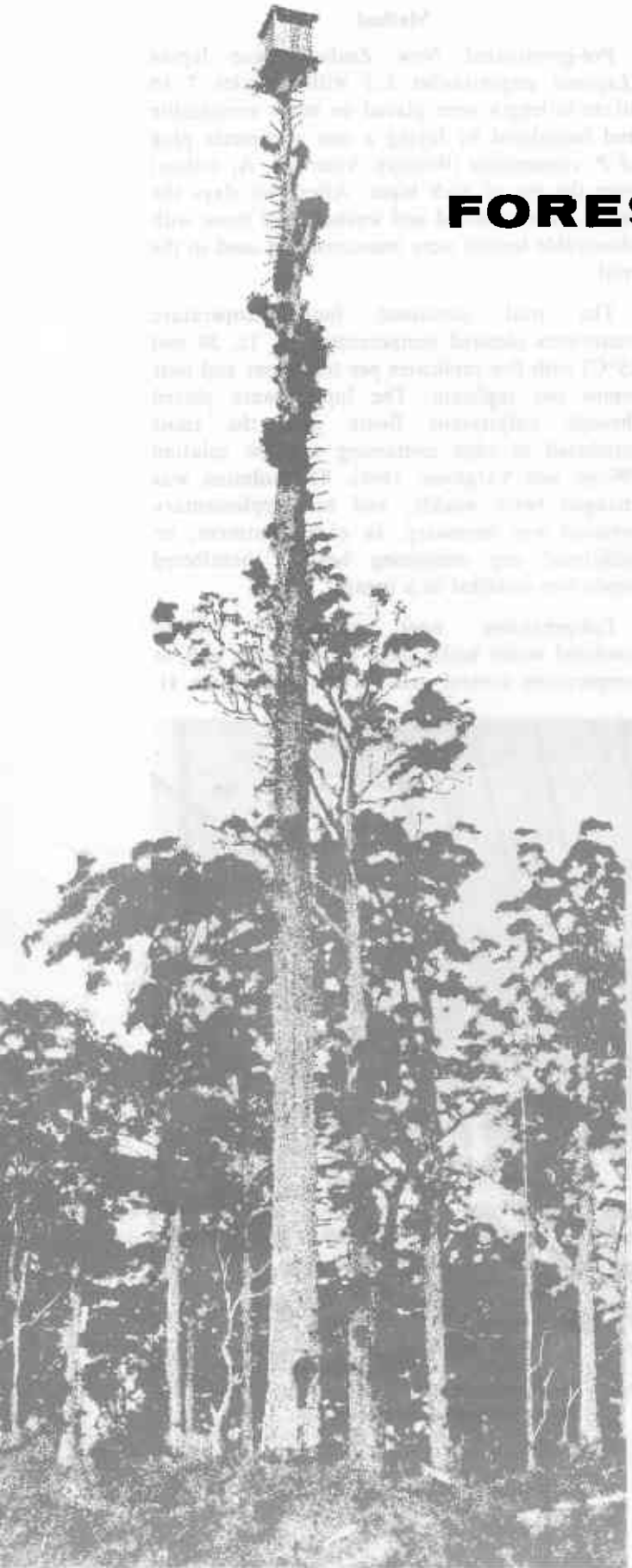

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THE EFFECTS OF TEMPERATURE ON THE INFECTION OF NEW ZEALAND BLUE LUPINS BY *Phytophthora cinnamomi.*

by

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SUMMARY

The effects of constant and fluctuating temperatures on the progression of lesions in root tissues and on disease expression were tested. Lesions progressed faster at higher temperatures (20.3 and 24.9°C), and the rate was less than the potential mycelial growth rate on agar. Some progression of lesions at 11.2 and 14.9°C was observed.

Seedling mortality, plant health and degree of root damage were greater at the higher soil temperatures (18.7 and 26.2°C), with some effects observable at 15.3°C. Fluctuations in temperature ranging from 6.1°C for 3 hours per day to 17°C for 6 hours per day had no observable effects on disease expression.

INTRODUCTION

Phytophthora cinnamomi Rands causes serious disease in a range of forest, horticultural and agricultural crops, including the jarrah forest and the woodland communities in the south-west of Western Australia. When defining the critical environmental limits for this fungus, Hepting (1964) listed soil temperature as a likely limiting factor.

