except the oldest two or three, became infected on their adaxial surfaces. Disease progress was similar to that described elsewhere (7); the abaxial surfaces did not become infected.

Measurements of leaf diffusive conductance to water vapor loss in the dark (L_l^d) were made with the gas exchange system described previously (7). The leaf to be examined was enclosed within a Plexiglas assimilation chamber just prior to the onset of the dark cycle to which the leaf was normally exposed. L_l^d was then evaluated during the first 4 hr of the normal dark period. After completion of these measurements, viscous flow conductance of the same leaf was determined by forcing the airstream to flow through the leaf blade. The flow-through procedure and units of measurement were as described previously (8) except that trans-leaf pressures as high as 49 millibars were required to obtain measurable flow rates through leaves in darkness.

The effect of water stress on leaf conductance to water vapor loss in the light (L_l) was examined on mildewed and healthy plants

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0031-949X/82/07072304/$03.00/0

under the same growth chamber conditions in which the plants were grown. Stress was induced by withholding water from the soil for a period of 5 days during which time measurements were made. L_1 was estimated with a diffusion porometer (10) for both the adaxial and abaxial surfaces of young fully expanded leaves. That segment of the leaf on which measurements were made was excised immediately afterwards for water potential (Ψ) determination with a thermocouple psychrometer. The leaf tissue was placed in a psychrometer chamber with the infected adaxial surface against the wall of the chamber and the uninfected abaxial surface facing the thermocouple junction. At the same time, a soil sample was removed from a depth of 5 cm and its Ψ determined with a thermocouple psychrometer. The leaf sample for which Ψ was determined was subsequently frozen at −20 C, thawed and returned to the psychrometer for estimation of its solute potential (Ψ_s); the turgor component (Ψ_p) was estimated by difference ($\Psi - \Psi_s = \Psi_p$). Possible effects of the disease on relationships between Ψ and Ψ_p in bulk leaf tissue were examined further in leaf disks brought to Ψ values near zero. Six disks from each nonstressed healthy and mildew-infected leaf were floated on distilled water for 4 hr, blotted dry, and frozen at −20 C, thawed and their Ψ_s values determined in the psychrometer.

RESULTS

Values of L_1^d at various times after inoculation, as determined at 2 hr after the onset of darkness are given in Fig. 1. Comparable data were obtained at 3 and 4 hr into the dark period. Three days after leaves were inoculated the adaxial surface had a uniform cover of mildew mycelium, and sporulation was evident at 6 days when L_1^d became elevated with further time after inoculation. Over the same time period, healthy leaves showed a uniformly low conductance to water vapor loss (Fig. 1). Measurements of viscous flow conductance were made on the same diseased and healthy leaves at the conclusion of diffusive measurements, 4 hr after the onset of darkness. Values for diseased leaves are shown in Fig. 1. For healthy leaves, the highest value obtained was 0.021 $(cm^4 \cdot s \cdot g^{-1})^{0.4}$; for most healthy leaves a measurable flow rate was not obtained because the leaf ruptured as a result of excessive trans-leaf pressure differential. In other words, their viscous flow conductance was consistently $\leqslant 0.021$ $(cm^4 \cdot s \cdot g^{-1})^{0.4}$.

The relationship between L_1 and leaf Ψ, as measured in the lighted growth chamber, is shown for healthy and mildew-infected leaves in Fig. 2. The data in Fig. 2 are taken from two separate experiments: one in which only one leaf on each of five mildewed plants was infected, and one in which entire plants were inoculated.

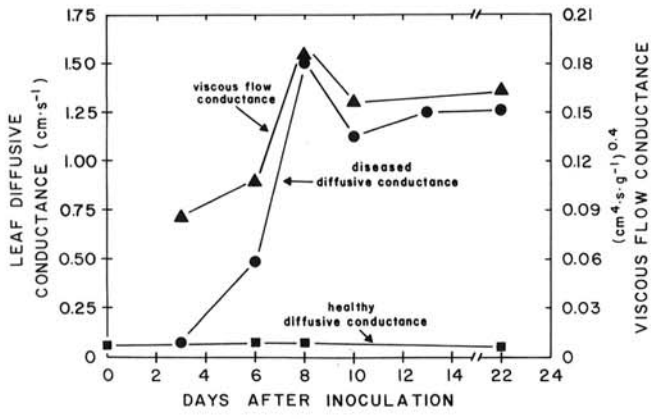

Fig. 1. Diffusive conductance (L_1^d) to water vapor loss in the dark (circles) and viscous flow conductance in the dark (triangles) for mildew-infected sugar beet leaves at various times after inoculation. Data for diseased leaves were obtained from two sets of plants, grown at different times, and each data point is the average of measurements on two plants. Data on the diffusive conductance of healthy leaves in the dark (squares) are based on a single plant for each day of measurement. For both diseased and healthy plants diffusive conductance data represent the summation of conductance values for both leaf surfaces.

