Some Effects of Uromyces phaseoli on the Transpiration Rate and Stomatal Response of Bean Leaves

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ABSTRACT

The transpiration rate and average stomatal aperture of primary bean leaves were significantly reduced during the fleck stage of rust development. A leaf disc assay for stomatal response showed that the pathogen inhibited stomatal opening in the light. Although inhibition was greatest during the fleck stage, the effect persisted through the remainder of the disease cycle. There was a linear decrease in the average stomatal aperture attained in the light as the infection density was increased up to 75 pustules/cm^2; at higher infection densities, there was no additional change in stomatal aperture. When the effect was maximal, the average stomatal aperture was about 30% of the healthy control. A significant reduction in stomatal aperture occurred up to 0.5 mm from the margin of isolated fungal colonies. Unlike the control, the stomata of diseased leaf discs did not respond to CO_2-free air.

At sporulation, the epidermis was ruptured and cuticular transpiration became paramount. This was evidenced by the high transpiration rate of rusted leaves in the dark, and by the lack of a correlation between stomatal aperture and transpiration after sporulation. Phytopathology 61:114-119.

Rust infections have generally been found to increase the rate of transpirational water loss from plants (12). Some workers, however, have observed that prior to sporulation rust infections actually reduce transpiration, and that only as sporulation occurs does transpiration increase (1, 9, 15, 21). This increase was most readily observed at night, and has usually been attributed to epidermal rupture.

The possible involvement of stomata in the observed transpirational alterations has only been briefly examined (8, 15, 21), and in the case of bean rust there are conflicting reports. Yarwood (21) observed the stomata over unopened pustules of bean rust to be closed relative to those in unparasitized tissues, and he attributed the reduction in transpiration during the early stages of the disease cycle to this lack of stomatal opening. In contrast, Sempio et al. (15) reported bean rust to cause an increase in stomatal aperture at the same time that it caused a reduction in transpiration. Only at the late stages of the disease cycle, when there was an increase in transpiration, did they find stomatal apertures to be reduced. In view of these contradictions and because there have been few detailed reports on the effect of diseases on stomatal behavior (6, 14, 20), this study was undertaken to examine the effect of rust infection on the transpiration and stomatal response of bean. Preliminary reports of this work have been presented (4, 5).

MATERIALS AND METHODS.—*Environmental conditions.*—Plants were grown and all experiments were done in a growth room having day and night periods of 16 and 8 hr, respectively. Light was provided by a mixture of cool-white fluorescent bulbs (1,100-1,500 ft-c at primary leaf height) and incandescent lights (100-150 ft-c). The air temp was 27 ± 1 C during the day and 17 ± 1 C during the night. Air turbulence was just enough to cause leaf movement. The calculated saturation deficit (sd) was 12.0 ± 3.5 mm Hg during the day and 6.5 ± 2.0 mm Hg during the night (17).

The snapbean, *Phaseolus vulgaris* L. 'Topcrop', and the fungus, *Uromyces phaseoli* (Pers.) Wint. *typica* Arth. race 32, were used. One week after seeding, plants with uniform primary leaves were transplanted into 500-cm^3 waxed paper cups containing vermiculite. They were watered daily with a nutrient solution at pH 4.5 containing 1.5 mM NH_4NO_3, 1.5 mM Ca $(NO_3)_2$, 1 mM KNO_3, 1 mM KH_2PO_4, and 1 mM $MgSO_4$. The solution also contained 0.5 ml of trace element solution (11) and 0.06 ml of chelated iron solution (Versenol FL, Dow Chemical Co.) per liter.

Two weeks after seeding, both surfaces of the fully expanded primary leaves were sprayed with a suspension of uredospores in 10^{-4} M *n*-nonyl alcohol (7). Except where noted, the spore concn was adjusted to yield between 55-65 pustules/cm^2 of leaf area. Control plants were sprayed with the *n*-nonyl alcohol solution, and both treatments were incubated for 12 hr in a 20-C mist chamber before being returned to the growth room.