Temperature can influence several aspects of fungal growth and infection. Podger (1968) recorded optimal mycelial growth on agar between 20 and 32°C, with sub-optimal growth at 15°C and slow growth at 10°C. Chee and Newhook (1965b) observed optimal growth rates between 26 and 28°C, with slow growth at 12°C. Sporangial production was also investigated and was found to be optimal between 22 and 28°C and negligible below 15°C or above 30°C. The germination of chlamydospores was very high within the range 18 to 30°C but fell off sharply below 15°C or above 33°C (Mircetich and Zentmyer, 1966). When infected Douglas-fir seedlings were grown in controlled-temperature tanks, no mortality was observed below 15.5°C; 11 per cent died at 18°C, and over 80 per cent mortality was recorded at 24 and 27°C (Roth and Kuhlman, 1966).

Shea (1975) showed that the period of coincidence of moisture and temperature regimes suitable for *P. cinnamomi* in the jarrah forests varied considerably between sites. Measurements of soil temperature indicated that canopy cover, tree species, litter cover and (to a lesser extent) aspect have significant effects on the length of time that soil temperature is within the limits for mycelial growth or infection. Silvicultural manipulation of the forest so as to depress soil temperature during the critical spring months (when soil temperature and moisture are at an optimum for fungal activity) was seen as a means of reducing the impact of *P. cinnamomi* on some sites.

The trials described in this paper investigated:

1. the effects of temperature on mycelial growth within root tissues:
2. the effects of temperature on the host-pathogen interaction on two different soil types;
3. the effects of fluctuating temperature on disease expression.

1. TEMPERATURE AND MYCELIAL GROWTH

Method

Pre-germinated New Zealand blue lupins (*Lupinus angustifolius* L.) with radicles 7 to 10 cm in length were placed on moist vermiculite and inoculated by laying a one centimeter plug of *P. cinnamomi* (Western Australia, A_2 isolate) over the tip of each lupin. After two days the lupins were removed and washed, and those with observable lesions were measured and used in the trial.

The trial contained four temperature treatments (desired temperatures 10, 15, 20 and 25°C) with five replicates per treatment and four lupins per replicate. The lupins were placed through polystyrene floats with the roots immersed in cups containing nutrient solution (Wong and Varghese, 1966). The solution was changed twice weekly, and no supplementary aeration was necessary. In each treatment, an additional cup containing healthy, uninfected lupins was installed as a control.

Temperatures were maintained within insulated water baths using cooling coils and/or temperature control units as required (Plate 1).

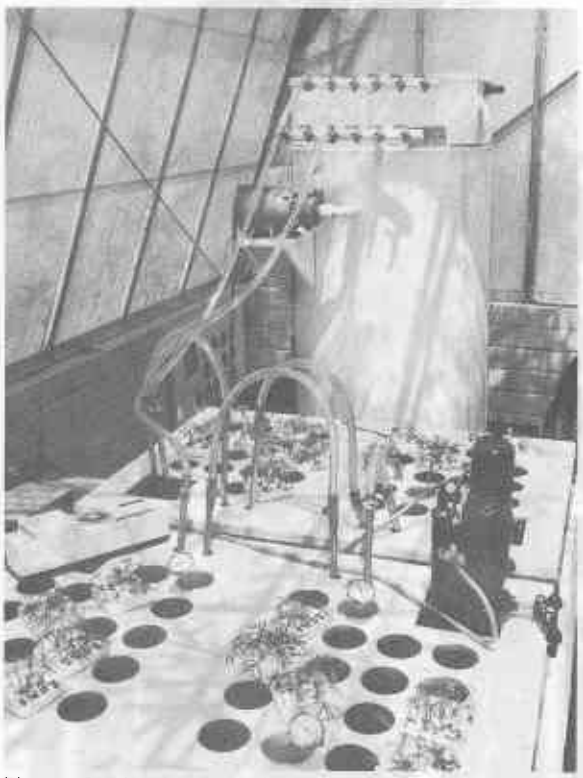

Plate 1 Insulated water baths showing cooling unit, coils and Haake temperature control units.

The baths were located in a temperature-controlled glasshouse (21 to 27°C), and treatment temperatures recorded twice daily. The lengths of lesions along the radicles were measured after 4, 8, 15 and 26 days. At the completion of the trial, pieces were plated on 3P agar (Eckert and Tsao, 1962).

Results

Excellent temperature control was maintained, and the treatment means (n = 40) were as follows: 11.2, 14.9, 20.3 and 24.9°C, with only minor fluctuations about the mean value.

