Detrimental Effect of Rust Infection on the Water Relations of Bean

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ABSTRACT

Bean plants (*Phaseolus vulgaris* L.) infected with the rust *Uromyces phaseoli* became unusually susceptible to drought as sporulation occurred. Under the conditions used (1,300 ft-c, 27 C, and 55% relative humidity) such plants wilted at soil water potentials greater than −1 bar, whereas healthy plants did not wilt until the soil water potential fell below −3.4 bars. Determinations of leaf water and osmotic potentials showed that an alteration in leaf osmotic potential was not responsible for the wilting of diseased plants. When diffusive resistance was measured as a function of decreasing leaf water content, the resistance of healthy leaves increased to 50 sec cm⁻¹ by the time relative water content decreased to 70%, whereas the resistance of diseased leaves remained less than 8 sec cm⁻¹ down to 50% relative water content. Apparently, water vapor loss through cuticle damaged by the sporulation process, together with the reduction in root to shoot ratio which occurs in diseased plants, upset the water economy of the diseased plant under mild drought conditions.

Diseases which do not characteristically cause wilting are not generally thought to affect host water relations adversely. However, there are indications that foliar infection, particularly by rust fungi, can cause unusual levels of plant water stress to occur. For example, rusted bean and corn leaves were found to have lower water contents than healthy leaves under conditions conducive to water stress (19). In terms of yield, a brief drought was also found to be more damaging to rust-infected than to healthy wheat plants (4). Furthermore, rust infection has been shown to alter osmotic potential (16) as well as transpiration rate (8) and root growth (20) or function (4). However, no one has demonstrated just how susceptible rust-infected plants are to drought or exactly what mechanisms are involved. The experiments described here were prompted by this lack of knowledge about a type of disease that is omnipresent, and by the observation that rust-infected bean plants wilted long before

healthy plants when water was withheld. A preliminary report of this research has been published (7).

MATERIALS AND METHODS

Biological Materials. Except where noted, bean plants (*Phaseolus vulgaris* L.) of the variety Topcrop were grown in vermiculite, and inoculated 12 days after seeding with race 32 of *Uromyces phaseoli* (Pers.) Wint *typica* Arth. (5, 8). Healthy and diseased plants shared the same root container. Inoculations were done by first spraying the leaves of control plants with a 10^{-4} M *n*-nonyl alcohol solution and then covering these plants with a plastic bag. A suspension of uredospores in the same solution was subsequently sprayed on the remaining plants. Measurements were made between 8 and 12 days after inoculation when primary leaves of diseased plants had 55 to 65 sporulating colonies per cm² of leaf area. By this time, the first trifoliate leaf was fully expanded and was always free of rust infection.

Plants were maintained in a controlled environment chamber with 16-hr days at 27 ± 1 C and 1,300 ± 200 ft-c of light, and 8-hr nights at 17 ± 1 C. The relative humidity was 55 ± 5% at all times, and air turbulence was just enough to cause leaf movement. Plants were watered daily with a half-strength nutrient solution.

Water and Osmotic Potentials. A Peltier-cooled thermocouple psychrometer was used to measure leaf water and osmotic potentials (1). Osmotic potential (ψ_π) was determined with the same tissue as leaf water potential (ψ_{leaf}), after it had been frozen and thawed. Leaf pressure potential (ψ_p) was taken to be $\psi_{leaf} - \psi_\pi$ (10). ψ_π was also measured by freezing point depression, after leaves were brought to full turgor and blotted dry. The freezing point depression of expressed sap was measured (5), and ψ_π at 25 C was calculated using a correction for supercooling (1). The water potential of the vermiculite medium, hereafter referred to as ψ_{medium}, was estimated by lining the psychrometer chamber with a piece of blotting paper, which had been buried in the vermiculite prior to the most recent application of water. A large volume of distilled water, rather than the usual nutrient solution, was applied at this time.

Root to Shoot Ratio. The total leaf area of plants was measured with a planimeter 9 days after inoculation. The plants were then uprooted under water, and the remaining vermiculite was washed from the roots. The weights of individual root systems were determined, after they had been blotted dry in a standardized manner. The root to shoot ratio of a plant is expressed as its root weight per unit leaf area (g cm⁻²).

Transpiration. Trifoliate leaves of the bean variety Blue Lake were used in the transpiration experiment. Diseased leaves had

30 sporulating colonies per cm² of an unknown race of *U. phaseoli* var. *typica*.

