Thermal Inactivation of Phytophthora cinnamomi for Control of Fraser Fir Root Rot

D. M. Benson

Assistant Professor, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607.
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ABSTRACT

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Thermal inactivation of two isolates of *Phytophthora cinnamomi* (from a root of Fraser fir and the other from a rhododendron root) was determined by exposing agar disks that contained mycelium of the isolates to hot water at various temperatures for different periods. The ED_{50} values (time required for 50% inactivation) were interpolated at 39 C as 26.3 and 51.7 min, and at 43 C as 2.7 and 3.3 min for the two isolates, respectively. Temperature coefficients (Q_{10}) were 304 and 970 for the Fraser fir and rhododendron isolates, respectively. Dormant seedlings of healthy Fraser fir tolerated hot water at 45 C for 120 min with only slight injury, but infected seedlings were severely injured by that treatment. Therapy of inoculated seedlings was successful at 45 C for 15 min if given within 14 days of inoculation. Therapeutic treatment of naturally-infected seedlings at 45 C for 15 to 120 min was unsuccessful.

Additional key words: thermotherapy, hot-water therapy.

Phytophthora cinnamomi Rands causes a root rot of Fraser fir [*Abies fraseri* (Pursh) Poir] which is a serious problem in production of this major Christmas tree species in North Carolina. Typically, nursery beds are direct-seeded and seedlings develop for 3 yr prior to being transplanted into new beds for an additional 2 yr. Then the seedlings are transplanted to the field and grown for an additional 7 to 8 yr to produce saleable Christmas trees. Fraser fir is susceptible to *P. cinnamomi* throughout the production period. In the nursery, control has been aimed at eliminating the inoculum through preplant fumigation since no therapeutic control measures are available once infection takes place. However, preplant fumigation does not give complete control due to variation in soil drainage and the long time seedlings are in the beds. A method of eradicating *P. cinnamomi* from seedlings in an early stage of disease development is needed.

Several diseases caused by *Pythium* spp. and *Phytophthora* spp. have been controlled by thermotherapy. For example, Pythium root rot of *Aloe variegata* L. was controlled by hot-water treatments at 46 C for 20-30 min (1), *Phytophthora richardiae* was inactivated in calla rhizomes (*Zantedeschia* sp.) by a 60-min soak at 50 C (4), *Phytophthora citrophthora* was eliminated from lemon fruits in 2 min at 48.9 C (2), and green tomato fruits required treatment at 60 C for 1.5 min to eliminate *P. parasitica* (7).

Recently, the merits of analyzing inactivation data in terms of the temperature coefficient (Q_{10}) (the ratio of activity at any temperature to the activity at a temperature 10 C lower) were pointed out (8). Once the Q_{10} value for inactivation of a particular pathogen has been determined, exposure time at selected temperatures can be calculated.

The purpose of this study was to determine thermal inactivation values for *P. cinnamomi* in culture, and the value of hot-water therapy for Fraser fir seedlings infected by this pathogen. A preliminary report has been presented (3).

MATERIALS AND METHODS

Laboratory studies.—Two isolates of *Phytophthora cinnamomi*, one (isolate 103) from Fraser fir and the second (isolate 114) from *Rhododendron* sp. were used. These isolates were obtained from infected plants in North Carolina. Agar disks (6-mm diameter) containing chlamydospores and hyphae were taken from the margins of 3- to 7-day-old cultures grown on V-8J agar at 25 C. Plastic centrifuge bottles, 6 × 12.5 cm, were perforated with >120, 4-mm-diameter holes to ensure rapid movement of water into the bottle. The bottles were maintained in separate 250-ml beakers of water at ambient temperature as twenty disks were added. The disks were treated in a circulating water bath (± 0.1 C) between 39 and 44 C and at times ranging from 2.0 to 90 min.

The bottles were plunged in an ice bath for 30 sec immediately after exposure and then placed back into the beakers of water at ambient temperature. The bottles were drained just before the agar disks were transferred to petri dishes containing V-8J agar. Inactivation of *P. cinnamomi* was based on the failure of the fungus to grow from the disks.

