Diffusive Resistances of Two Sugarbeet Cultivars in Relation to Their Black Root Disease Reaction

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ABSTRACT

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Leaves of sugarbeet (*Beta vulgaris*) seedlings, 4 weeks old, were inoculated with zoospores of *Aphanomyces cochlioides,* and diffusive resistances to vapor flow were determined at intervals with a diffusion porometer. Two cultivars were used: the highly susceptible SP63269-0, and the less susceptible SP6822-0. Five days after inoculation, diffusive resistances of leaves of cultivar SP63269-0 averaged 4.5 sec cm^{-1}, whereas resistance of SP6822-0 averaged 2.6 sec cm^{-1}. Noninoculated control plants of both cultivars averaged 1.6 sec cm^{-1}. Inoculated plants of SP63269-0, and those from which water was withheld, had a similar relation between leaf diffusive resistance and leaf water potential. Whole-plant resistances to water transport, calculated from steady-state transpiration data, were 10-fold larger for inoculated plants than for control plants 9 days after inoculation. The increased diffusive resistance of infected plants appears, therefore, to be caused by decreased water supply to leaves.

Characterizations of foliage wilting associated with infections by root-rotting fungi have been reported by several investigators (5, 10, 12). Infection of pine seedlings by *Verticicladiella wagenerii* caused decreases in leaf water content, transpiration rate, and stomatal aperture (10). Likewise, infection by a *Phytophthora* sp. caused reduced leaf water potentials in rhododendron (5).

Black root, a disease caused by the water mold, *Aphanomyces cochlioides* Drechs., is a major sugarbeet disease in humid areas of the USA. Sugarbeet lines differ in susceptibility to the black root syndrome, which includes seedling blight, foliage wilting, and root rot. Increased resistance to the pathogen is a major objective in the development of improved cultivars for the humid area.

The purpose of the work described in this report was to determine the effects of *A. cochlioides* infection on diffusive resistance of leaves to vapor flow in two sugarbeet cultivars that differ in susceptibility to the fungus.

MATERIALS AND METHODS.—*Test plants.*—The two sugarbeet cultivars used in this study included the moderately resistant SP6822-0 (designated Line R in this report) and the more susceptible SP63269-0 (designated Line S). Seedlings were grown in 9-cm diameter styrofoam pots containing a steam-sterilized mixture (1:1:1, v/v) of peat, vermiculite, and arcillite (a granular calcined montmorillonite clay commercially obtainable from greenhouse supply outlets). After a preliminary 2.5- to 3.0-week period in the greenhouse, seedlings were thinned to one plant per pot, and transferred to a growth chamber maintained at 26 C during a 14-hour light period and at 21 C during a 10-hour dark period. Light intensity was approximately 18.3×10^3 lumens/m^{-2} (fluorescent) and 1.1×10^3 lumens/m^{-2} (incandescent) at plant height; relative humidity was maintained at 60% ± 5%.

Inoculation of plants.—Test plants were inoculated 4-7 days after transfer to the growth chamber. *Aphanomyces cochlioides* zoospore inoculum was produced in vitro in accordance with a previously described method (13), using a replacement solution prepared by the method of Yang and Schoulties (15).

Zoospore suspensions were diluted with replacement solution to allow application of 1×10^6 spores in 25 ml of solution per pot. An equal amount of sterile replacement solution was applied to each control pot.

Aboveground symptoms of infection in plants of Line S were generally evident within 3-5 days after inoculation, and in Line R, by 7 days. Subsequent determinations of disease severity were expressed according to the following numerical index: 1 (blackening near base of hypocotyl); 2 (more than one-half of the hypocotyl blackened); 3 (most of hypocotyl blackened and generally reduced to a thread); 4 (plant moribund); 5 (plant dead). The ratings also indicate relative degree of root damage resulting from the infection.

Measurements of transpiration.—Plants, pots, and soil were enclosed to the plant crown with polyethylene to prevent water loss from the soil surface and the pots. Steady-state transpiration rates were determined from weight loss per pot when soil was moist, and expressed as g (water loss)·$100 cm^{-2}$ (leaf area)·hr^{-1}. Leaf areas for whole plants were measured from leaf outline. Three experiments, comprising four plants per treatment, were conducted.