The equation for transpiration, E (g cm⁻² sec⁻¹), is generally written in the form

$$E = \frac{c_w - c_a}{r_a + r_1} \qquad (1)$$

where c_w and c_a (g cm⁻³) are the water vapor concentrations at the surface of the mesophyll cell walls and in the ambient air, respectively, r_a (sec cm⁻¹) is the boundary layer resistance and r_l is the resistance of the leaf. The parameters in equation 1 were obtained from measurements of transpiration in a leaf chamber. The value of c_w was assumed to be the saturation vapor concentration at leaf temperature, which was measured with a thermocouple in the leaf blade. Values of $(r_a + r_l)$ were thus obtained from measurements of leaf temperature, transpiration rate, and ambient vapor concentration (15). Small fans in the chamber made r_a constant and relatively small. Its value was found to be 0.23 sec cm⁻¹ using wet blotting paper ($r_l = 0$), instead of a real leaf. For any set of measurements of $(r_a + r_l)$, r_l was then obtained by subtraction of r_a.

Transpiration measurements were made under a mercury vapor lamp which provided a radiation flux density of 20 mw cm⁻² in the 300 to 700 nm wave band. The chamber was equipped with a β-gauge for the measurement of leaf water content (13), and water stress was induced during the measurements by slowly chilling the roots to 0 C.

RESULTS AND DISCUSSION

Relative Susceptibility to Drought. When water was withheld from the vermiculite, diseased plants wilted more rapidly than healthy plants (Fig. 1, Table I). When plants were well watered, leaves of healthy and diseased plants appeared turgid and had similar values of ψ_{leaf} and ψ_p. However, when water was withheld, both the noninfected trifoliate and infected primary leaves of diseased plants wilted and had suffered major reductions in ψ_{leaf} and ψ_p by the time ψ_{medium} had dropped to −0.8 bars. In contrast, the same treatment had little effect on healthy plants, and the vermiculite had to dry until ψ_{medium} was −3.4 bars before healthy plants were even slightly wilted. By this time diseased plants were very severely stressed as evidenced by their low ψ_{leaf} values.

Leaf Osmotic Potential. Additional psychrometer determinations of ψ_{leaf} and ψ_π are shown in Figure 2. Although there is some variation in the data, it can be seen that the relationship between ψ_{leaf} and ψ_π was the same in the rust-free trifoliate leaves of healthy and diseased plants. While fewer data points were obtained for healthy and diseased primary leaves, those obtained did fit the relationship shown in Figure 2.

Measurements of freezing point depression also showed little difference in ψ_π between healthy and diseased plants. Sap from fully turgid leaves had average values of ψ_π as follows: healthy plant primary leaf, −7.5 bars; healthy plant trifoliate leaf, −7.2 bars; diseased plant primary leaf, −8.0 bars; and diseased plant trifoliate leaf, −7.1 bars. These values approximately equal the
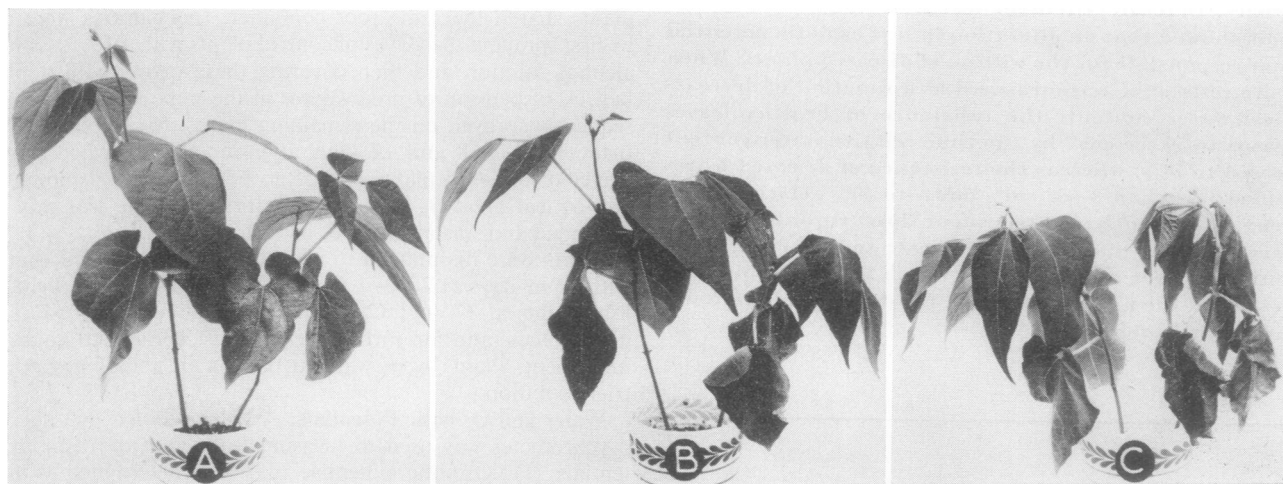

FIG 1. The condition of healthy and diseased plants after water was withheld for approximately 2 (A), 36 (B), and 60 hr (C). Cups B and C were selected for photography when the diseased and healthy plant, respectively, first appeared wilted. The diseased plant is on the right in each cup.

