Water Relations in Safflower During Wilting Induced by Phytophthora Root Rot

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ABSTRACT

The possible causes of wilting in safflower plants infected with *Phytophthora drechsleri* were examined. Determinations of leaf water and solute potentials showed that wilting was due to water stress. The diffusive resistance of leaves to water vapor loss following inoculation was as high as, or higher than, the diffusive resistance of healthy leaves at the same water status and there was no alteration in the transpirational behavior of infected plants which could cause water stress. Measurements of recovery rates of plants from water stress were used to examine resistance to water uptake. Infection caused a marked increase in resistance to water uptake through root systems, but also caused a marked increase in resistance to water movement through the xylem of stems. The influence of infection on xylem resistance extended above the highest point of stem invasion by the pathogen.

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Wilting is a common symptom of Phytophthora root rot in safflower and other plants (e.g., 6, 11, 13, 16, 18, 19). Review of the literature (9), however, indicates the mechanisms by which root-rotting organisms alter host water relations have not been examined in a physical manner. Limited root distribution in soil may account for the wilting induced by some root pathogens, but there remains the possibility that root-rotting organisms influence water relations parameters other than root system size. For example, there is limited evidence that Phytophthora root rot increases resistance to water movement within infected tobacco plants (16, 18). The present study examines the influence of Phytophthora root rot in safflower on host cell turgor, transpiration, and resistance to water movement.

MATERIALS AND METHODS.—*Biological materials.*—Safflower plants (*Carthamus tinctorius* L. 'Nebraska 10') were grown in 2-liter crocks of steamed soil. Four plastic tubes, 3 cm diameter and 6 cm long, were placed in each crock and were filled with soil so that the soil surface within the tubes was 4 cm above the surface of the bulk soil. Seeds were planted just below the soil surface in the tubes and 1 week after seeding plants were thinned to one per plastic tube. Plants were maintained in a 25 C greenhouse until 3 weeks after planting when they were moved to a controlled environment chamber. The chamber had 14-hour periods with 85 W m^{-2} (300-700 nm) of fluorescent and incandescent light at 27 ± 0.1 C and 75 ± 3% relative humidity. The temperature was 21 ± 0.1 C and relative humidity was 85 ± 3% during the 10-hour dark periods.

Plants, 3-4 weeks old, were inoculated with zoospores of *Phytophthora drechsleri* Tucker. Motile zoospores were obtained by the method of Barash et al. (1) and 4 × 10^5 spores were pipetted onto the bulk soil of each inoculated crock. Crock drain holes were then plugged, and just enough water was added to saturate the bulk soil. After saturation for 12 hours, crocks were allowed to drain and were subsequently watered as needed. Care was taken not to get zoospores or surface water in the plastic tubes in which plants were growing. This precaution prevented direct infection of the hypocotyl. A darkening of root tips 1-2 days after inoculation was the first visible symptom of infection. Wilting, which was the predominant symptom in the tops of infected plants, usually occurred 5-7 days after inoculation.

Safflower plants were also grown in nutrient solution. Seeds were germinated in vermiculite and 1-week-old seedlings were individually transplanted into 2-liter crocks of aerated nutrient solution. Plants in solution were maintained under the same environmental conditions and were used for experimentation at the same

age as were the plants grown in soil. Inoculation was accomplished by pipetting 2×10^4 motile zoospores into the nutrient solution. Following inoculation, the surface of the nutrient solution was maintained at the level of the highest branch root. Symptom development in solution-grown plants was almost identical to that in soil-grown plants.

Leaf water status.—Relationships between leaf water potential (ψ), solute potential (ψ_s), and relative water content (RWC) were determined as leaf water stress developed following inoculation of plants in well-watered soil. Water stress was induced in uninoculated plants by withholding water from the soil. In addition to sampling leaves as water stress increased during light periods in the controlled environment, some leaves were sampled during recovery from water stress. Recovery was initiated by moving plants into a saturated atmosphere and watering the soil or excising the stem or leaf base under water. At the time of sampling, a leaf was transferred to a humid chamber where it was subsampled for the measurement of ψ and RWC. The isopiestic technique (5), with a correction for heat of respiration (2), was used to determine leaf ψ. After freezing and thawing, the same tissue and psychrometer methods were used to determine ψ_s. Leaf disks were used to determine RWC by the methods already described (2, 9).