Greenhouse studies.—Five-yr-old Fraser fir seedlings were collected from the State Forest Service nursery at Crossnore, North Carolina, from beds infested with *P.*

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cinnamomi and from pathogen-free beds. Seedlings were stored no longer than 1-14 days in plastic bags at 4 C before treatment. For injury studies, trees from pathogen-free areas were collected during the dormant period (October to March) except in one case where trees which had broken dormancy (trees with new growth present) were used. Dormant seedlings were completely immersed whereas seedlings which had broken dormancy were treated by immersing only the roots in hot water. Seedlings were treated at 45 C (±0.5) for 15 and 120 min.

For therapy studies, either inoculated or naturally-infected seedlings were used. Seedlings from pathogen-free beds were potted individually in a fumigated medium of sand:soil:peat (1:1:1, v/v) in 15-cm-diameter pots and placed in the greenhouse. The growing medium was infested with oat grain cultures of *P. cinnamomi* 30 days after potting. Thirty oat grains were placed in each of three, 6-cm-deep holes spaced around the plant between the stem and edge of pot. Seedlings were removed 7, 14, and 28 days after infestation of the medium. The roots were dipped in water at ambient temperature to dislodge soil particles and then given hot-water therapy at 45 C for 15 min. After treatment, roots were cooled in water at ambient temperature and repotted in pathogen-free medium. There were six plants per treatment. Seedlings were observed for the next 2 mo for symptoms of Phytophthora root rot. Then root segments were placed on Eckert and Tsao's PPP medium (6) containing 10 mg pimaricin per liter to determine the number of plants infected by *P. cinnamomi*.

Naturally-infected seedlings were classified according to the stage of root rot development prior to treatment. Class I represented 0-30% of the root tips necrotic and no foliar symptoms, Class II represented 31-70% of the root tips necrotic and/or chlorotic foliage, and Class III represented 71-100% of the root tips necrotic and/or flagging of foliage. Four experiments were conducted using this classification for seedlings that had been exposed to hot water treatment for time periods ranging from 15 to 120 min. After exposure, seedlings were potted in the growing medium and placed in the greenhouse. For the next 2 to 4 mo seedlings were rated (disease severity index) for response to therapy as 1 = no visible foliar symptoms, 2 = visible symptoms ranging from chlorosis to flagging, and 3 = dead plant.

RESULTS

Laboratory studies.—Thermal inactivation of *P. cinnamomi* was inversely related to the exposure time and the treatment temperature. For instance, at 39 C the percent inactivation increased from 0% to 100% as exposure time was increased from 9 to 90 min for both isolates. Similarly, at 44 C, complete inactivation of both isolates occurred after 4.5 min. Isolate 114 was more tolerant of treatment at 39 and 43 C than was isolate 103.

Two temperatures (39 and 43 C) were selected for experiments designed to investigate more precisely the effects of time on the inactivation of each isolate. Exposure time ranged from 13.3 to 65.0 min and 2.0 to 5.0 min at 39 C and 43 C, respectively. Data from several trials were pooled and regression lines were constructed by plotting exposure time as a logarithmic function. Time required (dosage) for 50% inactivation (ED_{50}) at 39 C was 26.3 min and 51.7 min for isolates 103 and 114, respectively (Fig. 1). The ED_{50} dosages at 43 C were 2.7 min and 3.3 min for isolates 103 and 114, respectively. Slope of the regression lines for both isolates and temperatures was not different ($P = 0.05$) according to the analysis of covariance. Position of the regression lines was different. Isolate 114 was more tolerant of the dosage than isolate 103 at both temperatures since the displacement of the curve from the origin for isolate 114 was greater than that for isolate 103 as determined by analysis of covariance. Isolate 114 had a significantly greater growth rate on cornmeal agar than isolate 103 at 25 C (6.1 mm/day vs. 4.3 mm/day, respectively).

