Impact of Root Infection by *Phytophthora cinnamomi* on the Water Relations of Two *Eucalyptus* Species that Differ in Susceptibility

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ABSTRACT

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Water relations changes associated with infection by *Phytophthora cinnamomi* were studied in inoculated plants of *Eucalyptus sieberi* (susceptible) and *E. maculata* (field resistant). Plants were inoculated and maintained in root boxes in a controlled environment. Growth of inoculated roots ceased and root lesions formed 8–10 hr after inoculation. In *E. maculata*, neither the pathogen nor the initial lesion extended within the root and no other symptoms developed. In *E. sieberi*, the lesions extended and shoot wilting symptoms were observed 3–15 days later. Along with lesion extension, a major reduction in the net hydraulic conductivity of root systems developed 2–14 days after inoculation, although the fungus was present in only 8–15% of the root system. Shoot wilting occurred when root conductance was reduced to ~9% that of the controls. Leaf conductance to water vapor loss, transpiration, leaf xylem water potential, and leaf relative water content were all reduced significantly 24 hr or more after major reductions in root conductance were detected. These changes in water relations were absent in infected *E. maculata*.

Susceptible plant species infected by the root pathogen *Phytophthora cinnamomi* Rands exhibit water stress symptoms before death. Diseased forest trees wilt, have low leaf xylem water potential, and may develop small leaves (21,28). Physiological studies have shown *Persea americana* Mill. (23), *Rhododendron catawbiense* Michx. (7), and *Isopogon ceratophyllus* R.Br. (5) to be severely water stressed as a result of infection. The cause of these water deficits is unknown, but they could be due to a dysfunction of the tissues involved in water transport and/or its regulation. The water stress may be either the cause of death or a secondary symptom associated with infection.

The aim of this study was to investigate water stress and subsequent changes in host water relations associated with different stages of infection of selected susceptible and field resistant *Eucalyptus* species.

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MATERIALS AND METHODS

Seedlings of *Eucalyptus sieberi* L.A.S. Johnson (susceptible) and *E. maculata* Hook. (field resistant) were grown in pots of nonsterile sand and watered with quarter-strength nutrient solution (1) until 4

mo old. The pots were plastic, 15 cm deep, 8 cm in diameter, and had been cut vertically in half to allow the attachment of a transparent polymerized methyl methacrylate Perspex (Plexiglas, ~6 mm thick) front to the cut edges to enable observation of root growth and lesion development. A detachable Perspex inoculation window in the front enabled inoculation of the underlying root tips with minimum disturbance. The Perspex front and the window were covered with foil and the pot was wrapped in black plastic to prevent light from reaching the roots. Plants were grown in a glasshouse at 20 ± 3 C in ~14 hr daylight and 10 hr dark for 3 mo, then transferred to a growth chamber with a 14-hr light (photosynthetically active radiation, 900 $\mu E \cdot m^{-2} \cdot sec^{-1}$, at 24 ± 1 C) and a 10-hr dark period at 19 ± 1 C.

Plants were inoculated with an axenically prepared zoospore suspension (3) prepared from a culture of an isolate of *P. cinnamomi* (A_2 mating type) obtained recently from an *I. ceratophyllus* plant growing in the Brisbane Ranges, Victoria. Roots were inoculated by removing the detachable inoculation windows and placing 0.1 ml of suspension containing ~10^2 zoospores on each of three root tips of each plant. A piece of plastic film (Parafilm M®, Dixie-Marathon, Greenwich, CT 06830) was placed under each root tip to prevent the inoculum from being dispersed into the sand by capillarity (4). Control plants were inoculated with distilled water. The inoculum remained on the root tips overnight. Subsequent lesion development was measured by tracing onto a transparent overlay the length of discolored root underlying the Perspex front. When measurements were completed, all parts of the root system (whether lesioned or not) were segmented (1 cm segments), surface sterilized in 70% ethanol for 1 min, then plated onto selective agar medium (16) to determine the percentage of root system infected.

After inoculation, the inoculation window was resealed to the pot front with a silicone rubber washer and a spring clip. The pot was watered and allowed to drain to field capacity. The pot drainage holes were then sealed with putty, and each pot was placed in a plastic bag with the stem protruding through a putty seal.