Table I. *Water Potentials in the Root Medium* (ψ_{medium}) *and Leaves* (ψ_{leaf}) *with Corresponding Leaf Pressure Potentials* (ψ_p)
Determinations were made under the conditions shown in Figure 1 on four separate occasions. The data represent mean values and total variation for the four determinations.

ψ_{medium}	Plant	Primary Leaf		Trifoliate Leaf	
		ψ_{leaf}	ψ_p	ψ_{leaf}	ψ_p
		bars			
0	Healthy	−6.3 ± 0.3	+3.3 ± 0.2	−7.3 ± 0.3	+2.7 ± 0.3
0	Diseased	−8.0 ± 0.5	+2.1 ± 0.1	−7.6 ± 0.8	+3.5 ± 0.2
−0.8 ± 0.1	Healthy	−7.8 ± 0.3	+2.6 ± 0.4	−9.4 ± 0.5	+2.0 ± 0.1
−0.8 ± 0.1	Diseased	−14.9 ± 0.3	−0.1 ± 0.1	−12.6 ± 0.4	+0.8 ± 0.2
−3.4 ± 0.2	Healthy	−12.3 ± 1.7	+0.8 ± 0.3	−12.2 ± 0.8	+0.6 ± 0.3
−3.4 ± 0.2	Diseased	−22.5 ± 3.1	−0.4 ± 0.1	−17.5 ± 4.0	−0.1 ± 0.6

projected value of ψ_π at $\psi_{\text{leaf}} = 0$ in Figure 2. Apparently rust does not predispose plants to drought by increasing leaf ψ_π. The differences observed between healthy and diseased plants in ψ_π ($\psi_{\text{leaf}} - \psi_\rho$ in Table I) can be attributed to the variation in leaf water content associated with differences in ψ_{leaf} (Fig. 2).

Root to Shoot Ratio. Root to shoot ratios of healthy and diseased plants were 1.14 and 0.85 g cm^{-2} × 10^{-2}, respectively (0.15 × 10^{-2} = LSD at 5%). Increased root resistance might therefore contribute to the wilting of diseased plants. However, a cessation of root function, like that reported in heavily rusted wheat plants (4), was not evident in this study. Rust-infected bean plants did not wilt unless water was withheld or transpirational demands were increased.

Transpiration. Transpiration was implicated as a cause of wilting when it was observed that diseased plants in solution culture wilted when placed in front of a fan while comparable healthy plants remained turgid. The exact role of transpiration in wilting was examined by measuring the diffusive resistance of a leaf, r_l, as a function of its decreasing relative water content (θ).

The value of r_l in a healthy leaf increased markedly when θ dropped below 85% ($\psi_{\text{leaf}} < -11.0$ bars) and increased to nearly 50 sec cm^{-1} by the time θ dropped to 70% ($\psi_{\text{leaf}} \simeq -15.0$ bars) (Fig. 3). This increase in r_l with leaf water stress is similar to that found previously in bean (14). In contrast to healthy leaves, the r_l value of a leaf with 30 sporulating colonies per cm^2 remained less than 8 sec cm^{-1}, even when θ was reduced to 51% ($\psi_{\text{leaf}} \simeq -25.0$ bars). When the measurements were repeated in the dark, the values of r_l in a comparable healthy or infected leaf at a given value of θ were essentially twice those shown in Figure 3 for the two conditions.

Stomatal closure in response to leaf water stress is responsible for the increase in r_l with the decrease in the water content of healthy leaves (14). This closure helps to maintain a balance between the rates of water supply and water loss and thus to prevent injurious water loss. Evidently, such limitation of water loss does not occur in rust-infected leaves after the time of sporulation. Thus, the diseased plant suffers from a continuous water loss which is of serious consequence when the transpirational demand exceeds the capacity of the plant-soil system to supply water. In addition, the capacity to supply water is reduced in rust-infected plants, as evidenced by the reduction in root to shoot ratio.

Silicone rubber impressions of leaf surfaces, made at the end of the transpiration measurements, indicated that the stomata were closed. This observation and the small increase in the r_l of diseased leaves with water stress or darkness, indicate that the cuticular component of r_l was very small. The cuticle is physically damaged at the time of sporulation, and apparently there is a significant water vapor flux through the sporulating colonies (see also 8, 18).

