**Temperature-Growth Relationships of Phytophthora cinnamomi in the Secondary Phloem of Roots of Banksia grandis and Eucalyptus marginata**

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**ABSTRACT**


The form of the temperature-growth rate relationships of *Phytophthora cinnamomi* in excised roots of *Banksia grandis* and *Eucalyptus marginata* was skewed toward optimal temperatures (25–30 C) and could be divided into two linear components. There was a linear increase in growth rate as temperatures increased from 10 to 30 C and a decrease for temperatures greater than 30 C. Most of the variation in fungal growth in field-inoculated intact roots of *E. marginata* was explained by variation in maximum daily temperature. Introduction of nonlinear components into the regression model did not improve the variation explained over that obtained for simple linear relationships. For the susceptible host *B. grandis*, there was close correlation between lesion lengths predicted from temperature-growth relationships in excised roots and observed lesion lengths in the field. Closest agreement between observed and predicted lesion lengths in *E. marginata* occurred for winter-inoculated roots. For roots of *E. marginata* inoculated in summer there was divergence between observed and predicted lesion lengths. Observed lesion lengths did not increase as optimal temperatures for fungal development increased. Host resistance or the physiological status of tissue could have inhibited fungal growth.

Additional key words: epidemiology, forest site type.

Most studies relating the influence of temperature to the epidemiology of *Phytophthora cinnamomi* Rands have concentrated on development of the pathogen in the soil and infection of roots (3,5,6,11,13,15,22,24) with little attention being given to growth in host tissue. Knowledge of the effect of environment on growth in host tissue is needed for accurate estimation of temporal changes in disease development. Grant and Byrt (2) compared the effect of temperature on growth of *P. cinnamomi* in roots of 9-11 wk-old seedlings of *Eucalyptus calophylla* R. Br. and *E. marginata* Donn ex Smith. Within the temperature range 14–28 C, length of lesions in seedling roots of *E. calophylla* increased up to 4 days after zoospore inoculation but did not increase significantly between 4 and 8 days. Excluding the latent period, lesion extension in seedling roots of *E. marginata* inoculated at 14–28 C increased nearly linearly with time during the first 8 days after inoculation, and the rate of lesion extension increased with increasing temperature. In intact and excised roots of *Abies fraseri* (Pursh.) Poir., growth rate of *P. cinnamomi* increased almost linearly as temperatures increased from 10 to 28 C (10).

We determined temperature-growth relationships of *P. cinnamomi* in media and secondary tissue, primarily the phloem, of roots of mature trees of *Banksia grandis* Willd. and *E. marginata*. Whereas *P. cinnamomi* extensively colonizes secondary phloem and xylem of *B. grandis* (12), lesion development in roots of *E. marginata* can be restricted due to host resistance (20). Temperature-growth relationships in excised roots under controlled conditions were compared with growth of *P. cinnamomi* in intact roots in the field.

**MATERIALS AND METHODS**

**Isolates and media.** In a growth comparison experiment, isolates, media, and temperatures were the independent variables (with four levels of each), and growth rate was the dependent variable. Each treatment was replicated three times.

The four isolates of *P. cinnamoni* were obtained from the CSIRO Division of Forest Research. CSIRO culture number, host isolated from, and location in Western Australia are: SC77, *Hovea elliptica* (Sm.) DC., Walpole; SC72 (IMI 264384), *Hibbertia subvaginata* F. Muell., Mandurah; SC179, *Persoonia longifolia* R. Br., Gnggara; SC191, *Hypocalymma cordifolium* (Lehm.) Schau., Harvey. Isolates were maintained on Difco cornmeal agar at 25 C. The media used were: Difco cornmeal agar (CMA), water agar (WA) (15 g of Bacto-agar in 1 L of distilled water), Difco lima bean agar (LBA), and half-strength Difco potato-dextrose agar (PDA) (19.5 g of PDA and 7.5 g of Bacto-agar in 1 L of distilled water).

A 3-mm-diameter agar disk was cut from the margin of a 3-day-old colony of *P. cinnamomi* and transferred to the center of an 85-mm-diameter petri dish containing 30 ml of the same medium as the original culture. The media were incubated at 12, 20, 25, and 30 C. Colony diameter was measured with calipers at the same time each day. For temperatures between 20 and 30 C, colony diameters on CMA and LBA were measured for 4 days and on PDA and WA for 9 days. At 12 C, colony diameters on CMA and LBA were measured for 11 days and on PDA and WA for 21 days. The plug diameters were subtracted from colony diameter and growth rates calculated. For each temperature-medium combination, significance of differences in growth rate between isolates was determined with one-way analysis of variance. Spearman's coefficient of rank correlation (8) was used to determine the correlation between growth and temperature for each isolate-medium combination.