Diffusive resistance.—A porometer (8) was used to measure the diffusive resistance of leaves to water vapor loss in the light in the controlled environment chamber. Immediately after each porometer measurement on an attached leaf of a soil-grown plant, disks were cut from the leaf area which had been exposed to the porometer cup and were used to measure RWC.

Rate of recovery.—Rates of recovery from water stress were used to compare resistances to water movement in healthy and infected plants. Rate of recovery was measured as the rate of increase in leaf RWC by the beta-gauging technique. The beta-gauge consisted of a 300 μCi Promethium-147 source mounted 2.5 cm above the leaf (this distance being adjusted to yield the desired initial count rate) and a thin-window Geiger-Müller tube mounted 1.2 cm below the leaf. The count rate varied between locations on the same leaf and between different leaves at the same water content (see also 14). Therefore, considerable care was taken to hold the leaf in a constant position during all measurements and to obtain a calibration curve (RWC vs. count rate) for the same leaf area as was measured during recovery. Leaves were held
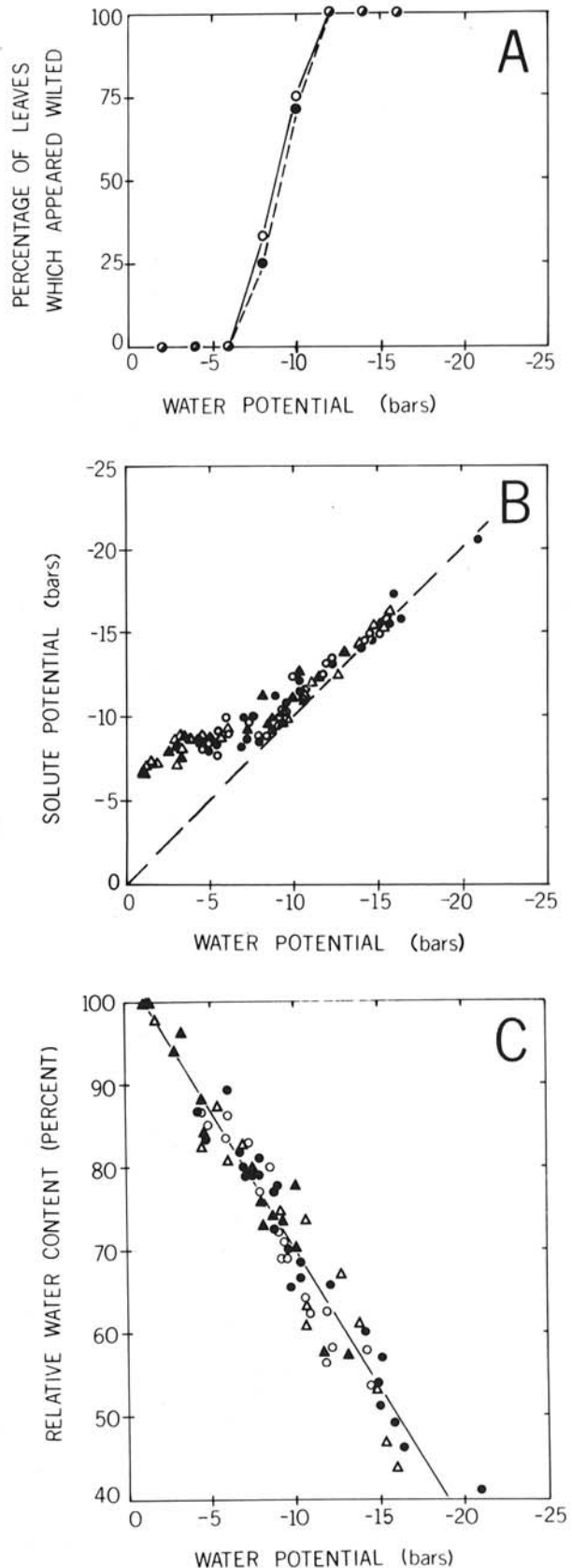
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Fig. 1-(A to C). Percentage of leaves which appeared wilted A), leaf solute potential B), and relative water content C) plotted as functions of decreasing leaf water potential. Open and closed symbols represent healthy and infected plants, respectively. Circles represent data obtained during the development of water stress and triangles represent data obtained during recovery from water stress. Percentages of leaves which appeared wilted A) were calculated after water potentials were rounded off to the nearest even bar. Each point in the plot of solute vs. water potential B) represents one leaf and the vertical distance between a point and the dashed line with a slope of one is the pressure potential. Each point in the plot of relative water content vs. water potential C) represents one leaf and the solid line was drawn according to the regression equation RWC = 103.8 + 3.365 ψ (r = 0.966).
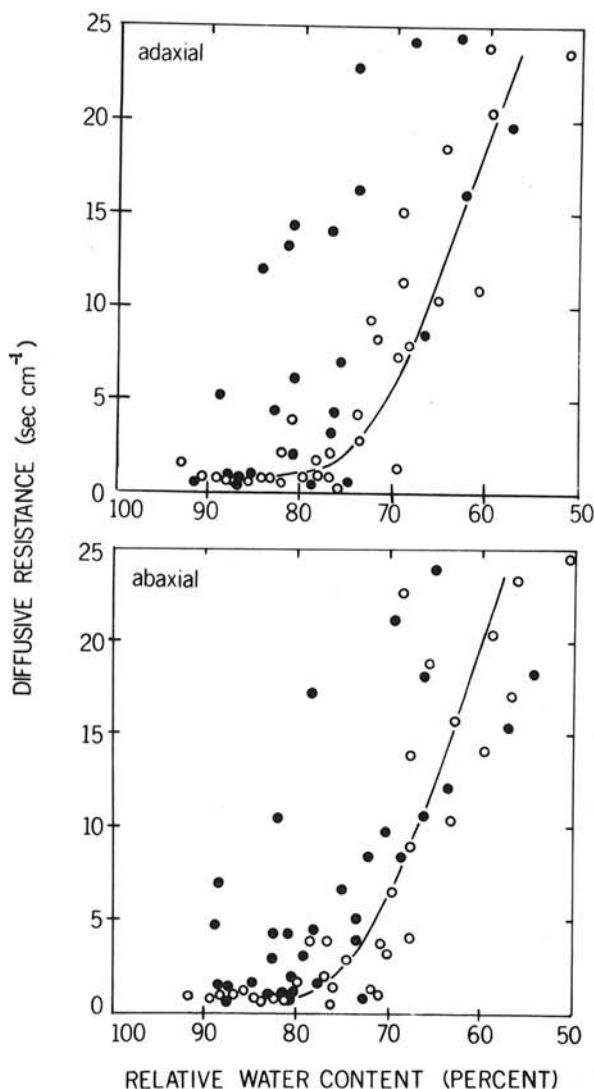
Fig. 2. Diffusive resistances of the adaxial and abaxial leaf surfaces plotted as functions of decreasing relative water content. Open and closed circles represent healthy and infected plants, respectively. Lines are drawn to show the trend of the data for healthy plants. Each point represents one porometer and water content measurement.