For both experiments, a total of eight mildewed and six healthy plants were used; water was withheld from the soil beginning at 8 days after inoculation. In all cases, mildew was restricted to the adaxial surface of the inoculated leaf. The uninfected abaxial surface of mildewed leaves and both surfaces of healthy leaves had progresssively lower values of L_1 as leaf Ψ dropped. The mildew-infected adaxial surface, on the other hand, showed comparatively little change in L_1 down to leaf Ψ values as low as −39 bars.

With only a single leaf on a plant infected, the relationship between leaf Ψ and soil Ψ was the same for mildewed leaves and comparable healthy leaves on uninoculated plants (Fig. 3A); when entire plants were inoculated so that all but the very oldest leaves were infected, mildewed leaves had a much lower Ψ than did healthy leaves on uninoculated plants at soil $\Psi \leqslant -6$ bars (Fig. 3B).

The relationship between Ψ_p and Ψ for healthy and mildew-infected leaves from both sets of water stress experiments is shown

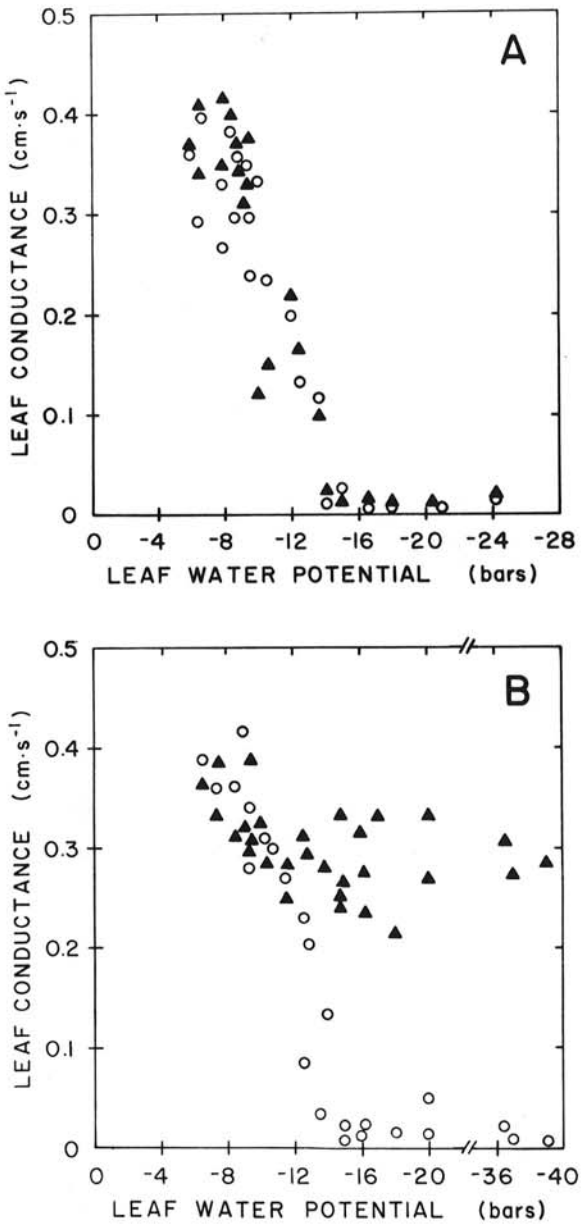

Fig. 2. The relationship between leaf conductance to water vapor loss (L_1) in the light and leaf water potential (Ψ) for the adaxial (triangles) and abaxial (circles) surfaces of **A**, healthy and **B**, powdery mildew-infected leaves. Mildew was restricted to the adaxial surface of infected leaves. Stress was induced by withholding water from potted plants in a growth chamber, beginning at 8 days after infected leaves were inoculated. Each data point represents one measurement with a porometer and one psychrometer measurement.

in Fig. 4. Linear regression of Ψ_p on Ψ predicts zero turgor at −14.2 bars for healthy leaves ($r^2 = 0.90$) and −15.0 bars for mildew-infected leaves ($r^2 = 0.83$). This is consistent with observations of the leaf Ψ at which visual wilting of these leaves occurred. Visual wilting was first evident near the end of the third day after watering was stopped (Figs. 2 and 3). Values of Ψ_s for floated disks from mildew-infected leaves were −15.7, −10.5, −13.5, −13.0, −15.2, −15.0, and −15.6 bars at 9, 11, 13, 18, 20, 22, and 26 days after inoculation, respectively. Values of Ψ_s for similarly treated disks from healthy leaves at ages corresponding to 0, 10, and 20 days after inoculation were −15.2, −12.9, and −14.8 bars, respectively. In all cases, the values of Ψ_s represent an average of the six leaf disks, since all six were measured simultaneously within a single psychrometer chamber.