Diffusive resistance measurement.—The transpiration equation is:

$$E = \frac{c_w - c_a}{r_a + r_l},$$

where E (grams·cm^{-2}·sec^{-1}) is the transpiration rate per unit leaf area; c_w and c_a (grams·cm^{-3}) are the water vapor concentrations at the surfaces of the mesophyll cell walls and of the bulk air, respectively; r_a (sec·cm^{-1}) is the boundary layer resistance and r_l (sec·cm^{-1}) is the diffusive resistance of the leaf to vapor flow. The leaf diffusive resistance is comprised of the stomatal and cuticular resistances in parallel. This equation was used to estimate the average leaf diffusive resistances of whole plants by substracting the boundary layer resistance r_a from (r_a+r_l). The boundary layer resistance (0.204 sec·cm^{-1}) was estimated from the vapor loss from wet filter paper cut into the shape of a soybean leaf (4, 8); i.e., r_l is 0 for wet filter paper. Resistance determinations were corrected for leaf temperature as measured with a copper-constantan thermocouple. Measurements were taken during the period when the lights were on.

In some experiments, relative diffusive resistances of individual leaves were determined with a diffusion porometer (11). Resistance values were corrected for diffusion temperatures. Primary leaves were used for these measurements since these were the first to show signs of wilt after inoculation. Measurements were taken during the light period. Plants used were kept well watered, since even mild water stress can cause stomatal closure.

Water potential values were measured with a thermocouple psychrometer using an isopiestic technique (2). Measurements were corrected for heat of respiration.

Resistances to liquid water transport were calculated according to the equation:

$$T = -\frac{(\psi_L - \psi_s)}{R},$$

where T is the transpiration flux at the steady state (grams·cm^{-2}·sec^{-1} of the leaf area), ψ_s is the water potential of the soil immediately next to the root (bars, assumed to be 0 here since the soil was moist) (3), ψ_L is the water potential of the leaf evaporating surface (bars), and R is the resistance to water transport (bar·sec·cm^{-1}) (3, 4). The soil component of the resistance pathway was considered to be negligible, since the soil was at field capacity (3, 9).

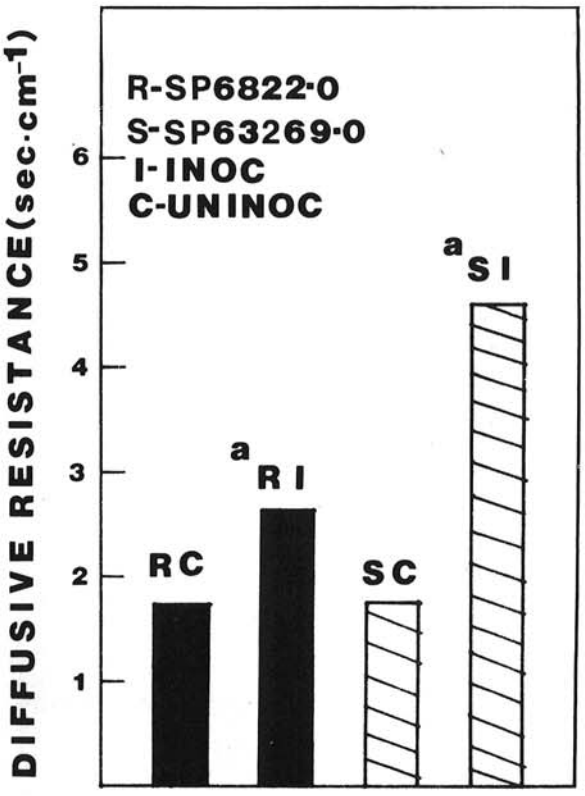

Fig. 2. Diffusive resistances of individual leaves of control (C) and inoculated (I) plants of sugarbeet cultivars SP6822-0 (R) and SP63269-0 (S) 5 days after inoculation with *Aphanomyces cochlioides*. Results are expressed as averages of four experiments. Treatments labeled "a" differ significantly (P = 0.01) from each other.