rigid in the beta-gauge between two acrylic sheets (Plexiglas®, Rohm and Haas Co.) which were covered with 0.2 mm foam foot pads. The pads were coated with a thin layer of silicone rubber to prevent leaf water loss. The acrylic sheets and pads had a 1.2 cm diameter hole in the center, the entire area of which was exposed for beta-gauging. Guides and clamps in the assembly assured a constant and repeatable leaf position.

Plants grown in nutrient solution were used to measure rate of recovery from water stress. To induce water stress, solution was drained from the roots and plants were allowed to wilt to the desired extent in the controlled environment chamber. The experimental leaf was then stretched flat between the acrylic sheets with the hole for beta-gauging midway along the leaf and midway between the midvein and leaf margin. The beta-gauge assembly was fully enclosed and the leaves and stem outside the beta-gauge were covered with plastic wrap to prevent water loss. Recovery of intact plants was initiated by submerging the roots in water. Recovery of excised plant parts was initiated by cutting the stem or leaf base under degassed water. When recovery measurements were complete, a 2.0 cm disk centered on the hole for beta-gauging was cut from the leaf. The disk and remaining plant parts were then weighed and brought to full turgor by floating on water. The following day, the leaf disk was blotted dry and placed in a polyethylene holder and weighed. The holder was open in the center and designed to clamp gently down on 0.2 cm of the disk margin. A special guide supported the holder so that the leaf tissue could be exposed to the beta-gauge in precisely the same position as was used during recovery. Count-rate was related to leaf disk weight and ultimately RWC by allowing the disk to dry in the holder and periodically measuring the weight and corresponding count rate.