DISCUSSION

Since the increase in L_l^d of diseased leaves (Fig. 1) was accompanied by increases in viscous flow conductance of the same leaves, it may be concluded that stomata on diseased leaves did not fully close in the dark. Failure of stomata to close in darkness has been reported for other mildew-infected leaves (2,12). However, Mignucci and Boyer (13) apparently did not find such an impairment of stomatal function in mildew-infected soybean leaves. Because their stomata remain open in the dark, mildewed sugar beet leaves will have a higher transpiration rate than darkened healthy leaves. However, mildew infection results in decreased stomatal conductance in lighted leaves (7) so transpiration during daylight hours should be less for mildewed than for healthy leaves. Using our leaf chamber measurements of transpiration for both lighted and darkened leaves on well-watered plants and assuming 12 hr of daylight, healthy leaves would lose up to 55% more water per unit leaf area in a 24-hr day than mildew-affected leaves at 10 days after inoculation. Under field conditions, a mildewed leaf might lose more or less water than a comparable healthy leaf depending on a number of factors, including the position of the infected leaf in the canopy, how much of its surface area is colonized, the ambient humidity, and soil water potential. In our drought stress experiments (Figs. 2 and 3), wilting of healthy and mildewed leaves occurred about the same time after the start of the drying cycle, when only one leaf on each mildewed plant was infected. When entire plants were inoculated, mildewed leaves wilted up to 10 hr before healthy leaves.

It is not surprising that mildewed leaves dried out more quickly than healthy leaves since the mildew-infected, adaxial surfaces showed little change in L_l when subjected to water stress (Fig. 2B). In contrast, both surfaces of healthy leaves showed a precipitous drop in L_l with decreasing Ψ (Fig. 2A), reflecting stomatal closure in response to water stress. This is consistent with previous reports on the stomatal behavior of sugar beets and other field crops subjected to water stress under controlled environment conditions (9,11,18). The lack of any significant change in L_l on mildewed leaf surfaces could be explained by a failure of stomata to close or by a high rate of mycelial transpiration which is not affected by the water potential of the leaf, or some combination of the two. Data presented previously (7) suggest that in light only a small amount of the measured transpiration rate of mildewed leaf surfaces can be attributed to water loss by fungal mycelium. Accordingly, the inability of mildewed leaves to alter L_l when subjected to water stress probably results from a failure of stomata to close fully. This could explain previous observations that, following excision, mildewed leaves desiccate much more quickly than healthy leaves (15). Inability of stomata to close in response to water stress has been reported in other plant diseases, including late blight of potatoes (6) and leaf blotch of barley (1). Stomata on the uninfected abaxial surface of mildewed sugar beet leaves were capable of closing in response to lowered leaf Ψ (Fig. 2B), when the drying cycle began at 8 days after inoculation. However, if water was withheld beginning at 15 days after inoculation, stomata on the uninfected abaxial surface did not close in response to lowered leaf Ψ (*unpublished*).

When only one leaf per plant was infected, the diseased leaf did not have a lower Ψ than leaves on healthy plants at the same soil Ψ (Fig. 3A). This is probably because a small fraction (less than 10%) of the total leaf area was diseased. When stress was induced, healthy leaves ceased to transpire at a significant rate. So mildewed