The conclusion that continuous water loss through damaged portions of the cuticle is the primary cause of wilting suggests that the susceptibility of a plant to drought will be somewhat proportional to the number of sporulating rust colonies on the plant. Thus, even rather sparse rust infections may be of consequence under arid conditions. Other pathogens that are thought to increase cuticular water loss (11, 18) probably also adversely affect host water relations. An inhibition of stomatal closure would affect plant water relations as rust infection does, and there is evidence that a toxin produced by a plant pathogen causes such an inhibition (17). In comparing the effects of diseases on water relations, it is interesting to note that the mechanism which upsets the water economy of rust-infected plants is just the opposite of the restriction of water flow to leaves which occurs in plants with vascular wilt disease (6).

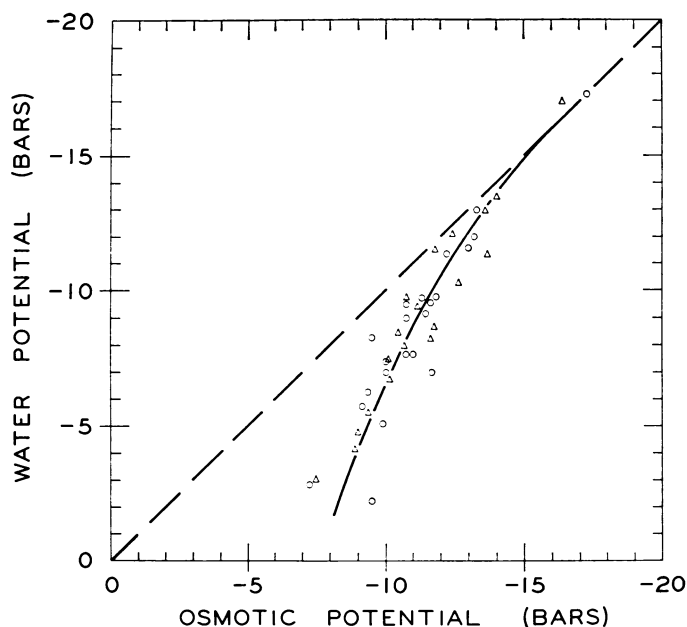There is evidence that plant water stress reduces photosynthe-

FIG. 2. Water potential plotted as a function of osmotic potential in the rust-free trifoliate leaves of healthy (circles) and diseased plants (triangles). The difference between the dashed line with a slope of one and the smooth curve through the data gives the average pressure potential. Water potentials below −6 bars were obtained by withholding water from healthy and diseased plants for 0 to 100 and 0 to 60 hr, respectively, whereas water potentials above −6 bars were obtained by holding well watered plants at 100% relative humidity for 5 to 120 min.
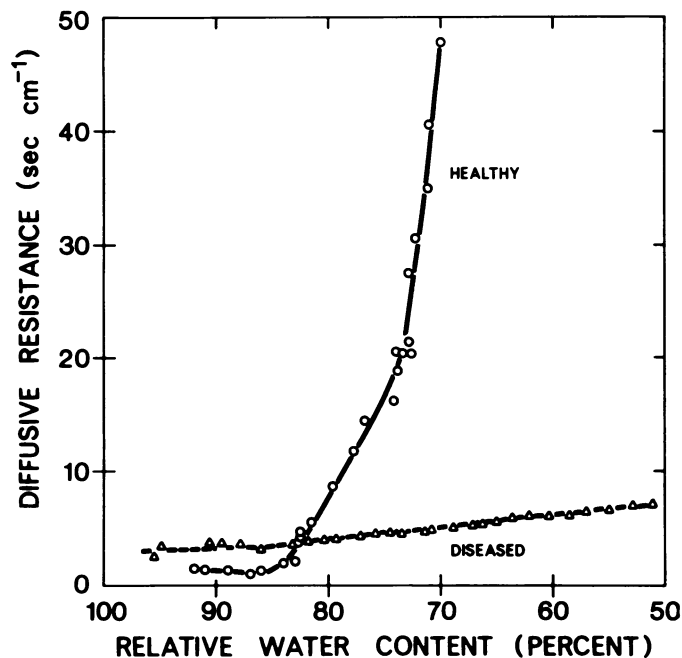
FIG. 3. Diffusive resistance of healthy and diseased leaves plotted as a function of decreasing relative water content. The measurements on healthy and diseased leaves required 10 and 5 hr, respectively.

sis (3), protein synthesis (12), growth (2), and crop yield (9). Because of these reductions and because rust infection can cause unusual levels of water stress to occur throughout the plant, the effect of rust on water relations may well be the most damaging effect on the disease on the host plant.

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