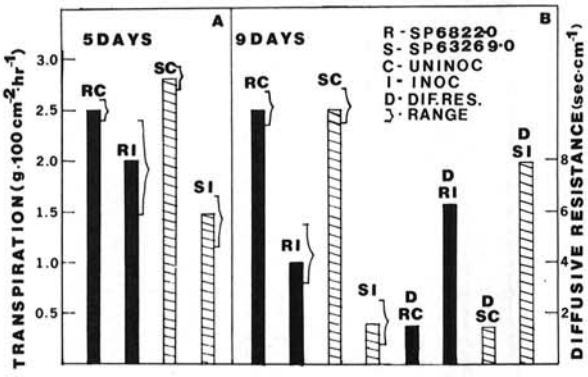

Fig. 1-(A, B). Average transpiration rates of four control (C) and four inoculated (I) whole plants of sugarbeet cultivars SP6822-0 (R) and SP63269-0 (S) **A)** 5 days and **B)** 9 days after inoculation with *Aphanomyces cochlioides*, and average whole-plant diffusive resistances (D) to water vapor 9 days after inoculation (Fig. 1-B).

RESULTS.—The whole-plant transpiration rates of two cultivars of sugarbeet seedlings 5 and 9 days after inoculation with *A. cochlioides* were lower than those of noninoculated controls, which were similar for the two cultivars (Fig. 1-A, B). Line R was less affected by the fungus than was Line S; the difference between the lines was more pronounced 9 days after inoculation. Average leaf diffusive resistances of whole plants 9 days after inoculation (Fig. 1-B), which were calculated using the transpiration rates presented in Fig. 1-A, B, were higher for inoculated than for noninoculated plants. Also, after inoculation, Line R had slightly lower diffusive resistances than did Line S. The noninoculated whole plants of both cultivars had similar average leaf diffusive resistances. The transpiration data presented in Fig. 1-A, B are from one experiment. Two additional experiments were conducted, and similar results were obtained.

Diffusive resistances of individual leaves (measured

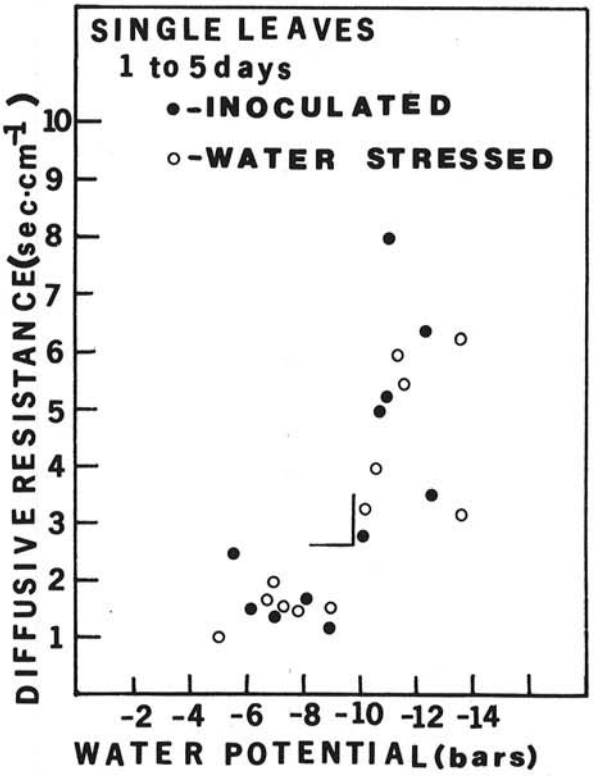

Fig. 3. Leaf diffusive resistance and water potential of primary leaves in seedlings of sugarbeet cultivar SP63269-0 (Line S), based on determinations made from plants 1-5 days after inoculation with *Aphanomyces cochlioides,* and from plants from which water was withheld.

