Effect of Phloem Water Relations on the Growth of Phytophthora cinnamomi in Eucalyptus marginata

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ABSTRACT


The growth rate of Phytophthora cinnamomi in the secondary phloem of Eucalyptus marginata was determined, in part, by the water status of the tissue. Phloem of trees with the greatest water deficits was the least susceptible to invasion by the fungus. The effect of tissue water status on growth of P. cinnamomi was investigated by establishing 10 plots of 15 saplings each in different rainfall zones of the northern jarrah forest in Western Australia. Stem phloem of each sapling was inoculated with P. cinnamomi in early summer, and fungal growth was monitored for 3 mo by means of a Plant Impedance Ratio Meter. Phloem relative water content (RWC) was determined on the same dates that fungal growth was estimated. At some plots, phloem RWCs decreased appreciably as summer progressed. When phloem RWCs were below 85%, lesion extension ceased even though summer temperatures were highly favorable for fungal growth. Mean predawn leaf water potentials (at selected plots) in late summer varied between −0.63 and −2.5 MPa. Excised phloem pieces were used to determine the relationship of RWC to phloem water potential. RWC was related linearly to water potential over the range 75-100% RWC, corresponding to water potentials of −1.5 to 0 MPa, respectively. Fungal growth in excised stem blocks was also correlated with phloem water potential and RWC.

Low tissue water potentials have never been shown to inhibit colonization of host plants by pythiaceous fungi. Dunlavy (2) suggested that most agricultural crop plants probably wilted at water potentials less negative than those likely to affect growth of Phytophthora spp. Plant species endemic to the southwest corner of Australia, however, are adapted to withstand summer drought and may show water potentials considerably more negative than −1.5 MPa for long periods (5). We have investigated the hypothesis that phloem water potentials affect rate of phloem invasion by Phytophthora cinnamomi Rands in Eucalyptus marginata Sm. (jarrah). Although the concept of predisposition is well understood, an alternative concept useful in explaining inhibition of pathogens due to the influence of environmental factors on the trees has never been developed.

The outcome of interactions between E. marginata and P. cinnamomi is determined by how fast the fungus can grow and how quickly the tree can respond. Our main objective has been to identify the environmental factors that either directly or indirectly affect the growth rate of the fungus in the host tissues. Temperature affects growth of P. cinnamomi directly; growth is optimal at 28°C (3,21). During previous field experiments we observed intermittent growth of P. cinnamomi in E. marginata (16,17) even though temperatures were high and relatively constant (mean daily temperatures >22°C). After observing renewed lesion extension following heavy summer rains (16), we investigated the effect of tissue water status on fungal growth. A relationship between lesion length and phloem relative water content (RWC) was determined for trees grown at one site (14). This result led us to compare the susceptibility of E. marginata to P. cinnamomi growing on a range of site types. Further relationships between the susceptibility of phloem tissue to invasion and the water status of the trees were sought. Predawn leaf water potentials and tissue water potentials were measured so inhibition of fungal growth could be considered in terms of water potential rather than RWC alone.

MATERIALS AND METHODS

Field study. Ten jarrah forest plots differing in understory composition were chosen at three localities. The plots, all in the northern jarrah forest of Western Australia, were classified according to Havel's site vegetation classification (6,7). Table 1 describes the plots, and Table 2 gives the mean monthly temperatures and rainfall.

Havel (6) described 19 continuum segments (site vegetation types), derived by principal component analysis, in terms of composition, structure, and environmental features. Only five types concerned us: P. s. T. W, and Z (Table 1). Plots where indicator species of more than one group were present, representing the continuum in vegetation between sites, are
TABLE 1. Description of plots established in the northern jarrah forest of Western Australia to determine effect of phloem tissue water status on growth of *Phytophthora cinnamomi*