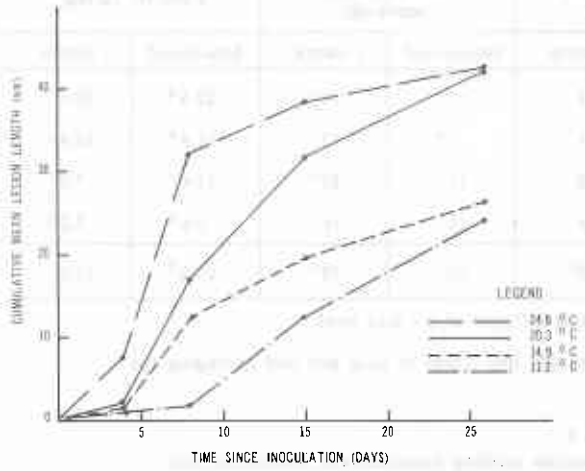

Figure 1 Cumulative mean lesion lengths in radicles of New Zealand blue lupin seedlings infected with *Phytophthora cinnamomi* and grown in water baths at different temperatures.

In all cases, the controls grew well and remained unlesioned. None of the inoculated lupins died, but lesion lengths progressed with time (Fig. 1), and *P. cinnamomi* was readily recorded by plating. After 4 and 15 days, the differences between treatments were not significant; at 8 days the progression of lesions was greater at 24.9°C ($p < 0.01$) and less at 11.2°C ($p < 0.05$). By the 26th day, the progression at the two higher temperatures (24.9 and 20.3°C) was greater than at the two lower ones ($p < 0.05$).

2. TEMPERATURE, SOIL TYPE AND HOST-PATHOGEN INTERACTION

Method

In this trial, four temperature treatments (desired temperatures as for Trial 1) and two soil types (lateritic silt and sand) typical of the jarrah forest were used. Inoculation was achieved by using naturally infected soils which yielded a high recovery of *P. cinnamomi* on lupin baiting (Chee and Newhook, 1965a). Controls were grown in naturally healthy soils from which *P. cinnamomi* could not be recovered by baiting.

The soils were sieved and placed in undrained plastic containers. Five lupins with radicles 2 to 7 cm in length were pricked into each. The pots were watered to saturation and thence as required, using distilled water. Temperature control was achieved by immersing the lower portions of the pots in the water baths described in Trial 1. Soil temperatures in the various treatments were recorded twice daily.

The trial thus consisted of a 2 × 2 × 4 factorial, and 2 replicates were used. After a period of 43 days, the mortality, health and oven dry weight of the live plants and the degree of root rot (rated on a scale from 1 to 6, where 1 = healthy plant and 6 = dead plant) were recorded. Analysis of variance was computed using the appropriate transforms where required.

Results

The comparisons between the desired temperatures and the temperatures achieved are shown in Table 1. Temperature means were significantly different ($p < 0.01$), but exact temperature control was not possible.

An inoculation-soils interaction was observed (Table 2). In the diseased soils, deaths in the silt exceeded those in the sand. The reverse trend applied when seedling health was used as the criterion for comparison. There were no deaths in the controls.

Several inoculation-temperature interactions are shown in Table 3. More dead and fewer healthy seedlings occurred in the inoculated 18.7 and 26.2°C treatments. The degree of root rot in the inoculated 15.3, 18.7 and 26.2°C treatments exceeded that of all other treatments. It is obvious that inoculation has some effect in the 15.3°C treatment; this is also reflected by the significantly different number of seedlings classified as healthy in that treatment. *P. cinnamomi* was readily recovered from seedlings grown in diseased soil, but not from those grown in healthy soil.

TABLE 1
Comparison of temperature desired with temperature achieved.

Temperature Desired (°C)	Temperature achieved °C (n = 104)		
	Mean	Standard Deviation	Range
25	26.2	0.8	25.5-27.0
20	18.7	1.2	16.5-20.5
15	15.3	0.9	15.0-16.0
10	9.2	1.7	7.5-12.0

TABLE 2
Interaction between inoculation, soil type and seedling health and survival.

Experimental Treatments	No. of dead seedlings		No. of unhealthy seedlings		No. of healthy seedlings	
	Silt	Sand	Silt	Sand	Silt	Sand
Inoculated	17^{a}	9^{b}	10	9	13^{x}	22^{y}
Control	0^{c}	0^{c}	4	4	36^{z}	36^{z}

Data with different superscripts are significantly different at $p < 0.01$ level.

TABLE 3
Interaction between inoculation, temperature and three parameters of seedling health.

Experimental Treatments (°C)	No. of dead seedlings		No. of unhealthy seedlings		No. of healthy seedlings		Root rot rating*	
	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control
26.2	13^{a}	0^{b}	5	1	2^{a}	19^{c}	22.4^{a}	10.4^{b}
18.7	9^{a}	0^{b}	9	3	2^{a}	17^{c}	21.8^{a}	10.4^{b}
15.3	3^{b}	0^{b}	5	0	12^{b}	20^{c}	17.4^{a}	7.6^{b}
9.2	1^{b}	0^{b}	0	4	19^{c}	16^{c}	5.6^{b}	5.2^{b}
Totals	26^{x}	0^{y}	19^{x}	8^{y}	35^{x}	72^{y}	67.2^{x}	33.6^{y}

Data with different superscripts within each parameter are significantly different at $p < 0.01$ level.