**Growth studies using excised roots.** In an experiment run in conjunction with the media experiment previously described, isolates and temperatures were the independent variables and growth rate in excised roots of *E. marginata* the dependent variable. The isolates and temperatures used were the same as those described previously. In subsequent experiments, using excised roots of *B. grandis* or *E. marginata*, temperature and time of sampling were independent variables and growth of isolate SC72 of *P. cinnamomi* the dependent variable. Isolate SC72 was chosen because it was used in previous studies (13,18). Each treatment was replicated three times with six roots per replicate.

Roots of *B. grandis* and *E. marginata* were dug from the field, cut into 20-cm long sections, washed, and brought into the
laboratory for inoculation. The diameter over bark of excised roots varied from 2 to 10 mm (average 6 ± 1 mm) for B. grandis and 2 to 6 mm (average 4 ± 1 mm) for E. marginata. A 7-mm-diameter agar disk was cut from the edge of a 3-day-old culture of Phytophthora cinnamomi growing in CMA and placed on one freshly cut end of each root. The ends were covered with aluminum foil and the root pieces buried in moist vermiculite so that they were well separated from each other and incubated at different constant temperatures.

Four, 8, and 12 days after inoculation, six root pieces were removed from the vermiculite and cut into 5–10-mm sections, starting at the uninoculated end. The pieces were sequentially plated onto PDA with added pimaricin, vancomycin, penicillin, and vancomycin, benzene, and hymexazol. The plated sections were incubated at 25°C for 3 days, and the length of root regression and correlation coefficients were determined.

Starting at the uninoculated end, the pieces were sequentially incubated at different constant temperatures. The lesion extension rates were calculated by averaging the lesion length above the inoculation point and dividing by the number of days since inoculation. Appropriate regression and correlation coefficients were determined.

**Temperature.** Excised root temperatures were controlled in incubators and recorded with thermometers placed next to the roots buried in the vermiculite. In the field, holes 5 mm in diameter and 50 mm long were drilled at an angle into the phloem of roots of control trees. A thermistor probe was inserted into each hole, the hole sealed, and the probe connected to a recorder. The change in root temperature with time of the year is given in Figure 1.

**RESULTS**

Variation between isolates in the temperature-growth relationships in different media and excised roots of E. marginata.

With the exception of growth on WA at 20°C, there were significant differences (P = 0.01) in growth rate between the isolates of Phytophthora cinnamomi on the four media incubated at 12, 20, 25, and 30°C. There were no significant differences in growth between isolates in excised roots of E. marginata incubated at the four temperatures.

By pooling the data of isolates and temperatures for each medium, growth rates in excised roots of E. marginata were significantly linearly correlated with those on media (Fig. 2). Growth rates of the isolates on LBA were most similar to those in excised roots, whereas growth rates on the other media were less than in excised roots (Fig. 2).

For the relationships between temperature and growth of most isolates on the four media, the Spearman's coefficient of rank correlation was not significant (P = 0.05), but it was significant for the relationship between temperature and growth of isolate SC72 on PDA and CMA. For the temperature-growth relationships in

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**Fig. 1.** Seasonal changes in the temperature of roots of Eucalyptus marginata and times when the roots were inoculated with Phytophthora cinnamomi. Dots indicate mean daily temperature, the continuous line a running mean of 5 days and 11 to 113 inoculation times.

**Fig. 2.** Relationship between growth rate of Phytophthora cinnamomi in excised roots of Eucalyptus marginata and in various media. The symbol X indicates overlap of more than one data point, continuous lines are the regression equations fitted to the data, and the broken line the 45° bisector.
excised roots of *E. marginata*, the Spearman’s coefficient of rank correlation was highly significant (*P* = 0.01) for all isolates.

**Temperature-growth relationships of an isolate in excised roots.** At all temperatures, growth of SC72 in excised roots of *E. marginata* was linear with time during the 12-day period between inoculation and harvest (Fig. 3). At each assessment time, the temperature-growth curve could be divided into two parts: a linear increase in growth as temperature increased from 10 to 30 C, then a rapid decrease in growth for temperatures greater than 30 C. Determination of the temperature-growth relationship at different times after inoculation (Fig. 3) was repeated using excised roots of *E. marginata* collected over 2 yr. Growth rates plotted against temperatures could be divided into two parts: a linear increase in growth rate as temperatures increased from 10 to 30 C and a rapid decrease for temperatures greater than 30 C. For temperatures between 10 and 30 C the linear regression line fitted to growth rates explained 84% of the variation between temperature and growth (*P* = 0.01, *n* = 23) and was of the form:

\[ y = -1.788 \pm 0.735 + 0.363 \pm 0.035 T \]  

(1)

where *y* is the growth rate for the interval 10–30 C, and *T* is temperature.