<table>
<thead>
<tr>
<th>Plot no.</th>
<th>Site type</th>
<th>Forest division</th>
<th>Forest block</th>
<th>Average annual rainfall (mm)</th>
<th>Landscape</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>Jarrahdale</td>
<td>Ashendon</td>
<td>1,150</td>
<td>Convex, lower slope</td>
<td>Light gravel sand</td>
</tr>
<tr>
<td>2</td>
<td>T</td>
<td>Jarrahdale</td>
<td>Canning</td>
<td>1,150</td>
<td>Flat</td>
<td>Gravelly sand with late reoters</td>
</tr>
<tr>
<td>3</td>
<td>Z</td>
<td>Jarrahdale</td>
<td>Canning</td>
<td>750</td>
<td>Crest and slopes of convex hill</td>
<td>Gravelly sand</td>
</tr>
<tr>
<td>4</td>
<td>P-S</td>
<td>Dwellingup</td>
<td>Whitaker</td>
<td>1,100</td>
<td>Parallel lower slope (linear)</td>
<td>Sandy gravel</td>
</tr>
<tr>
<td>5</td>
<td>S-T</td>
<td>Dwellingup</td>
<td>Whitaker</td>
<td>1,100</td>
<td>Parallel upper slope (linear)</td>
<td>Gravelly sand with late reoters</td>
</tr>
<tr>
<td>7</td>
<td>W-S</td>
<td>Dwellingup</td>
<td>Driver</td>
<td>1,200</td>
<td>Gentle, upper slope</td>
<td>Sandy gravel with late reoters</td>
</tr>
<tr>
<td>8</td>
<td>S</td>
<td>Dwellingup</td>
<td>Driver</td>
<td>1,200</td>
<td>Concave, lower slope</td>
<td>Gravelly sand</td>
</tr>
<tr>
<td>9</td>
<td>W-P</td>
<td>Dwellingup</td>
<td>Keats</td>
<td>1,200</td>
<td>Concave, mid slope</td>
<td>Gravelly sand and surface laterite</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>Dwellingup</td>
<td>Keats</td>
<td>1,200</td>
<td>Upper slope</td>
<td>Gravelly sand</td>
</tr>
</tbody>
</table>

*Continuum segments, derived by principal component analysis of vegetation, described by Havel (6) in terms of composition, structure, and environmental features.

Fifteen saplings (7–12 cm diameter over bark, 1.3 m above ground level [DBHOB]) at each plot were inoculated with *P. cinnamomi* during the last 2 wk of January 1985 (midsummer). *P. cinnamomi* (culture 1/MI 264384, A2 compatibility type) was grown on cornmeal agar. Four-day-old cultures were mashed and the inoculum was drawn into a disposable syringe without needle. A square of inner bark was cut from the stems 1.5 m above ground level with a sharp scalpel, and the wound was filled with 0.8 ml of inoculum from the syringe, then taped over.

Inner bark was sampled to determine the RWC of the phloem tissue on the date of inoculation and on subsequent visits to the trees to measure lesion lengths. The bark pieces were cut between 0.2 and 0.5 m above ground level, well away from the developing lesions.

Use of a Plant Impedance Ratio Meter (PIRM) for estimating position of lesion fronts. Lesion extension above and below the inoculation points was estimated 2 wk after inoculation by means of a PIRM (patent pending, Biosensors, Melbourne). Subsequent extension was estimated at less than monthly intervals for a further 3 mo.

The PIRM was used to measure electrical admittance of the bark tissues at two frequencies, 1 kHz and 10 kHz. As admittance is the inverse of impedance, impedance ratios were calculated directly by dividing the high frequency admittance by the low frequency admittance. (Greenham and Daday [4] first proposed that the ratio of impedance values at low and high frequency alternating current could be used to rate the physiological status of tissues.)

The PIRM has a two-needle probe (needles 0.8 mm in diameter, 5 mm apart) that was inserted into the bark. Lesions were detected by probing in a vertical line. A series of ratios was determined at 2-cm intervals, working toward the inoculation point. Lesion lengths were estimated both above and below the inoculation points by noting the position along the stems where the impedance ratios decreased. To avoid spreading *P. cinnamomi* into unaffected phloem, the probe needles were sterilized with 80% alcohol after each contact with lesioned tissue.