* Root rot rating was obtained by summing the root rot scores for the five lupins in each pot and averaging the values for each treatment.

TABLE 4
Effects of soil type and temperature treatment on various seedling parameters in inoculated soils.

Experimental Treatments	No. of dead seedlings	No. of unhealthy seedlings	No. of healthy seedlings	Total oven dry weight of live seedlings (g)	Root rot* rating
Soils					
Silt	20^{x}	14	66	50.4	62.4^{x}
Sand	10^{y}	14	76	56.8	49.8^{y}
Temperatures (°C)					
26.2	13^{a}	5	2^{a}	3.9	22.4^{a}
18.7	9^{a}	9	2^{a}	6.2	21.8^{a}
15.3	3^{b}	5	12^{b}	10.1	17.4^{a}
9.2	1^{b}	0	19^{c}	13.6	5.6^{b}
26.2 (6 hrs)	1^{b}	2	17^{c}	11.8	6.2^{b}
26.2 (3 hrs)	0^{b}	0	20^{c}	14.2	6.6^{b}
18.7 (6 hrs)	0^{b}	0	20^{c}	11.8	8.0^{b}
18.7 (3 hrs)	1^{b}	2	17^{c}	11.7	7.2^{b}
15.3 (6 hrs)	1^{b}	3	16^{c}	13.0	7.8^{b}
15.3 (3 hrs)	1^{b}	2	17^{c}	10.9	9.2^{b}

Data with different superscripts within each parameter are significantly different at $p<0.01$ level.

*See Table 3

3. FLUCTUATING TEMPERATURE AND DISEASE EXPRESSION

Method

This trial was carried out in conjunction with Trial 2 using the same procedures except that, due to limitations of space, only diseased soils were used. The same temperature treatments (9.2, 15.3, 18.7 and 26.2°C) were used, with the addition of six fluctuating temperatures. To simulate fluctuations in temperature, pots were removed from the 9.2°C treatment and transferred to each of the other treatments for periods of 3 or 6 hours each week day. They were then transferred back to the 9.2°C treatment for the remainder of the day. The trial thus consisted of a 2×10 factorial with 2 replicates and 5 lupins per replicate.

Results

Results are shown in Table 4. In comparing soils, irrespective of temperature, more deaths and a greater degree of root damage were observed in the silt. The two other parameters used (plant health and oven dry weight) showed similar trends, but the differences were not significant (at $p < 0.01$).

Within the temperature treatments, there were more deaths and less healthy seedlings at 26.2 and 18.7°C. These treatments had lower oven dry weights, but the differences were not significant. The observed root rot in the constant 26.2, 18.7 and 15.3°C treatments was greater than in all others. Short-term (up to 6 hours) fluctuations in temperature (up to 17°C) appear to have no effect on any of the parameters which were assessed.

DISCUSSION

Once infected, the observed progression of lesions within root tissues was faster at the higher temperatures. The trend with increasing temperature was similar to those reported for mycelial growth rates, but the actual rate was much less than the potential growth rate on agar (Podger, 1968; Chee and Newhook, 1965b). As time progressed, so the rate of extension decreased. A considerable degree of variation between lupins in the same treatment was noted, probably the result of differential host reaction to infection. Some progression of lesions at temperatures as low as 11.2 and 14.9°C occurred.

The greater impact of infection on the heavier-textured silt was not surprising in view of the general behaviour of this fungus in heavier-textured soils (Hepting, 1964; Shea, 1975). No temperature-soils interaction was observed. The effect of temperature on mortality was similar to that reported by Roth and Kuhlman (1966). However, in the current trial, a greater number of deaths was observed in the lower temperature range, namely 45 per cent at 18.7°C as compared with Roth and Kuhlman's result of 11 per cent at 18°C. Since the data refer to different plant species, such an interaction could be anticipated.

It is most interesting that fluctuations in temperature, which ranged from 6.1°C for 3 hours per day to 17°C for 6 hours per day, had no apparent effect on any disease parameter in a highly susceptible species. The fungal processes of growth and infection are not favoured by temperature fluctuations, and the pathogen is apparently unable to benefit from an optimal temperature when this is applied for a relatively short period of time. Diurnal fluctuations in temperature are normal, especially in a Mediterranean climate such as that in Western Australia.

The data suggest that the opportunity to control disease development by the manipulation of canopy cover, understorey density and litter depth is a real possibility, especially since short-term fluctuations above critical values appear to have little effect on disease expression.

ACKNOWLEDGEMENT

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