The linear regression model fitted to growth rates at temperatures greater than 30 C explained 58% of the variation (*n* = 4). From a plot of the data, estimated optimum and maximum temperatures were 29 and 34 C, respectively. Extrapolating equation 1 gave a minimum temperature of 4.9 C.

There was a significant positive linear correlation (*P* = 0.01) between temperature within the range 10–30 C and growth rate of isolate SC72 in excised roots of the susceptible host *B. grandis* (*R* = 0.98, *n* = 7). The regression equation was of the form:

\[ y = -2.610 \pm 0.616 + 0.454 \pm 0.030 T \]  

(2)

where *y* is the growth rate for the interval 10–30 C, and *T* is temperature.

For the temperature-growth relationship in roots of *B. grandis* the slope of the regression line was significantly greater than that for roots of *E. marginata* (eqs. 1 and 2). Extrapolating equation 2 gave a minimum temperature of 5.8 C.

**Temperature-growth relationships for intact roots of *E. marginata*.** Variation in maximum temperature explained most of the variation in lesion linear extension rate of isolate SC72 in field inoculated intact roots of *E. marginata* (Table 1). Only a third of the variation in lesion extension was explained by variation in minimum temperature, whereas average temperature was intermediate between maximum and minimum temperatures. Introduction of nonlinear components into the model did not improve the variation explained over that obtained for simple linear relationships. The slopes of the regression lines relating rate of lesion extension to maximum, minimum, and average temperature were very similar to each other but significantly less than those for excised roots (Table 1, equation 1). Extrapolating the equation with average daily temperature as the independent variable gave a minimum temperature of 3.2 C for lesion extension.

Use of temperature-growth relationships derived from excised roots to predict lesion length in intact roots of *B. grandis* and *E. marginata*. Lesion length was estimated each day by substituting daily average temperature recorded in roots in the field (Fig. 1) into the appropriate temperature-growth equation obtained for excised roots of *B. grandis* (equation 2) or *E. marginata* (equation 1). Total lesion length was then calculated by summing the daily estimated lesion lengths over the period from inoculation to harvest.

Lesion length predicted from the temperature-growth relationship for excised roots of *B. grandis* was highly correlated with the lesion lengths observed in intact roots inoculated in the field (Fig. 4). The predicted lesion lengths were very similar to observed lesion lengths less than 300 mm; the points fall close to the 45° bisector, where predicted and observed values are equal.

In intact roots of *E. marginata*, observed lesion lengths were relatively constant during the first summer, decreasing to a minimum in winter (June, July), followed by a gradual increase to a maximum in spring (early October), then in the second summer, fluctuated around a mean of 68 mm per 6-wk assessment period (Fig. 5). In comparison to that observed, lesion lengths predicted from the excised root temperature-growth equation (1) showed a sinusoidal pattern (Fig. 5), with predicted lesion length being minimal in June–July, increasing to a maximum in summer followed by a decline.

The plot of observed and predicted lesion lengths for *E. marginata* (Fig. 6) was very different to that already described for *B. grandis* (Fig. 4). Observed lesion lengths were not as long as

![Fig. 3. Growth of *Phytophthora cinnamomi* in excised roots of *Eucalyptus marginata* incubated at different temperatures and assessed 4, 8, and 12 days after inoculation.](image)

**TABLE 1. Regression coefficients and percentage of variation explained for the linear relationship between the temperature variables indicated and the variation in lesion linear extension rate of *Phytophthora cinnamomi* in intact roots of *Eucalyptus marginata* in the field**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Range (C)</th>
<th>Regression coefficient</th>
<th>Variation explained (%) (<em>R</em>² × 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AVTEMP</strong></td>
<td>8.4–22.3</td>
<td>0.22</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td><strong>MAXTEMP</strong></td>
<td>11.0–26.3</td>
<td>0.06</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td><strong>MINTEMP</strong></td>
<td>6.1–19.0</td>
<td>0.44</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td><strong>MAXTEMP + MINTEMP</strong></td>
<td>as above</td>
<td>0.03</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td><strong>MAXTEMP + MINTEMP + MAXTEMP</strong></td>
<td>as above</td>
<td>-0.92</td>
<td>0.19 ± 0.23</td>
</tr>
<tr>
<td><strong>MINTEMP</strong></td>
<td>as above</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*AVTEMP = Average hourly root temperature during period from inoculation to assessment.*

*± Standard error.

*Asterisks * or ** indicate a significant value for the correlation coefficient at *P* = 0.05 or *P* = 0.01, respectively.

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those predicted. The divergence between observed and predicted values was least in winter, intermediate in autumn and spring, and greatest during summer.