Data were collected on the range of ratios for healthy and lesioned tissue throughout the experiment, and the accuracy of our estimations was determined at the end of the experiment when all stems were harvested. Lesion lengths were estimated with the PIRM, then the stems were dissected. Discoloration of the phloem (lesion length) was recorded as described previously (16). Some samples of discolored phloem were plated onto cornmeal agar to confirm that *P. cinnamomi* was present.

RWC. Tissue blocks were cut from the inner bark. The blocks of secondary phloem included all cell layers between the cambium and the most recent phellogen; in vigorous trees, this tissue was up to 1.2 cm thick. Dead outer bark (rhytidome) and rough edges were trimmed off with a sharp blade before being weighed for fresh weight (FW). The trimmed blocks were hydrated in distilled water for about 18 hr, blotted dry, and reweighed to obtain turgid weight (TW). Samples were then dried at 70°C and dry weights (DW) measured. RWC was calculated according to the formula

\[
\text{RWC} = 100 \times \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}
\]

TABLE 2. Mean monthly temperature and rainfall in the northern jarrah forest of Western Australia during January–April 1985

<table>
<thead>
<tr>
<th>Element</th>
<th>Jarrahdale forest division (plots 1–3)</th>
<th>Dwellingup forest division (plots 4–10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Maximum</td>
<td>29.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>13.6</td>
<td>18.9</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

**RWC = 100 \times (FW-DW)/(TW-DW) (19).** Error in determining RWC in the field was thought to be at least ±2%.

Water potentials. Predawn leaf water potentials were measured in March with a pressure chamber (10). Twigs were cut with long-handled clippers and tightly wrapped in plastic film to prevent evaporation before and during measurement of balance pressure.

Phloem water potentials were measured by means of a Wescor W33-T Dewpoint Microvoltmeter (1) fitted with a C-52 sample chamber and 2-mm-deep soil planchets (Wescor, Logan, UT). Samples of phloem about 1 mm thick were cut from the bark with sharp razor blades. Care was taken to place the functional sieve tube zone adjacent to the cambium from the samples. Samples were then wrapped in plastic film and pressurized for 20 min before being mounted in the sample chambers. Sap squeezed from cells during cutting was resorbed during the pressurization period.

Growth of *P. cinnamomi* in excised phloem samples. Jarrah stems 12–15 cm DBHOB were cut into lengths of about 2 m and kept in water overnight to eliminate preexisting water deficits. Stem segments having a range of water deficits were prepared from the hydrated stems as follows: The stems were cut into 12– or 24-cm sections, which were cut radially to produce wedges of wood overlain by bark. The wedges were rinsed in water before being placed on a rack in the sun to dry for varying periods. When the desired water deficits had developed, the segments were wrapped in plastic and left to equilibrate overnight at room temperature.

The equilibrated stem pieces were inoculated at midpoint by a method similar to that used in the field. The inoculated pieces were placed individually in plastic to prevent further water loss, then incubated at 25°C for 6–7 days. Growth of *P. cinnamomi* was assessed by noting extent of phloem discoloration. Samples with high RWC showed faint discoloration only, although plotting confirmed extensive invasion of the phloem by the fungus.

**RESULTS**

Field inoculations. Two weeks after inoculation, the fungus had established in the trees at all sites but fungal growth rate varied widely among the lowest RWCs (Figs. 1–3). Lesion lengths (means for 10 trees at each site) were determined with the PIRM at three sites before the final measurement of lesions. The mean

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RWCs recorded during February, the hottest month, are shown in Figures 1–3.