Reisolation of the pathogen.—Stems of the plants used to measure rate of recovery were surface-sterilized and were aseptically cut into 2 mm segments. Segments were transferred to petri dishes containing cornmeal agar supplemented with 10 μg/ml pimaricin (Delvocid®, Gist-Brocades nv, Delft, Holland) and 300 μg/ml vancomycin hydrochloride (Vancocin®, Eli Lilly and Co.). Stem segments were incubated on agar for up to 8 weeks at 25 C and were periodically examined for growth of *P. drechsleri*.

RESULTS AND DISCUSSION.—Figure 1-A shows that Phytophthora root rot induced leaves to visibly wilt at the same leaf ψ values as did withholding water from healthy plants. Furthermore, Fig. 1-B shows that the relationship between ψ and ψ_s was the same in leaves of healthy and infected plants. The turgor of leaf cells, expressed as pressure potential (ψ_p), is commonly calculated as the difference between the ψ and ψ_s values of leaf tissue (2, 9, 12). Comparison of Figs. 1-A and 1-B shows that visible wilting of leaves on both healthy and infected plants generally occurred at $\psi_p \leqslant +1$ bar. The ψ and ψ_p values at which safflower leaves visibly wilted (Fig. 1-A, B) are similar to the leaf ψ and ψ_p values correlated with the onset of wilting in several other herbaceous crop species (2, 8, 10, 12, 17). The wilting of infected safflower plants was as reversible as the wilting induced in healthy plants. Reference to Fig. 1-C shows that disease development did not alter the relationship between RWC and leaf ψ. It can be concluded from the data in Fig. 1 that wilting induced by Phytopthora root rot in safflower is due to water stress and is not due, even in part, to a change in the solute content of leaf cells or the elastic properties of cell walls.

The leaf water loss and low ψ values induced by Phytophthora root rot could be due to a change in transpirational behavior and/or a change in resistance to water uptake. The influence of root rot on the transpirational behavior of safflower was examined by measuring the diffusive resistance of leaves to water vapor loss as water stress developed following inoculation. Parallel measurements were made on healthy plants from which water was withheld. The diffusive resistance of leaves on healthy plants increased markedly when RWC dropped below 70% (Fig. 2). This increase is due

primarily to stomatal closure and is similar to the increase in diffusive resistance with decreasing ψ observed in leaves of other plants (e.g., 8, 9, 10, 17). Even though the data in Fig. 2 are somewhat scattered, it can be seen that the diffusive resistance of leaves following inoculation was almost invariably as high as, or higher than, the diffusive resistance of leaves on healthy plants at the same RWC value. Transpiration rate/unit leaf area is inversely related to diffusive resistance, and the transpiration rate of infected plants is not expected to exceed that of healthy plants at the same ψ value and ambient conditions. Therefore, there is no alteration in the transpirational behavior of infected plants which could cause depression in leaf ψ. In fact, the data in Fig. 2 suggest root rot causes some inhibition of stomatal opening which would tend to maintain high ψ values in leaves.

Representative curves relating leaf RWC to time after recovery from water stress was initiated are shown in Fig. 3. Although the recovery experiments were patterned after the experiments by Boyer (3, 4), the shape of the recovery curves, even when the degree of recovery was calculated in proportion to initial and final RWC values, did not generally conform to the logarithmic increase in leaf ψ with time which is expected from Boyer's (3, 4, 15) analysis of the diffusion of free energy. Furthermore, the shape of the recovery curves was somewhat variable between leaves and there was considerable water uptake by the fleshy stems of safflower plants during recovery. For the reasons above, Boyer's (3, 4, 15) analysis for leaves of plane geometry was not used to calculate a resistance to free energy transfer from the half-time of recovery. Rather, the initial slopes of the recovery curves obtained for healthy and infected plants under the same conditions are compared. Such a comparison of rates of water movement through the same plant parts and into leaves at the same ψ value should provide a reasonable estimate of the relative resistance to water movement between the source and leaf tissues. The fully turgid weights of stems and leaves of infected plants were 8-15% less than those of healthy plants, but this difference in plant size is not large enough to seriously complicate a comparison of initial rates of recovery.