Transpiration.—Transpiration was measured by two methods. The first method, a gravimetric analysis, involved sealing the tops of cups containing two plants with paraffin on the 1st day after inoculation (9). Nutrient solution was added at the beginning of each subsequent day, and the wt losses during the following day and night periods were measured separately. To avoid complications from growth in leaf area, the trifoliate leaves were removed as they started to expand.

The second method utilized a recording hygrometer to determine the rate of water vapor loss from an individual intact primary leaf. Each midday, a leaf was inserted into a chamber and a tight seal made around the petiole. Air of known humidity was passed into the chamber, and the absolute humidity of the air leav-

ing the chamber was measured continuously during the next 24-hr period. The major components of the system were surrounded by water at 27 ± 0.2 C.

Stomatal response.—A modification of the leaf disc assay described by Zelitch (22) was used to measure stomatal response. Between 1 and 2 hr after the beginning of the light period, intact plants were placed in the dark at 28 C and 0 sd for 30 min. Discs were then cut from the primary leaves and floated, abaxial side down, on nutrient solution. The containers of solution were placed in a water bath at 28 ± 0.2 C in the dark for an additional 30-90 min. The contents of the water bath were then exposed to light in the growth room for 90 min, unless otherwise stated. Discs were blotted dry, and silicone rubber impressions made of the abaxial surface. The apertures of 10 stomata were measured at random on the positive replica of each disc. The average stomatal aperture reported is the mean for five discs, one from each of five plants. When an analysis of variance and an F-test showed significance between treatments at the 1% level, but not between replicates, Tukey's "honestly significant difference" procedure was used to calculate a value for judging the significance of all differences within the experiment (18).

RESULTS.—The transpiration rate of diseased leaves measured gravimetrically was significantly less than that of healthy leaves (LSD calculated for each day) when fleck symptoms were apparent on the 4th through the 6th days after inoculation (Fig. 1). During this period there was no difference between the treatments at night. With the onset of sporulation the situation was reversed, and diseased leaves transpired at a significantly higher rate than healthy leaves on the 7th through the 9th days. They also had a significantly higher transpiration rate on the 6th and subsequent nights.

Measurements made under steady-state conditions with the recording hygrometer confirmed the results obtained gravimetrically. Moreover, measurements made when the illumination was changed showed additional differences between the treatments. For example, on the 5th night after inoculation, the transpiration rate of diseased leaves declined to a constant level more slowly than did that of healthy leaves and rose more slowly on the following morning (Fig. 2-A, B). In going from the 9th night to the 10th day after inoculation, diseased leaves showed little change in transpiration with changes in illumination (Fig. 2-C, D).

The stomatal apertures of transpiring leaves were measured by making silicone rubber impressions randomly throughout the light period of the 5th and 9th days after inoculation. On the 5th day, healthy and diseased leaves had average stomatal apertures of 1.9 and 1.0 μ, respectively (LSD = 0.4 μ); on the 9th day, the corresponding apertures were 1.1 and 0.7 μ (LSD = 0.3 μ). The stomata of both treatments appeared to be closed during the ensuing dark period.

The leaf disc assay allowed measurements of stomatal response to be made in the absence of water stress. An assay done 5 days after inoculation showed that the pathogen caused a partial inhibition of stomatal