At the Jarrahdale P, T, and Z sites (Fig. 1), lesion extension was most rapid in the P and T trees during January and February (up to day 46). Mean RWCs at the end of January were 90.6 ± 0.4% (P site) and 88.4 ± 0.6% (T site), and only small decreases occurred in these values during February (Fig. 1). At the eastern low rainfall site (Z site), mean RWCs dropped from 87.8 ± 0.7% to 82.7 ± 0.5% during the last 2 wk of January (the first 2 wk of the experiment). The mean RWC determined on 18 February was 83.4 ± 0.5% (Fig. 1). The fungus stopped growing in most of the Z trees during the first month of the experiment. There was little tangential spread of the fungus from the inoculation points, and lesions were narrow strips of discoloration above and below the inoculation points. Comparison of the final lesion lengths further emphasized the difference in susceptibility of the trees to invasion by the two sites. Mean lesion length for the Z trees (with low RWCs) was 15.2 ± 1.9 cm, compared with 6.4 ± 0.9 cm and 8.4 ± 0.5 cm for the T and P trees, respectively (both of which had higher RWCs).

A similar description can be given of the results for the sites north of Dwellingup (Fig. 2). Fungal growth was limited in trees with low RWCs at the P-S site compared with trees at two “wetter” sites (S and T). The P-S site was less than 1 km from the S-T site, so rainfall would have been similar (unlike the Z site of the Jarrahdale group, which was in a low, 750-mm annual rainfall zone). The mean lesion length recorded at the end of the experiment for the P-S site was 21.4 ± 3.7 cm, compared with 54.8 ± 8.4 cm for the S-T site and 65.2 ± 6.5 cm for the S site. Mean plume RWC (20 February) was 82% for the P-S trees, 90% for the S-T trees, and 89% for the S trees. The results from these three sites were consistent with those for the Jarrahdale sites and apparently confirmed our hypothesis that fungal growth was limited in trees with RWCs below 85% (14).

The four plots (7–10, Table 1) chosen south of Dwellingup were paired: upslope S sites and downslope W-P and W-S sites. Some understory species were growing on the lower sites, suggesting those sites were water gaining rather than well drained. We had expected the fungal growth rate to be slower in the S site trees than in the P and W-S site trees, but no such separation in susceptibility of the trees to invasion was found (Fig. 3). Plume RWCs remained constant throughout summer for the two plots. Mean RWCs from 17 January to 18 March were 88–90% for site 7 (W-S site type), 88–90% for site 8 (S-S site type), 88–91% for site 9 (W-P site type), and 91–92% for site 10 (S site type).

Throughout the experiment, estimating the position of lesion fronts with the PIRM depended on recognizing during probing the point of greatest change in impedance ratios. In all trees, an obvious change in ratios was noted and was taken to indicate that necrotic tissue had been reached. Healthy bark probed some distance from the inoculation points gave ratios in the limited range of 1.18–1.40 (mean 1.265 ± 0.004, n = 150), whereas ratios recorded for lesions were within the range of 1.04–1.16 (mean 1.129 ± 0.003, n = 150) at the first probing date, 2 wk after inoculation. The mean change in ratios along lines of probing, i.e., lesion fronts, was 0.136 ± 0.004 (n = 150) for the first date. Once lesions stopped extending, the change in ratios between healthy and dead tissue became greater. Ratios for healthy tissue were similar throughout the experiment but approached 1.0 for lesions aged (ratios for lesions were within the range 1.07–1.18 before harvest).

At the end of the monitoring period, all stems were cut both transversely and longitudinally through the inoculation points, and some outer bark was chiseled away to reveal the dark lesions. Lesion lengths had been estimated with the PIRM just before harvest. Differences between the lesion lengths measured in the dissected stems and the estimates made with the PIRM were not large. At the Jarrahdale Z site, for example, the mean length of lesions in trees measured above the inoculation points was 7.9 cm, compared with the PIRM estimate of 6.7 cm. The greatest difference in the mean length of lesions (above inoculation points) determined by the two methods was 4.3 cm; this was for trees at the W-P site in Dwellingup, which had relatively long lesions (mean 62.2 cm, Fig. 3). Overall, the linear regression for lesion lengths estimated with PIRM against the lesion lengths measured in the dissected stems had an R² = 0.89 (n = 147). There were two sources of error. Lesion extension was not always in line with the inoculation point, and toward the end of the experiment the positions of some fronts were missed. The performance of the PIRM was satisfactory, although distance between probing points meant we consistently underestimated lesion length (mean error 1.29 cm); this did not influence our interpretation of the results, however.