It can be seen in Fig. 3 that the initial rate of recovery of an intact plant which had wilted because of disease development was approximately one-eighth of the rate for an intact healthy plant. Fig. 3 also shows that cutting off the roots and supplying water directly to the stem base only eliminated a portion of the unusual resistance to water uptake in infected plants. When water was supplied directly to the stem base, the initial rate of recovery of an infected plant was less than half the initial rate of an intact healthy plant and only one-sixteenth of the initial rate of a healthy plant from which the roots were cut. In contrast to an infected plant, rate of recovery of healthy plants was nearly the same when water was supplied directly to the base of the stem or leaf (Fig. 3). Therefore, resistance to water movement through a healthy stem must be small in comparison to resistance to water uptake in leaves. Rates of recovery of excised leaves from healthy and infected plants were nearly the same (Fig. 3) and root rot did not increase resistance to water uptake within leaves. The experiment in Fig. 3 was repeated on three separate occasions with similar results.
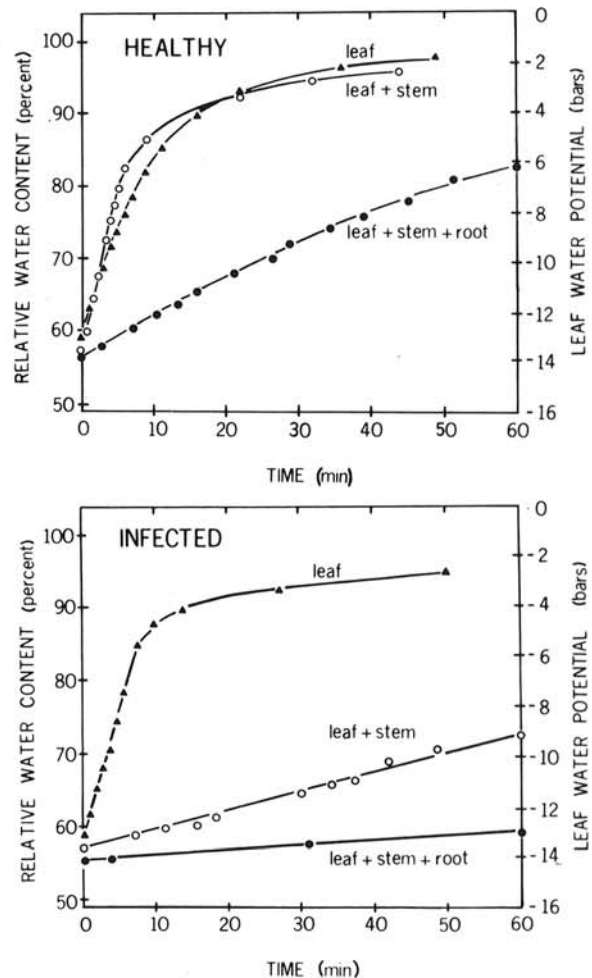

Fig. 3. The relative water contents of leaves determined during recovery from water stress. Recovery of healthy and infected plants was initiated at time zero by supplying water to the roots (enclosed circles) or directly to the base of the stem (open circles) or leaf (triangles). The right hand axis gives the equivalent water potentials calculated from the regression equation in Fig. 1-C.