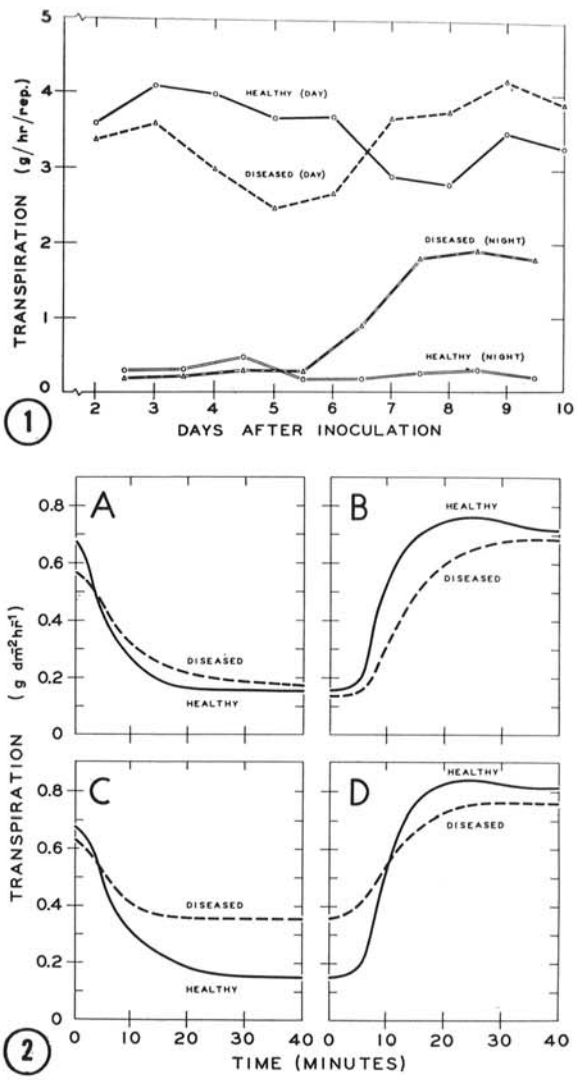


Fig. 1-2. **1)** The transpiration rates of healthy and diseased leaves measured gravimetrically at different times after inoculation. **2)** The transpiration rates of healthy and diseased leaves measured continuously during the first 40 min of the night or day period. The 0 time corresponds to the beginning of the 5th night (A); the 6th day (B); the 9th night (C); and the 10th day after inoculation (D).

opening in the light (Fig. 3). The period of illumination required for a significant increase in stomatal aperture was 60 min in diseased leaf discs, but only a little over 10 min in healthy leaf discs. Maximum apertures were obtained in 90 min of illumination, and were 2.2 and 6.8 μ in diseased and healthy leaf discs, respectively. Increasing the intensity or duration of illumination did not significantly alter the max aperture of either treatment. When illumination was discontinued after 120 min, the stomata of both treatments started to close immediately.

Daily measurements of stomatal response showed that inhibition of stomatal opening by the pathogen first became significant 4 days after inoculation (Fig.