Throughout the experiments, the aim was to correlate the proportion of root system infected with stages in disease development and any changes in the component factors affecting plant water relations. Measurements were taken for both control and infected plants during 15 days following inoculation. The number of replicate seedlings tested is given in Fig. 1. Leaf area was measured with a Lambda Li-3000 leaf area meter (LI-COR, Inc. [formerly Lambda Instrument Corp.], Lincoln, NE 68504) and transpiration was recorded from daily loss in weight calculated as milligrams of water per square millimeter of leaf area per day. Water loss was subsequently replaced daily through the stem seal by syringe. Leaf water vapor conductance was measured with a vapor diffusion porometer (model MK II; Delta-T Devices, Cambridge, UK CB5 OE5) (15,24). Both surfaces of three leaves per plant were measured daily at noon.

Leaf water potential was determined with a pressure bomb (20). Leaves, one to three per plant depending on size, were excised, immediately placed in plastic bags in the dark, and assayed within 1 hr. Leaf relative water content was determined (2).

Root and stem hydraulic conductivity was measured by using a pressure chamber based on the design of Mees and Weatherley (13). The pressure bomb was used in the following way for root, stem, or leaves. The root system was excised with 3 cm of stem, gently washed free of sand, and placed in nutrient solution in a large test tube inside the bomb. The stem protruded through a silicone rubber seal. Bomb pressure was kept at 0.5 ± 0.02 MPa (5 bar) and the flow rate of exudate was measured several times during a 4-hr period by means of a pipette attached to the stem.

Flow rates of nutrient solution were measured both through the root system and through stem segments without roots. The osmolarity of the nutrient solution and of the exudate were determined with a vapor pressure osmometer (model 5100B; Wescor Inc., Logan, UT 84321) calibrated against sodium chloride standards, and results were converted to water potential (19).

Root and stem hydraulic conductivities were determined from the equation:

$$J_v = L \Delta P x^{-1} \quad (1)$$

in which J_v is the hydraulic flux ($mm^3 \cdot mm^{-2} \cdot sec^{-1}$) based on the cross-sectional area of stem or root, ΔP is the pressure potential (kPa) applied across stem or root, x is the length (mm) of stem or root, and L is the hydraulic conductivity ($mm^2 \cdot kPa^{-1} \cdot sec^{-1}$) (14).

The osmotic potential of sap exuded from the roots and that of the nutrient solution accounted for less than 0.01% of the total water potential applied across the root system; therefore, osmotic potentials were ignored in calculations of root conductivity. Root length was measured by the line intersect method (26).

RESULTS

The first symptom observed in both species was a dark-brown lesion 7–10 mm long which formed overnight behind the tips of inoculated roots. Growth of inoculated roots ceased. In *E. maculata*, neither the fungus nor the initial lesion extended further than 10 mm and no other visible symptoms were observed. In *E. sieberi*, the lesions extended 12 mm a day on each of the three roots for the 2–3 days they were visible in the root boxes. Secondary symptoms of shoot wilting were observed in *E. sieberi* after a period which varied from 3 to 15 days after inoculation.

For convenience, symptom development in *E. sieberi* is classified in three clearly defined, successive stages (Figs. 1, 3, and 4).

Infection stage 1. Lesion formation, approximately 10 hr after inoculation.
Infection stage 2. Lesion extension associated with reduced root hydraulic conductivity.
Infection stage 3. Water stress in shoots which followed the continued and increasing reduction in root conductivity.

Inoculated plants of *E. maculata* displayed only stage 1 symptoms. Root conductivity of inoculated plants of *E. sieberi* (Fig. 1) showed a marked reduction during stages 2 and 3 of disease development compared with the controls. These differences were not due to changes in root length (Table 1). The decreased root conductivity is particularly remarkable in that the fungus was isolated from only 8–15% of the total root system of *E. sieberi* at stage 3 of disease (Fig. 2). At stage 2 of symptom development the