The location of the large resistance to water uptake in infected plants was examined further by cutting roots and stems under degassed water at various times after recovery of an intact plant was initiated. The results of one such experiment are shown in Fig. 4. Cutting roots from the stem at the point of the highest branch root caused a twofold increase in rate of recovery, an increase which is expected from the data for infected plants in Fig. 3. A second cut in the stem 6 cm above the first cut and 14 cm below the measured leaf also caused a marked increase in rate of recovery (Fig. 4), but did not restore rate of recovery to the rate observed in excised leaves or in healthy plants after excision of stems (Fig. 3). Evidently, root rot increased resistance to water movement through the remaining upper stem. The 6-cm stem piece from between the points of cutting (Fig. 4) had visible symptoms of infection in that the cortex and xylem were darkened in the bottom 2 cm. The pathogen was readily
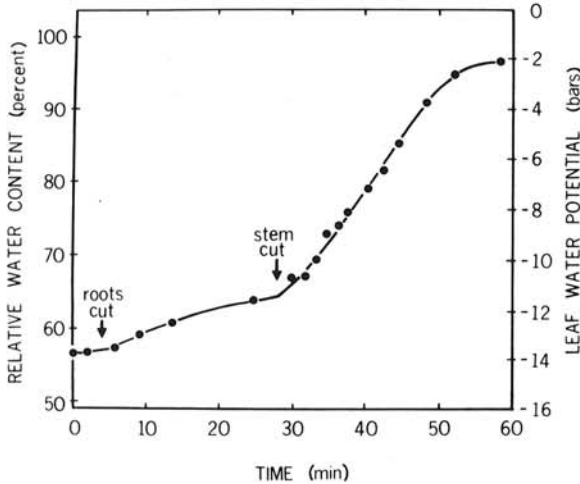
Fig. 4. The relative water content of a leaf determined during recovery of an infected plant from water stress. Recovery of the intact plant was initiated at time zero and the roots and stem were cut under degassed water at the times indicated by arrows. The right hand axis gives the equivalent water potentials calculated from the regression equation in Fig. 1-C.

reisolated from the bottom 2 cm, but was not isolated from the top 4 cm of the 6-cm stem piece or from the stem above the highest point of cutting during recovery (Fig. 4). Furthermore, plant parts above the highest point of cutting during recovery had no visible symptoms of infection other than those of water stress. Cutting the stems of two additional infected plants during recovery and plating the stems on agar yielded results similar to those for the plant in Fig. 4.

All of the results of the present study lead to the conclusion that increased resistance to water movement within the infected plant is responsible for the wilting induced by Phytophthora root rot in safflower. At the time infected plants began to wilt, the fresh weights of infected root systems were 43-56% less than the fresh weights of uninfected root systems and reduction in root surface area may be responsible, in part, for the increased resistance to water movement. However, the influence of infection on the rate of recovery of intact plants (Fig. 3) was much greater than the influence of infection on root system size. Recovery after excision of stems (Fig. 3 and 4) also indicates infection caused a marked increase in xylem resistance. In contrast to healthy plants, most infected plants did not recover from wilting when the stem base was excised under water in the controlled environment chamber used for plant growth. Therefore, the resistance of xylem in diseased stems was frequently large enough to cause wilting. Interestingly, with the exception that the unusual xylem resistance occurs in the lower portions of the plant, the water relations of wilting induced by Phytophthora root rot in safflower are similar to the water relations of wilting induced by Fusarium wilt in tomato (8) and Verticillium wilt in cotton (9). It should be noted, however, that relationships between the severity of root symptoms and severity of wilting caused by root-infecting *Phytophthora* spp. appear to be highly variable (e.g., 13, 19). Thus, not all Phytophthora root rots may

have the same influence on resistance to water movement within the host as was found with *P. drechsleri* and safflower.

The mechanism by which Phytophthora root rot alters the xylem resistance of safflower is not known. Examination of free-hand sections showed that much of the xylem in infected roots and lower stems was discolored and contained foreign material. Stem xylem more than 4 cm above the highest point of invasion by *P. drechsleri* appeared normal but had an unusually high resistance to water flow (Fig. 4). The smaller size of xylem tissues in infected safflower cannot account entirely for the observed influence of infection on rate of recovery (Fig. 3 and 4). Calculation of stem resistance from the dimensions of the xylem and Poiseuille's law (7) indicates the influence of infection on the number and size of vessels increased stem resistance by only 18-22%. Wolf and Wolf (18) and Schramm and Wolf (16) suggested *P. parasitica* var. *nicotianae* produces a toxin which increases xylem resistance. The possible involvement of toxins in Phytophthora root rot of safflower has not been investigated. At the present time, it is not even known that increased xylem resistance is a specific response to infection of safflower by *P. drechsleri* or a more general response to root injury. Injuring root systems of safflower by dipping in hot water also causes xylem resistance in stems to increase during the following 2-4 days (Duniway, *unpublished*).

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