Values of ED_{50} at 39 and 43 C were used to calculate the Q_{10} for each isolate based on the formula presented by Yarwood (8). The Q_{10} was 304 for isolate 103 and 970 for isolate 114. The Q_{10} data were used to predict ED_{50} values at 41 C (8). Isolates 103 and 114 had predicted values for ED_{50} of 8.4 and 13.0 min, respectively, at 41 C. These mathematical predictions were confirmed by plotting the respective values for ED_{50} at 39 and 43 C for each isolate on semilogarithmic graph paper and fitting a straight line between the points to interpolate ED_{50} at 41 C.

The ED_{50} at 41 C also was obtained experimentally for both isolates. Isolate 103 had an observed ED_{50} of 8.2 min (0.2 min less than predicted) and isolate 114 had an observed ED_{50} of 11.1 min (1.9 min less than predicted).

Greenhouse studies.—Tolerance of Fraser fir seedlings to hot-water dips varied with stage of plant development and exposure time. For instance, after 60 min at 45 C injury was observed on 18% of the seedlings that had broken dormancy, even though only the roots had been treated. In contrast, dormant seedlings did not develop injury symptoms when completely submerged (Table 1). Usually buds died and/or needle necrosis appeared on dormant seedlings 1-3 days after exposure and root rot symptoms developed later. All seedlings that had broken dormancy developed injury symptoms whereas only 33% of the dormant trees were injured when exposed for 120 min at 45 C. However, after 120 min of exposure at 45 C naturally-infected dormant trees were as sensitive to

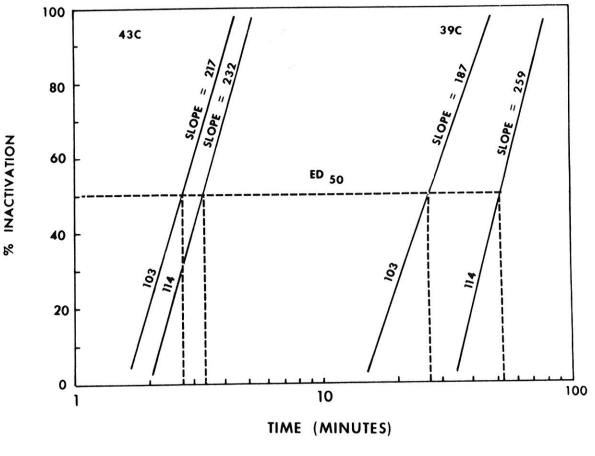

Fig. 1. Semilogarithmic plot of thermal inactivation data for *Phytophthora cinnamomi* isolates 103 and 114 at 39 and 43 C. All correlation coefficients were statistically significant, $P \leq 0.01$.

injury (81%) as healthy trees that had broken dormancy.

Survival of Fraser fir seedlings inoculated with *P. cinnamomi* was 67% if given therapy (45 C, 15 min) at either 7 or 14 days after inoculation. The effectiveness of therapy was demonstrated when the fungus could not be isolated from any of the surviving inoculated plants 50 days after exposure. All nontreated inoculated (control) plants died of root rot. No seedlings survived that were treated 28 days after inoculation.

Therapy of naturally-infected seedlings at 45 C for 15 to 120 min was not sufficient to eradicate *P. cinnamomi* from the roots except for one treatment (Table 2). After 60 min at 45 C fewer seedlings in infection Class III had symptoms than did controls. However, increasing exposure time to 120 min was ineffective.

DISCUSSION

The ED_{50} values determined in the laboratory experiments might have been somewhat lower if hyphae and chlamydospores had been exposed free of the agar substrate. However, the small size of the agar disks (6 mm diameter) should allow a rapid equilibration with water bath temperature. In preliminary studies, test tube agar cultures took several minutes to equilibrate. This was undesirable because of the unknown effect of elevated temperatures on inactivation prior to equilibration.

The inactivation curves for isolates 103 and 114 were parallel at 39 and 43 C. Therefore, the mechanism of inactivation in these two isolates should be similar if inactivation dosage-response relations are analogous to fungicide dosage-response relations (5).