The weather was hot during January, February, and March (Table 2), and the mean daily temperature did not drop below the optimum for fungal growth until April. Decreasing night temperatures, along with tree resistance, may have contributed to the general decrease in rate of fungal growth observed toward the end of the experiment (Figs. 1–3). Lesions had ceased to extend in some trees at all plots by the final harvest date, and standard errors increased accordingly.

**Tree water potentials.** Toward the end of the experiment, predawn leaf water potentials were determined in six of the plots (Table 3). Significant differences in predawn leaf water potentials existed between each of the Havel site types (6) at both the
Jarradale and Dwellingup study areas. The least water-stressed plots were the P site type in the Jarradale division (mean predawn water potential $-0.63$ MPa) and the S site type at Dwellingup ($-0.67$ MPa). The most severely water-stressed plot was the Z site type at Jarradale (mean predawn water potential $-2.5$ MPa); the lowest water potential of the study, $-3.3$ MPa, was recorded in an apparently healthy tree in this plot. The driest Dwellingup plot was the P-S site type (mean predawn water potential $-2.2$ MPa), with a minimum single tree water potential of $-2.7$ MPa. A significant correlation ($P<0.005$) between RWC and predawn leaf water potentials was found at the P-S site, where trees showed a range of water deficits (unlike those at "wetter" sites such as P). No wilting or shedding of leaves that might have indicated damaging water stress was seen in trees in any of the plots studied.

Final lesion length above the inoculation points correlated significantly ($P<0.001$) with predawn leaf water potentials measured in April for the six plots (Fig. 4). Only where trees had developed substantial water deficits could part of the variation in lesion lengths for the plot be explained in terms of tissue water potential. For example, lesion length was not correlated with predawn leaf water potentials at the P and S-T sites where water stress was slight (Fig. 4).

**Phloem RWC and phloem water potential.** The relationship between phloem RWC and water potentials determined by means of stem segments is shown in Figure 5. Phloem water potentials were related linearly to RWC over the range of $0$ to $-3.0$ MPa (100 to 45% RWC). Variability was high between individual samples and between samples from different stems. Variability probably arose from deficiencies of technique (e.g., accuracy with which functional phloem was removed and wound effects) [13] and from differences in bark structure related to dominance class of the tree and site factors.

**Fungal growth and phloem water content.** Lesion lengths in phloem of differing RWCs after 6 days of incubation are shown in Figure 6. Lesion lengths declined approximately linearly with decreasing bark RWC and water potential. Lesions failed to develop in bark when RWC fell to 70%, corresponding to a water potential of $-1.5$ MPa.

**DISCUSSION**

The main objective of our current research on *E. marginata* has been to recognize the factors that are most important in determining the severity of *P. cinnamomi* disease impact in the forest. In the state of Victoria, the importance of soil water as a factor has been recognized as "dieback" outbreaks caused by *P. cinnamomi* follow years of heavy summer rainfall [18]. In both Victoria and Western Australia, areas that are moist because of topography or impeded drainage are known to be areas of high disease impact [9,11,12,20]. The association of disease with high soil moisture availability has hitherto been attributed to production and dispersal of zoospores of the fungus [11,21]. Likewise, high temperatures favor sporulation and growth of the fungus.

The work reported here has identified plant water status as a factor affecting the susceptibility of *E. marginata* to *P. cinnamomi*. The relationship between relative water content (RWC) and water potential (WP) of phloem of jarrah stems. Circles are points obtained from stem segments; triangles are points obtained from saplings left to dry without recutting after falling. Equation of the regression line is $RWC = 96.4 - 17.4 WP$ ($R^2 = 0.68$).