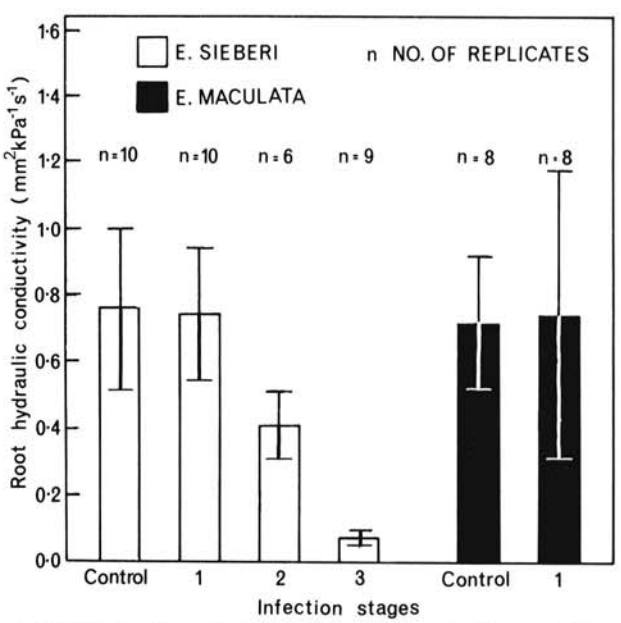

Fig. 1. Root hydraulic conductivity of *Eucalyptus sieberi* (susceptible) and *E. maculata* (resistant) for uninfected controls and for plants infected by *Phytophthora cinnamomi* at different stages of infection (see text for definition of infection stages). Tops of bars represent the means and the vertical lines the confidence limits, $P = 0.05$.

root hydraulic conductivity of *E. sieberi* was only 50% of the control (Fig. 1). By stage 3, it had fallen to ≤9% of the control value. There was no significant reduction in hydraulic conductivity of *E. maculata* roots, and only 2–4% of the root systems were infected.

Water stress did not appear in shoots of *E. sieberi* until root conductivity was significantly less than that of uninfected controls. At stage 3, wilting was observed in shoots, and leaf conductance (Fig. 3), leaf xylem water potential (Fig. 4), transpiration (Table 1), and leaf relative water content (Table 1) had declined significantly but still followed the same pattern with noon maxima. Leaf conductance was reduced 75%. Leaf xylem water potential changed from −0.4 to −1.2 MPa. The relative water content of the leaves fell from 88 to 54%, and shoot transpiration rate was only 26% that of the uninfected controls. These changes were absent in infected plants of *E. maculata*.

Stem conductivity of *E. sieberi* did not change with infection and in both control and infected plants it was three times greater than control root conductivity. In fact, rate of flow through stem segments of infected plants was at least six times that through roots of uninfected plants. Stems, therefore, did not contribute significantly to the reduced hydraulic conductivity measured in infected root systems.

TABLE 1. Transpiration, leaf relative water content (RWC), and root length of *Eucalyptus sieberi* and *E. maculata*. Measurements of uninfected control plants and of experimental plants inoculated with *Phytophthora cinnamomi*

Stage of infection	Replicates (seedlings)	Root length[a] (m)	Transpiration[a] ($mg \cdot mm^{-2} \cdot hr^{-1} \times 10^2$)	RWC[a] (%)
E. sieberi				
Control	10	2.0 ± 0.4	34 ± 13	87 ± 3
Infection stage:				
1. root lesions	10	2.2 ± 0.4	28 ± 12	85 ± 2
2. root conductance affected	6	2.0 ± 0.2	37 ± 9	88 ± 4
3. shoot symptoms	9	2.5 ± 0.5	9 ± 4	54 ± 7
E. maculata				
Control	8	2.6 ± 0.6	62 ± 18	90 ± 3
Infection stage:[b]				
1. root lesions	8	2.1 ± 0.6	57 ± 14	91 ± 4

[a] Mean and confidence limits, $P = 0.05$.
[b] Infection stages 2 and 3 are absent in *E. maculata*.

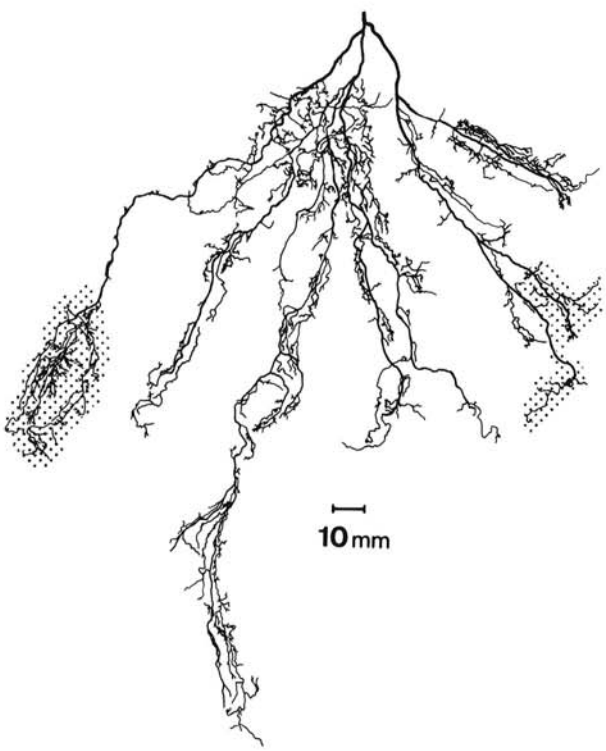

Fig. 2. Root system diagram of *Eucalyptus sieberi* (susceptible) with hydraulic conductance reduced 91% due to infection by *Phytophthora cinnamomi*. At infection stage 3 the pathogen was isolated only from the dot-patterned areas.