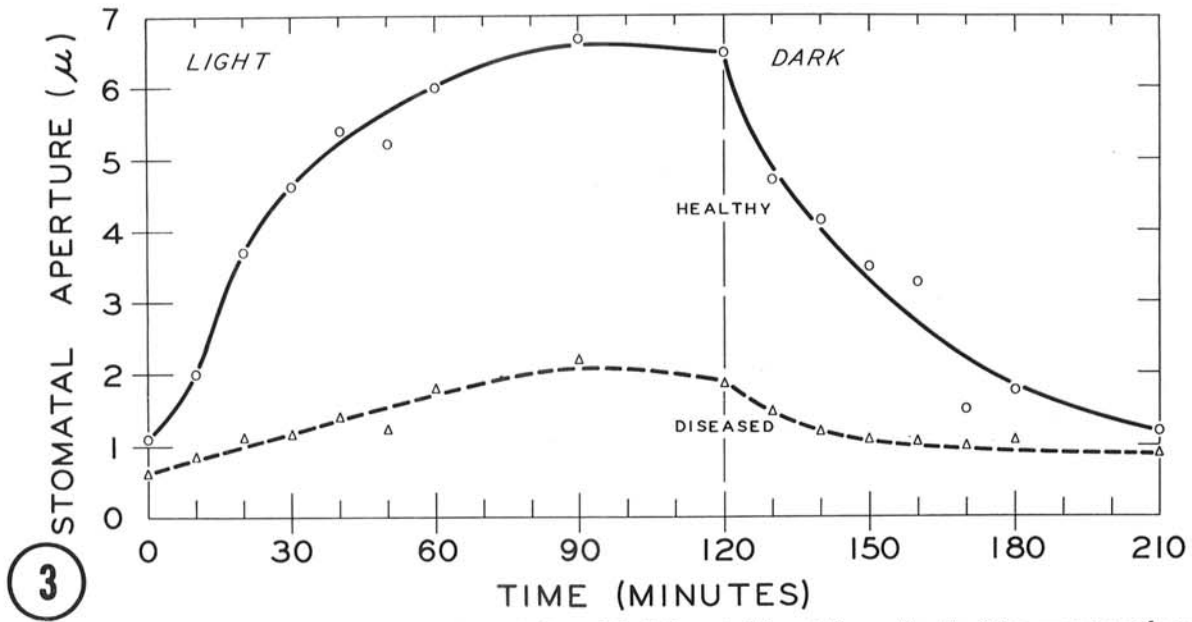


Fig. 3. Average stomatal apertures of healthy and diseased leaf discs at different times after the lights were turned on (at 0 min) and off (at 120 min). A difference of 1.1 μ between apertures indicates significance at the 1% level.

4). Although the inhibition was greatest 5 days after inoculation, the day chosen for subsequent experiments, a significant inhibition of lesser magnitude persisted through the remainder of the disease cycle.

In one experiment, leaf discs were floated abaxial side up on degassed water and exposed to normal or CO_2-free air in the light or dark. CO_2-free air was obtained by passing normal air through 5 N KOH and adjusting it to 28 C and 0 sd before it flowed through the experimental vessels (3). Silicone rubber impressions were made of the abaxial surface after a 90-min exposure. In the light, CO_2-free air did not induce a significant stomatal response in either healthy or diseased leaf discs. In the dark, CO_2-free air did induce a highly significant stomatal response in healthy leaf discs but not in diseased leaf discs (Table 1).

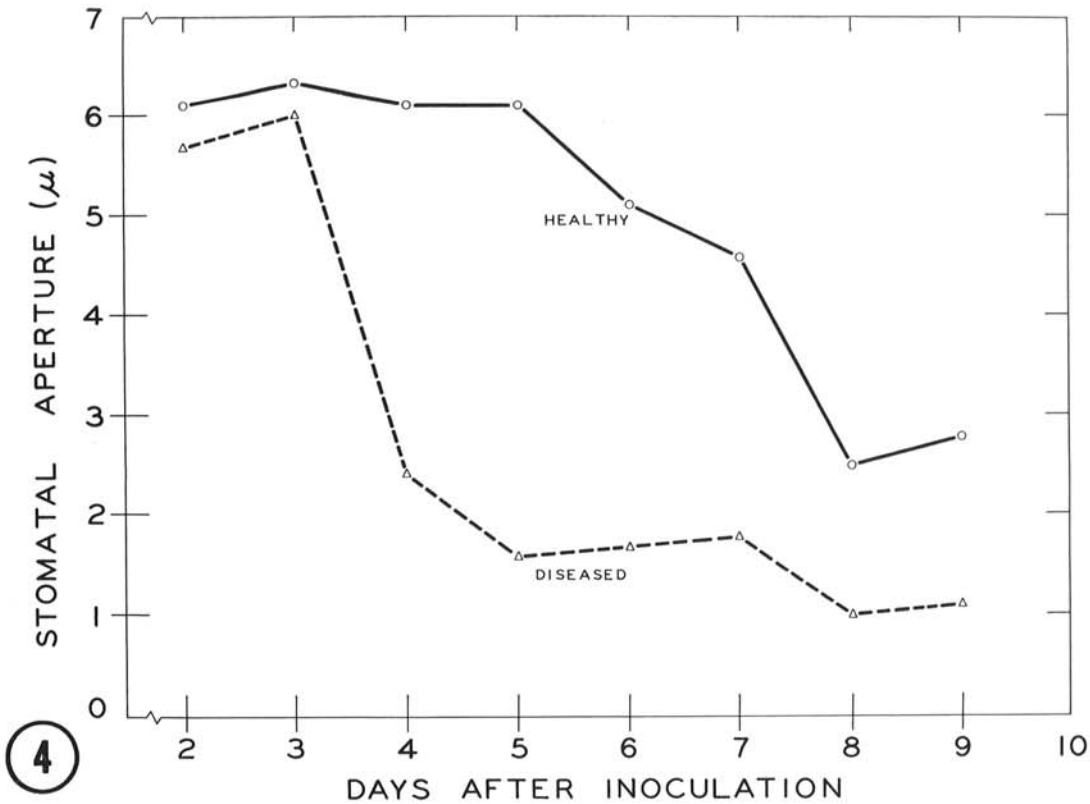
The average stomatal aperture attained in the light and the percentage of leaf area invaded by the pathogen were plotted as functions of infection density (Fig. 5). The percentage of area invaded was calculated from the number of colonies and average colony diam as measured on the discs after they had been cleared and stained (3). As the infection density was increased from 0 to 73 pustules/cm^2, there was a linear decrease from 4.9 to 1.0 μ in the average stomatal aperture attained in the light; with higher infection densities there was no additional decrease. A similar, but positive, relationship existed between infection density and the amount of leaf area invaded, except that the max was reached at an infection density of 108 pustules/cm^2.