TABLE 1. Calculated resistances to water transport of control and inoculated whole plants of sugarbeet cultivar SP63269-0 9 days after inoculation with *Aphanomyces cochlioides*

Treatment	Soil water potential (bars)	Leaf water potential (bars)	Transpiration ($g \cdot 100\ cm^{-2} \cdot hr^{-1}$)	Whole plant resistance ($bar \cdot sec \cdot cm^{-1}$)
Control	0	−7	2.8	0.9×10^6
Inoculated	0	−11	0.4	9.9×10^6

with a diffusion porometer) of the two cultivars 5 days after inoculation are shown in Fig. 2. The resistances of leaves obtained from noninoculated controls were lower than those from inoculated plants. In addition, leaves from Line R had lower diffusive resistances than leaves from Line S. The differences in diffusive resistance between leaves from inoculated and noninoculated plants, and from inoculated plants of the two cultivars, were significant, $P = 0.01$. The comparisons were based on mean data from four experiments. Each treatment/experiment consisted of six leaves (one from each of six plants).

Average root rot ratings, obtained 5 days after inoculation, were similar (approximately 1.5) for inoculated plants of both cultivars. Nine days after inoculation, disease ratings of Line S averaged 2.54, while disease ratings of Line R averaged 1.60. These differences in disease ratings were significant, $P = 0.05$.

Figure 3 shows the relation between leaf diffusive resistance and leaf water potential for the more susceptible Line S at 1 to 5 days after inoculation. These values are compared with those from noninoculated plants of the same cultivar 1-5 days after water was withheld from the soil. The values obtained from leaves of inoculated plants were similar to those from water-stressed plants. The leaf diffusive resistances were essentially unaffected by a drop in water potential to about −9 bars. Below −9 bars the diffusive resistances in both inoculated and water-stressed leaves increased sharply. Samples for water potential measurement were taken from the first leaves formed after the cotyledons immediately after determinations of diffusive resistances of the same leaves were made with a porometer. Each data point represents measurements from one leaf.

Resistances to liquid water transport were determined for inoculated and noninoculated plants of the more susceptible cultivar 9 days after inoculation (Table 1). Values used for these calculations were derived from data presented in Fig. 1, 2, and 3. The resistance to water transport of diseased plants increased to 9.9×10^6 $bar \cdot sec \cdot cm^{-1}$ by 9 days after inoculation. The resistance of healthy plants was calculated to be 0.9×10^6 $bar \cdot sec \cdot cm^{-1}$, or about one-tenth that of diseased plants.

DISCUSSION.—We have shown that sugarbeet seedlings inoculated with *A. cochlioides* zoospores have lower transpiration rates and larger diffusive resistances to water vapor flow than noninoculated seedlings. Furthermore, the differences between inoculated and noninoculated controls are more pronounced in the more susceptible of the two cultivars tested. Five days after inoculation, diffusive resistances of the first leaves above the cotyledons were significantly higher for the more susceptible cultivar than for the more resistant cultivar at a time when visual differences in disease symptom severity between the two cultivars were not apparent.

The similar relation between the leaf diffusive resistance and leaf water potential for diseased and water-stressed plants indicates that the increased diffusive resistance of diseased plants is probably initiated by a decreased water supply to the leaves (6, 7). This is further supported by the fact that calculated resistances to liquid water transport in the more susceptible cultivar increased after inoculation to about ten times that of healthy tissue. Increases in flow resistances of this magnitude should cause diseased sugarbeets to suffer more than healthy beets during brief drought periods. To check this possibility, leaf diffusive resistances of sugarbeets were measured in an Aphanomyces-infected nursery during a brief drought period in 1974. Leaves of susceptible cultivars had significantly higher leaf diffusive resistances than did leaves from more resistant cultivars.

The diffusive resistance parameter, which reflects small changes in stomatal aperture, may be useful in preharvest estimation of root rot severity. A root-rotting fungus might affect stomatal aperture indirectly by cutting off the water supply to the leaves, or by stimulating production of either a fungus or host metabolite, which,

when transported to the leaves, would affect stomatal aperture directly (1, 14). Whatever the cause, consistent correlations between infection levels and diffusive resistances might provide a quantitative, unbiased estimate of root rot severity readily obtained in situ.

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