DISCUSSION

Despite an adequate water supply in the soil, all infected plants of *E. sieberi* became severely water stressed. These experiments demonstrated that in every case, water stress was associated with gross impairment of root hydraulic conductivity. Infected plants of *E. maculata* were not water stressed and root conductivity was scarcely affected. In *E. sieberi*, shoot water relations (Table 1, Figs. 3 and 4) were not affected at stage 2 in symptom development, when hydraulic conductivity was reduced by 50% (Fig. 1), but shoot water relations were severely disrupted by stage 3 when root hydraulic conductivity was only 9% that of the control. The onset of changes in root conductivity was associated with lesion extension and was observed 2–14 days after inoculation in all plants. The range in time taken for failure in water transport probably reflects variability in seedling vigor and in susceptibility to the pathogen.

Water transport through the roots was reduced by 91% (ie, practically eliminated) when less than one-sixth of the root system was infected. Once water flow within the roots failed, water stress symptoms quickly developed in the shoots. The disproportion between the amount of root infected and the reduction in hydraulic conductivity indicates that the failure of water transport cannot be due directly to decay of the relatively small diseased portion of the root system. The decline of water potential in the leaves resulted in stomatal closure, measured as a decrease in leaf conductance. When uninfected plants are exposed to stressful conditions, stomatal closure helps to maintain reasonably high leaf water potentials because the reduced transpiration rate does not exceed the rate of absorption of water by the roots. A favorable water balance was not maintained in infected seedlings of *E. sieberi* because root conductance was so low that leaf water potentials

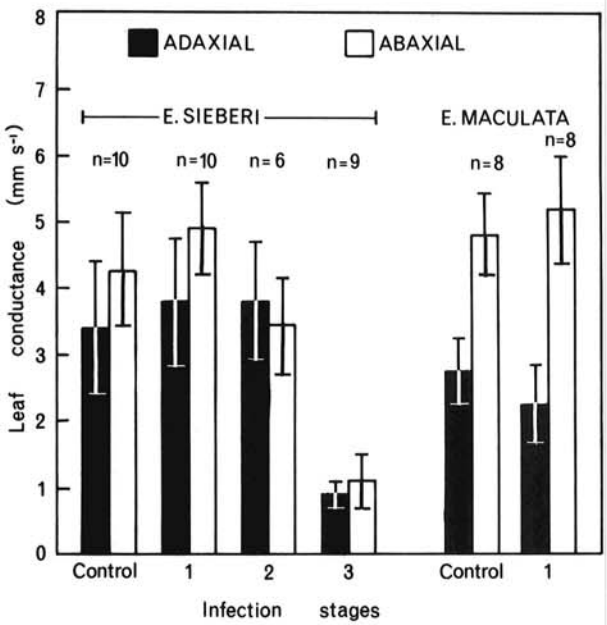

Fig. 3. Stomatal conductance of *Eucalyptus sieberi* (susceptible) and *E. maculata* (resistant) for uninfected controls and for plants inoculated with *Phytophthora cinnamomi* after development to three different stages of infection. Stippling indicates adaxial surface. Clear bars represent the abaxial surface. Tops of bars represent the means and the vertical lines the confidence limits, $P = 0.05$.

remained low and the plants subsequently died.

The experiments reported here were conducted with 4-mo-old seedlings grown under controlled conditions. Previous research demonstrated large and highly significant reductions in leaf relative water content, leaf xylem water potential, and transpiration in 1- to 2-yr-old container grown plants of *I. ceratophyllus* (6). Measurements recorded from mature forest trees of *E. obliqua* L. Hérit. showed plants infected by *P. cinnamomi* had lower leaf xylem water potential than uninfected trees (28). *P. cinnamomi* is reported to have similar effects on the water status of *E. marginata* Sm. growing on lateritic soils (21). The sudden-death syndrome recorded for trees of *E. baxteri* and *E. macrorhyncha* infected with *P. cinnamomi* in the Brisbane Ranges, Victoria (29), may have been due to failure of root water conductivity, thus preventing an adequate water supply to the crown during periods of rapid transpiration.