TABLE 1. Response of stomata on healthy and diseased leaf discs to light and CO_2-free air. Apertures with different superscripts are significantly different at the 1% level

Conditions	Stomatal aperture	
	Healthy	Diseased
	μ	μ
Light + normal air	6.3[a]	2.4[b]
Light + CO_2-free air	6.5[a]	3.3[b]
Dark + normal air	0.8[c]	0.7[c]
Dark + CO_2-free air	4.8[d]	0.8[c]

The fact that no additional decrease in stomatal aperture occurred as the percentage of leaf area invaded was increased from 50 to 75% suggested that the pathogen might inhibit stomatal opening some distance beyond the colony margin. Using 5 leaf discs containing only one colony, stomatal apertures were measured directly at various distances from the colony margin. The center of the fleck was marked with a fine needle on a silicone rubber impression and leaf disc while they were still attached. After clearing and staining, a disc was mounted abaxial side up on a slide with the same orientation as its positive impression. The distance from the mark to the furthest hyphal tip was then measured in four directions. Proceeding in the same manner on the matching impression, 10 stomatal apertures were measured per increment of distance given below. Average stomatal aperture varied significantly with increasing distance from the colony margin as follows: within the colony, 0.9 μ; 0-0.25 mm, 2.9 μ; 0.25-0.50 mm, 4.5 μ; and 0.50-5.00 mm, 5.6 μ.

Experiments of a different type demonstrated that this effect on stomata is not general in uninvaded regions of the host. There was no measurable reduction in average stomatal aperture of leaf discs taken at distances of more than 5 mm from the pustules of a dense infection. In addition, there was no difference between the average stomatal apertures of discs from