**DISCUSSION**

In roots of *B. grandis* and *E. marginata*, linear regression equations explained most of the variation in growth rate of *P. cinnamomi* with temperature. Estimates of the minimum, optimum, and maximum temperatures of 5, 29, and 34 °C, respectively, are very similar to values reported from other studies (23). As the form of the temperature-growth relationship can change with substrate (14,25), the use of linear regression temperature-growth relations in excised roots. This would not necessarily be the case for intact roots inoculated in the field and assessed 6 wk after inoculation.

In intact roots of *E. marginata*, the best agreement between predicted and observed lesion lengths occurred for roots inoculated in winter, suggesting that temperature had a major effect on lesion size during this period. In summer there was considerable divergence between predicted and observed values, even though optimal temperatures for fungal growth, as indicated by the predicted lesion lengths, were experienced during this period. Thus temperature was not the only factor affecting fungal growth in intact roots of *E. marginata* in summer. Lesion extension of *P. cinnamomi* in *E. marginata* can be inhibited by host responses such as the formation of periderms and phenol accumulation and oxidation (20). In mature tissue, temperatures that favor fungal growth can also favor host defense mechanisms that inhibit fungal growth. The observed plateau in lesion lengths in summer reflects the outcome of the balance between the effect of temperatures on the rate of fungal growth and host response.

Other factors than the interaction between temperature and host response may affect lesion extension of *P. cinnamomi* in intact roots of *E. marginata*. Relative water contents of the phloem are an important factor, with levels less than 80% probably limiting growth of the pathogen (17). However, phloem water content varies with timing of rainfall (17) and site (19) and, in trees growing on sites similar to the one used in this study, did not fall below levels that would inhibit growth of the pathogen in summer (19).
For the highly susceptible *B. grandis*, there was close correlation between lesion lengths predicted from temperature-growth relationships in excised roots and those observed in intact roots in the field, even though there was a limited number of inoculation times. Greatest divergence between observed and predicted lesion lengths occurred when the length was assessed after 22 wk instead of the standard 6 wk. We would expect that the longer the period of prediction the greater the cumulative effect of errors of measurement. In roots of *B. grandis*, host response probably had minimal effect on the temperature-growth relations; there was close agreement between observed and predicted lesion lengths in roots of *B. grandis* inoculated in spring and summer compared with the large differences for roots of *E. marginata* inoculated at the same time. Temperature was a major determining factor affecting growth of *P. cinnamomi* in intact roots of *B. grandis*.

Havel (4) divided the *E. marginata* forest into a number of site types recognized by the response of vegetation indicators to topography, soil type, drainage, and fertility. The site used in this study would be at the more fertile, better drained end of the continuum of sites recognized by Havel (4) and is probably not representative of the majority of forest site types of *E. marginata*. Work begun after the completion of this study (Shearer, unpublished) has shown that death of *E. marginata* after infection by *P. cinnamomi* is more likely to occur in sites that have poorer drainage and are more infertile than the site used in this study. Thus, without further verification, our data cannot be used to infer the seasonal behavior of *P. cinnamomi* in infected trees in other site types than the one we used.

Lesion extension of *P. cinnamomi* in intact roots of *E. marginata* did not vary greatly with season, a seasonal pattern different to that reported for other *Phytophthora*-host interactions (1,7). In stems sampled in different seasons, but inoculated and incubated under standard conditions, lesion extension of *P. cactorum* (Lebert & Cohn) Schroeter in apple (1) and *P. citricola* Sawada in walnut (7) varied greatly with season. In these studies, the season when the *Phytophthora* species most rapidly invaded the stem tissue was also the period when conditions were most favorable for pathogen development in the soil (1,7).

In upland areas of the *E. marginata* forest, development of *P. cinnamomi* in surface soil and growth in susceptible host tissue do not necessarily coincide. In the hot-dry summers, moisture potentials of surface soil are unfavorable for survival and sporulation of *P. cinnamomi* in upland areas (11,13), but as we have shown, temperatures are optimal for growth in host tissue. Thus, in summer, although activity of the pathogen in dry soil can cease, infection-centers will continue to expand through growth in roots of susceptible hosts.

In the cool-wet winters experienced in the *E. marginata* forest, interpretation of the effect of temperature on the epidemiology of *P. cinnamomi* was based on the assumption that 15 C was the critical limit below which sporulation of the pathogen and infection did not take place (11). However, the 15 C limit should not be applied to growth in host tissue. When temperatures are below 15 C, *P. cinnamomi* can infect roots (3,15,24), and we found that the pathogen could establish and grow, albeit slowly, in host tissue. Thus, for the accurate prediction of spread of *P. cinnamomi* in environments where low temperatures alternate with temperatures more favorable for growth, we would agree with the conclusion of Zentmyer (23) that more "definitive information is needed on the role of temperature in the range 14–16 C in relation to disease."

LITERATURE CITED