Decreased root hydraulic conductivity has been demonstrated previously for cotton plants infected by *Phymatotrichum omnivorum* (17), and for safflower infected by *Phytophthora cryptogea* (8). None of these infected plants wilted until root conductivity was reduced to a small fraction of that in uninfected controls. The hydraulic conductivity measured for infected roots of *E. sieberi* is comparable with that calculated on a leaf area basis for infected safflower roots (8).

The failure in water transport through the root system associated with decay in only a small proportion of that root system may be due to fungus-mediated hormonal imbalance, to plugging of xylem pit membranes by tyloses, or to macromolecules released due to the activity of fungal enzymes or toxins. Plant water transport may be hormonally regulated (11), and the dramatic reduction in root conductivity recorded when only a small portion of the root system is infected may be mediated by changes in root hormone concentrations associated with root tip necrosis, pathogen activity, or with root × pathogen interaction. Hormonal changes have been reported in tomato plants infected by *Verticillium* (18). Glucans (with molecular weights >30,000 daltons) derived from *P. cinnamomi* caused wilting in seedlings of *E. sieberi* (30). Molecules with molecular weights >20,000 daltons can block xylem vessels (27). Endopectin lyase, and high-molecular-weight polysaccharides produced by its action, occluded xylem vessels of tomato leaves and petioles infected by *Verticillium alboatrum* (25). *Phytophthora cinnamomi* is a root rotting pathogen, and in the experiments with eucalypts, stem conductivity was unaffected but a macromolecular fungal or host metabolite may have reduced root conductivity by being too large for dispersal through the xylem complex in the hypocotyl region between root and stem. Pectin lyase is produced by *P. cinnamomi* (J. M. Hinch, *unpublished*). Tyloses have been observed in *Eucalyptus* spp. (6,12), but are rare in *E. sieberi* (9,22). Duniway (8) suggested that toxins from root cells killed by a pathogen could induce changes in root conductivity. Fungal toxins may cause wilt by interfering with the leaf cell membranes (10), but the experiments with *P. cinnamomi* and eucalypts have shown that in this case root transport fails before wilting and before stomatal conductance is affected.

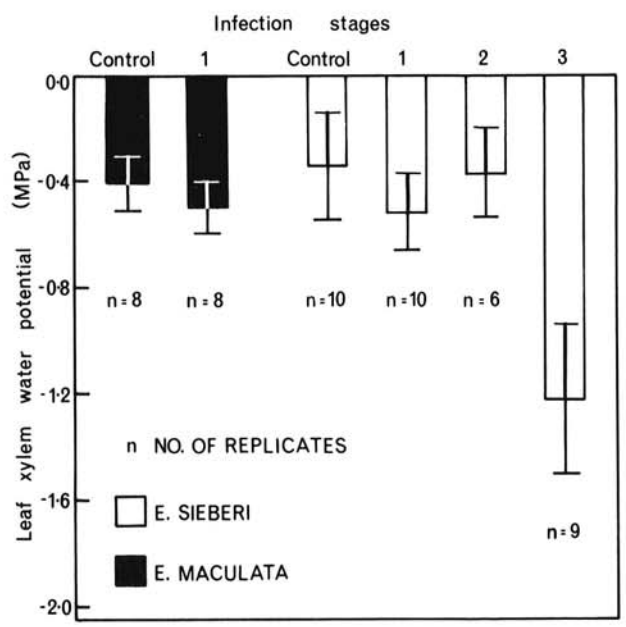

Fig. 4. Leaf xylem water potential of *Eucalyptus sieberi* (susceptible) and *E. maculata* (resistant) for uninfected controls and for plants infected by *Phytophthora cinnamomi* at different stages of disease development. Tops of bars represent the means and the vertical lines the confidence limits, $P = 0.05$.

Although the exact mechanism remains unknown, the experiments described in this paper have demonstrated failure in root water transport in a susceptible species, *E. sieberi*, when infected with *P. cinnamomi*. Further investigations are in progress to determine whether xylem dysfunction is caused by hormone imbalance or by embolism in vessels. The latter may result from high-molecular-weight compounds produced either by fungal enzymic degradation or during host interaction.

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