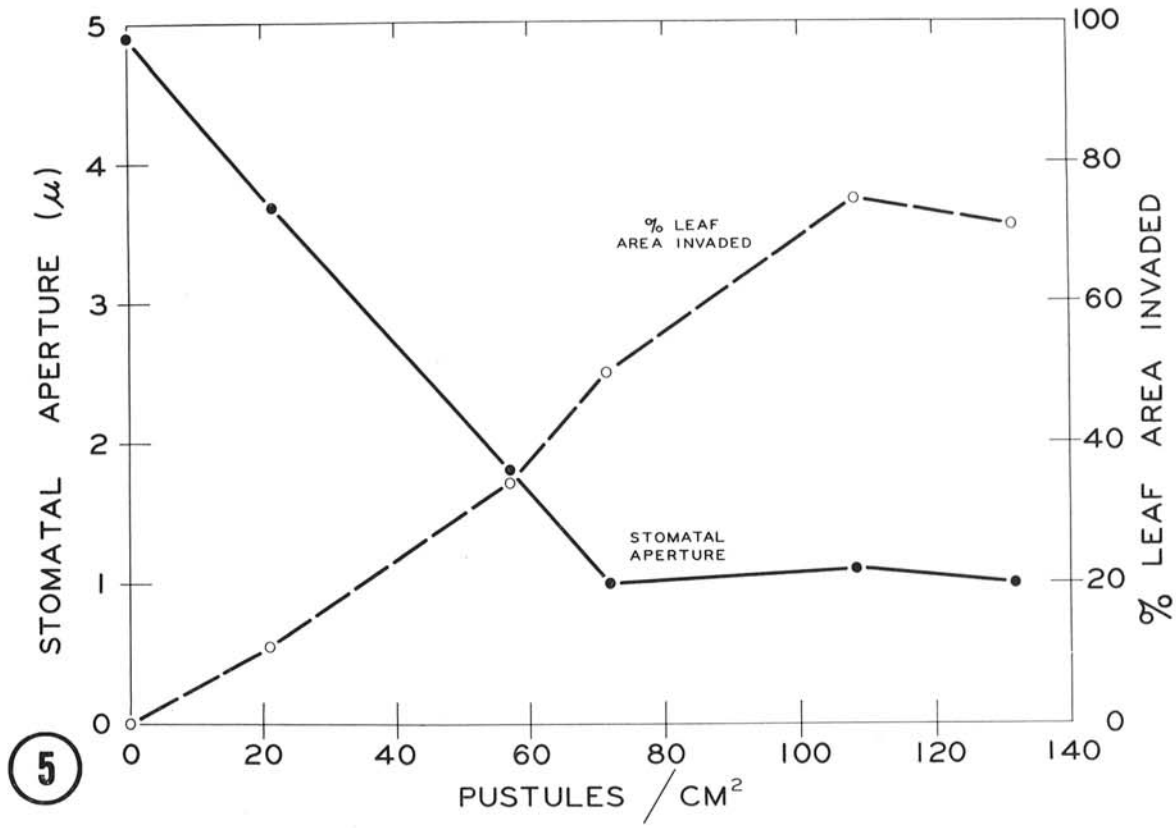


Fig. 4-5. **4)** Average stomatal apertures of healthy and diseased leaf discs in the light on different days after inoculation. A difference of 1.1 μ between apertures indicates significance at the 1% level. **5)** Average stomatal aperture obtained in the light and percentage of leaf disc area invaded by the pathogen plotted as functions of infection density. A difference of 0.9 μ between apertures indicates significance at the 1% level.

healthy trifoliate leaves above healthy or diseased primary leaves at 8 days after inoculation.

DISCUSSION.—The rate of diffusion of water vapor out of the leaf generally limits the rate of transpiration (17). In the experiments described here, leaf boundary layer resistance and the difference in vapor pressure between the leaf and ambient air, which is the sd where leaf and ambient air temp are equal, can be assumed to have been the same for healthy and diseased leaves. Therefore, in attempting to explain the observed differences in transpiration, one must examine the effects of the pathogen on stomatal and cuticular resistances.

The results presented here lead to the conclusion that inhibition of stomatal opening by the pathogen reduced the transpiration rate of leaves during the fleck stage. For instance, both the inhibition of stomatal opening in the leaf disc assay and the reduction in transpiration first became significant 4 days after inoculation. In addition, intact rusted leaves had significant reductions in both transpiration and stomatal aperture at 5 days after inoculation. Cuticular resistance cannot account for the reduction in transpiration because cuticular resistance is normally very much higher than, and parallel to, the resistance of open stomata.

While the conclusion that stomata are responsible for the reduction in transpiration agrees with that of Yarwood (21), it does not agree with that of Sempio et al. (15). It is difficult to explain the increase in stomatal aperture which they found associated with the reduction in transpiration. The stomatal aperture of individual transpiring leaves varied greatly during our experiments, and perhaps such variation confounded the results of their brief study.