**TABLE 3.** Mean leaf predawn water potentials at the end of March 1985 for saplings inoculated with *Phytophthora cinnamomi* at six plots in the northern jarrah forest of Western Australia*

<table>
<thead>
<tr>
<th>Plot no.</th>
<th>Site type</th>
<th>Number of saplings sampled</th>
<th>Water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P</td>
<td>7</td>
<td>$-0.63 \pm 0.04$</td>
</tr>
<tr>
<td>2</td>
<td>T</td>
<td>5</td>
<td>$-1.3 \pm 0.2$</td>
</tr>
<tr>
<td>3</td>
<td>Z</td>
<td>12</td>
<td>$-2.5 \pm 0.1$</td>
</tr>
<tr>
<td>4</td>
<td>P-S</td>
<td>8</td>
<td>$-2.2 \pm 0.3$</td>
</tr>
<tr>
<td>5</td>
<td>S-T</td>
<td>8</td>
<td>$-1.43 \pm 0.05$</td>
</tr>
<tr>
<td>6</td>
<td>S</td>
<td>8</td>
<td>$-0.67 \pm 0.06$</td>
</tr>
</tbody>
</table>

*Plots 1–3 are in the Jarradale forest division and plots 4–6, in the Dwellingup division. Means of sites within each division are significantly different ($P<0.05$).

Continuum segments, derived by principal component analysis of vegetation, described by Havel (6) in terms of composition, structure, and environmental features.
after infection. Growth of *P. cinnamomi* in stem pieces with RWCs of 85% was one-half that found in pieces with RWCs of 95% or greater. Fungal growth was halted completely at 70% RWC (Fig. 6), corresponding to a water potential of -1.5 MPa (Fig. 5). Results in the field were similar. Although *P. cinnamomi* established successfully in the phloem of *E. marginata* saplings after inoculation, spread of the lesions from the inoculation points was slowed by increasing water deficits during the summer (Figs. 1 and 2). Lesions stopped growing when phloem RWC fell below 85%. In contrast, experiments with *P. cinnamomi* growing in soil have shown growth to be reduced by water potentials of -1.05 MPa and prevented by potentials of -3.6 MPa (8). The greater sensitivity of *P. cinnamomi* to low water potentials in phloem than in soils may reflect the “balance of rates” between fungal invasion and host resistance.

Differences in bark RWC between sites were reflected in the predawn water potentials of trees in late summer. Both RWC and water potential measurements support Havel's (7) view that vegetation characteristics reflect the physical characters (both geophysical and climatic) of the site. As Havel site types indicate broadly defined areas of water availability to plants, and as plant water status has been shown to alter susceptibility to dieback, a partial link between susceptibility and site has been established.

Although *P. cinnamomi* is primarily a root-infecting fungus, stems were used for ease of experimentation. Roots vary and monitoring of lesion extension could have been difficult. Roots were inoculated at the Jarrahdale sites during February 1984, and growth of the fungus was significantly less in roots of the Z site trees than in roots of the P and T site trees. Further experiments on root tissues are necessary.

The PIRM proved satisfactory in monitoring the growth of *P. cinnamomi* in the *E. marginata* stems. This meter has the advantage over others (e.g., the Shigometer) in that the ratios used to determine lesion fronts apparently change independently of water content and ionic strength and reflect condition of cell membranes more accurately. The PIRM should prove useful as a nondestructive way to measure growth of other fungal pathogens that establish in the phloem of stems or roots.

Factors other than water availability will also affect the outcome of interaction between *P. cinnamomi* and *E. marginata*. Lesion lengths recorded on some sites varied greatly (although water was not limiting), and significant differences in mean lesion lengths have been recorded for sites of different nutritional status. Quantifying tree resistance and relating levels of passive or active resistance to phenotype, vigor, and growing conditions is difficult. *E. marginata* does have a range of resistance mechanisms that may be used effectively against the fungus (15,17), and we have observed that trees on some sites where moisture is not limiting survive even though infected by the fungus. Correlations between lesion length and site factors other than water availability must be sought. Such studies must be conducted before tree water deficits develop, because fungal growth is inhibited at low phloem water potentials regardless of the capacity of trees to resist.

LITERATURE CITED