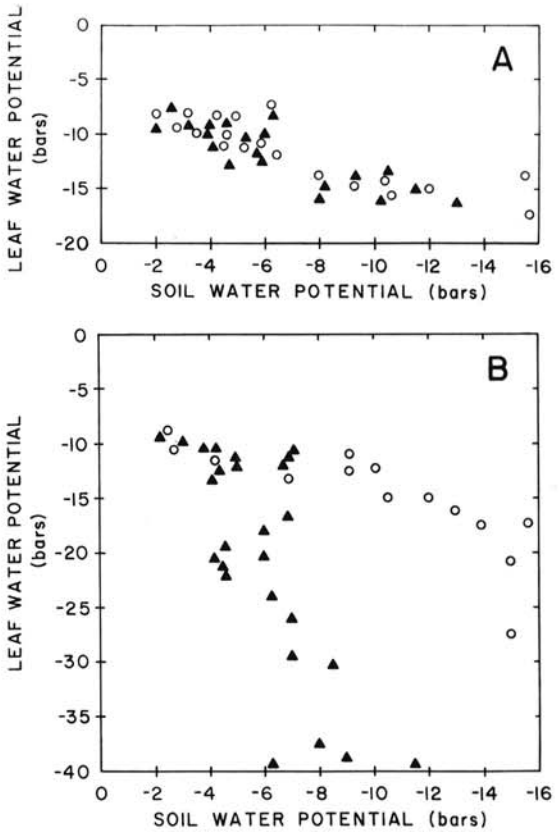

Fig. 3. The water potentials (Ψ) of healthy (circles) and powdery mildew infected (triangles) sugar beet leaves measured in a lighted growth chamber at various soil-water potentials, **A**, when only one leaf on each of the mildewed plants was infected and **B**, when the majority of leaves were infected on the mildewed plants. In both cases mildew was restricted to the adaxial surface of infected leaves. Healthy plants had no mildew-infected leaves. Data in (A) were obtained on five mildewed and three healthy plants and the data in (B) were obtained on four mildewed and four healthy plants.

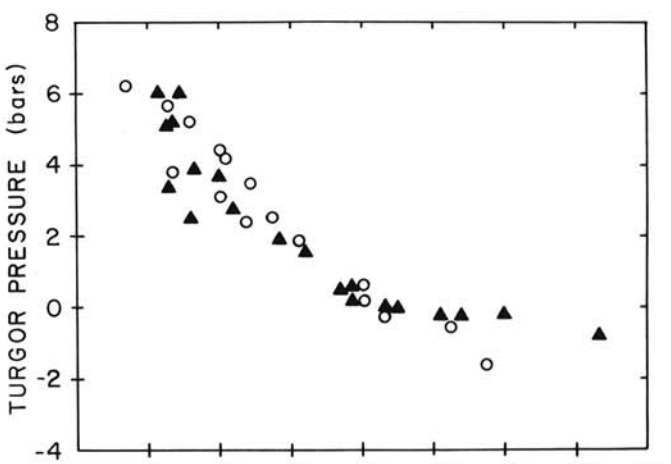

Fig. 4. Turgor pressure (Ψ_p) and the corresponding total water potential (Ψ) for healthy (circles) and powdery mildew-infected (triangles) leaves. Turgor pressure was estimated as the difference between the total water potential and solute potential determined for the same sample in a thermocouple psychrometer.

leaves, although continuing to lose water, were able to draw on a disproportionate share of the water supply available to the plant, thus preventing their Ψ from dropping much below that of leaves on healthy plants. When most of the leaf area on a given plant was infected, the diseased leaves had much lower Ψ values than did healthy plants after soil Ψ dropped below −6 bars (Fig. 3B). This is expected since nearly every leaf on the mildewed plant continued to lose water long after stomatal closure occurred on healthy leaves. The mildewed leaves which reached water potentials of −32 bars or less did not recover after rewatering the soil. All healthy leaves recovered when watering was resumed, although the lowest water potential recorded for a healthy leaf was −27.4 bars. Given the greater tendency of mildewed leaves to become desiccated under stress-inducing circumstances and the frequency with which field-grown sugar beets are subjected to water stress (14), mildew-infected leaves may have an abbreviated life span in the field.

There was no evidence that powdery mildew infection altered the intrinsic relationship between Ψ and Ψ_p in sugar beet leaves (Fig. 4), at least at leaf Ψ values higher than −20 bars. Both diseased and healthy leaves reached zero turgor at −14 to −15 bars Ψ. Presumably, the Ψ_s of mildew mycelium is lower than that of host cells and the presence of mycelium in the frozen and thawed leaf samples may have caused an underestimation of host cell Ψ_s, and hence an overestimation of Ψ_p. We assume that the magnitude of this error is negligible because, relative to the host tissue, the amount of fungal tissue present was quite small. Leaf disks taken from mildew-infected leaves up to 26 days after inoculation and floated on distilled water were capable of maintaining Ψ_s values comparable to those of healthy leaf disks. Thus, membranes in bulk leaf tissue seem to be functioning with respect to their ability to retain solutes. If a loss of membrane function had occurred in a significant portion of the floated tissue, loss of solutes to the surrounding distilled water would have lead to very high values of Ψ_s relative to Ψ_s values for floated healthy leaf disks (5). This contrasts with Ayres' (3) findings for mildew-infected pea leaves. He reported a substantial loss of membrane function and irreversible wilting of pea leaves at 10 days after inoculation with powdery mildew. Such premature desiccation, also reported for mildew-infected barley leaves (16), was not seen in mildew-infected sugar beet leaves; in the absence of an induced water stress, diseased sugar beet leaves remained turgid until at least 26 days after inoculation. It should be mentioned that our data do not preclude the possibility of a localized effect on the water relations of infected leaves, such as a loss of membrane function in guard cells as postulated by Ayres for mildew-infected peas (4). Our data indicate that the most significant result of mildew infection of sugar beets with regard to plant water relations is the inability of mildewed leaves to control their transpiration rate, especially under conditions of water stress.

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