The results show that as sporulation occurred, the transpiration rate of rusted leaves became nearly independent of stomatal aperture. A significant reduction in stomatal aperture and increase in transpiration were found with the same leaves 9 days after inoculation, and the increase in transpiration was evident on the same days as was an inhibition of stomatal opening in the leaf disc assay. The recording hygrometer showed that leaves with sporulating uredia transpired at nearly the same rate in the presence or absence of light, even though the stomata of diseased leaves were found to close in the dark. In fact, the diurnal variation in the transpiration rate of leaves with sporulating uredia found gravimetrically probably only reflects the diurnal variation in sd.

The independence of the transpiration rate and stomatal aperture after sporulation (see also 15) indicates that cuticular resistance was low. Johnston & Miller (12) followed a similar line of reasoning when they attributed the increased transpiration to water loss through the open uredia. While fungal tissues do have a transpirational resistance of their own (21), water vapor loss from fungal and host tissues in the uredia would still be expected to nearly saturate the air near these surfaces. Thus, with sporulation there is a decrease in the cuticular resistance of a leaf to water vapor loss, this decrease probably being somewhat proportional to the number and size of the epidermal ruptures over uredia. Indeed, microscopic examinations, made when the increase in transpiration at night first occurred, revealed small openings in the epidermis over a few of the uredia; as sporulation progressed, the openings became larger and more numerous.

Yarwood's hypothesis (21), that the increased transpiration after sporulation is a consequence of increased cell permeability, is not in keeping with the arguments presented here. Furthermore, his hypothesis does not take into account the likely effect of a permeability change on stomata. Although the role of guard cell permeability in the inhibition of stomatal opening reported here remains unknown, the increased permeability of rust-infected tissues found by Thatcher (19) would be expected to allow a leakage of solute from the guard cells and thus cause stomatal closure.

The leaf disc assay demonstrated that the pathogen reduced both the rate of stomatal opening (μ per min) and the final aperture attained in the light. Therefore, there was a partial inhibition of the net opening process (22). The reduced rate of stomatal opening in diseased leaves was also demonstrated with the recording hygrometer 6 days after inoculation. While the results showed that the stomata under the influence of the pathogen did close in the dark, it is difficult to assess the effect of the pathogen on the rate of stomatal closure. Perhaps the more rapid stomatal closure in healthy leaf discs can be attributed to the greater aperture attained during the preceding light period. Unfortunately, the rates at which transpiration declined on the 5th night after inoculation were most likely complicated by a turgor difference between the treatments. The healthy leaf transpired considerably more water during the preceding 16-hr period, and because of an absorption lag due to root resistance it would be expected to have a lower leaf water potential (13).

The stomata of diseased leaf discs, unlike those of healthy leaf discs, did not respond to CO_2-free air in the dark. Heath (10) has suggested that a lack of gas exchange between the air and substomatal cavity may prevent a stomatal response to CO_2-free air in the dark. This probably does not apply here because the stomata in both healthy and diseased leaf discs had virtually the same aperture in the dark, and thus, CO_2-free air would have had almost equal access to the substomatal cavities of both treatments. The absence of a CO_2-free air response in diseased leaf discs does suggest that the pathogen does not inhibit stomatal opening by increasing the CO_2 level in the leaf, an increase which could arise from the high respiration rate of rusted tissues (16).

Since stomata in uninvaded tissues less than 0.50 mm from a colony margin are affected by the pathogen, contact between the pathogen and guard cells is not a prerequisite for the observed inhibition. In fact, even in invaded portions of the leaf, very few guard cells were in contact with hyphae or appressoria (2). When values for individual stomatal apertures were rounded off to the nearest 0.5 μ, plots of the number of stomata as a function of aperture revealed a nearly normal distribution about the mean aperture for both healthy and

diseased leaf discs. Thus, it appears that the pathogen did not cause reductions in average stomatal aperture by rendering a fraction of the stomata completely nonfunctional. Rather, the stomata under the influence of the pathogen had a range of apertures, as did stomata under the influence of environmental factors or chemical inhibitors (22).

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