The variation in ED_{50} values and consequently Q_{10} values between *P. cinnamomi* isolates was unexpected. Yarwood found a five-fold variation in Q_{10} for tobacco mosaic virus when compared to earlier data [see Table 2 in (8)]. With the variation in ED_{50} values, the Q_{10} value for isolate 114 was slightly more than three times greater than the value for isolate 103. Rate of growth in culture was significantly greater for isolate 114 but the relationship of growth rate to inactivation is not known. It seems unlikely that ED_{50} values can be predicted for closely related fungal species since the variation within species was so great.

The comparison of predicted with observed ED_{50} values at 41 C demonstrated that prediction was most accurate when Q_{10} value was relatively low (isolate 103, $Q_{10} = 304$) rather than when it was several-fold higher (isolate 114, $Q_{10} = 970$). The usefulness of predicting ED_{50} for *P. cinnamomi* at other temperatures may lie in the practical application of hot-water therapy to other species of infected-nursery stock of temperatures other than those studied here.

The therapeutic index (TI) is the dose for injury to the host divided by the dosage for therapy of the pathogen (8). The dose for 81% injury in naturally-infected Fraser fir seedlings was 120 at 45 C (Table 1). The dosage for 81% therapy of isolate 103 at 45 C was 1.6 min (data not given); thus the TI is 75. At 45 C the relatively high TI compared to the 9.8 value given for therapy of bean rust (8) means a greater margin of safety in therapeutic treatments for Fraser fir.

Unfortunately, a serious limit to the use of hot-water therapy for Fraser fir seedlings and other woody plants infected with *P. cinnamomi* is the variability of root size, the type of fungal structure(s) present, and the stage of disease development. In the studies with inoculated seedlings, therapy was successful if given within 14 days of inoculation. Since a 15 min exposure at 45 C was therapeutic, it is likely that the pathogen still was limited to the smaller roots. Once the pathogen had invaded the larger roots, as in the naturally-infected fir seedlings,

TABLE 1. Tolerance of Fraser fir seedlings to hot water treatment at 45 C for various time intervals

Time (min)	Seedlings with injury symptoms[a]		
	Active, healthy[b] (%)	Dormant, healthy (%)	Dormant, infected (%)
5	...	0	...
30	0	0	...
60	18	0	...
120	100	33	81
180	100	18	...

[a]Injury symptoms varied in severity from a killing of buds to necrosis of the needles.

[b]Seedlings were considered active if buds had broken and new growth was present. Only roots of actively growing seedlings were immersed. Entire dormant trees were immersed.

[c]Three-dot leader indicates the treatment was not tested.

TABLE 2. Disease severity index for Fraser fir seedlings naturally infected with *Phytophthora cinnamomi* and treated with hot-water at 45 C for 15-120 min

Class[c]	Disease severity index 2 or 4 mo after treatment[a]							
	Experiment I		Experiment[b] II		Experiment III		Experiment IV	
	Control	15 min	Control	60 min	Control	90 min	Control	120 min
I	1.7	1.3	1.6	1.2	1.7	n.d.	1.6	1.9
II	2.2	1.9	1.4	1.4	3.0	2.3	1.8	2.1
III	3.0	2.9	1.8	1.0*[d]	3.0	2.5	2.8	2.6

[a]Disease severity indices were determined after 2 mo in experiment I and III and after 4 mo in experiment II and IV, after hot-water treatment. Disease severity ratings were 1 = no foliar symptoms, 2 = foliar symptoms or root rot, 3 = dead seedling.

[b]Seedlings in this experiment were collected during a period of active growth, so only roots were immersed.

[c]Seedling infection classes prior to treatment were, I = slight root rot, 0-30% of root tips necrotic, no foliar symptoms; II = moderate root rot, 31-70% of root tips necrotic, chlorotic folage; III = severe root rot, 71-100% of root tips necrotic, flagging of foliage evident.

[d]The starred mean was significantly different from the control at $P = 0.05$.

injury had occurred before the pathogen was inactivated. Under nursery management practices, the variation in extent of disease development might make therapy impractical on a large-scale basis.

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