Invasion of Phloem and Xylem of Woody Stems and Roots of *Eucalyptus marginata* and *Pinus radiata* by *Phytophthora cinnamomi*

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ABSTRACT

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Woody stems and roots of field-grown *Pinus radiata* (pine) and *Eucalyptus marginata* (jarrah) were inoculated with *Phytophthora cinnamomi*, and the progress of invasion was investigated by sampling wood and bark 1, 3, 6, and, in some cases, 12 wk after inoculation. *P. cinnamomi* rapidly invaded phloem and xylem of both species, and a hemibiotrophic infection developed in the phloem. In jarrah and in some pine stem and root samples, an inapparent infection was established in the xylem, but in other pine samples, invaded wood became dry and/or resin soaked. This fungus persisted longer in and was more frequently isolated from wood than from bark. In jarrah, xylem invasion was confined to a narrow band adjacent to the cambium, whereas in pine wood radial invasion was more extensive. In some pine stems, a segment of nonconducting wood formed adjacent to the inoculation point; this did not occur in jarrah. Tissue invasion in both species was less extensive after inoculation during autumn than during spring. In all experiments, invasion was restricted by host responses to infection.

Additional keywords: jarrah dieback, Monterey pine.

Jarrah dieback, caused by *Phytophthora cinnamomi* Rands, is the most important disease of the jarrah (*Eucalyptus marginata* Donn ex Smith) forest of Western Australia (18). About 14% of the forest area is infested, and such sites are recognized by mortality of many midstorey and understorey plants (9). Although some species are completely eliminated from infested areas, jarrah deaths are sporadic and site specific. Symptoms in jarrah include sudden wilting and death of groups of trees extending over several hectares, wilting and death of scattered individuals, or chronic crown decline (18). However, the estimated mortality rate is low, less than 0.05 trees per hectare per year (9).

Several hypotheses have been proposed to explain how and why jarrah dies on infested sites. Podger (18) isolated *P. cinnamomi* from soil, fine roots, root phloem, and wood of lignotuberous jarrah seedlings, and he proposed that death resulted from fine-root necrosis (17). Shea et al (20,21), however, proposed that wilting and death were the result of reduced water movement through infected roots, because they isolated *P. cinnamomi* from both bark and wood from root-collar cankers and cankers on major horizontal and vertical roots. When mature stems and roots were wound inoculated, necrotic lesions formed in the phloem but were rapidly confined by necrophylactic periderms; if xylem discoloration occurred, it occurred several months after cankers formed in the bark (27).

Some of the poor quality, wetter areas of the jarrah forest were cleared for *Pinus radiata* D. Don (pine) plantations in the mid- and late-1970s. Many of these sites were infested with *P. cinnamomi*, and pine deaths have occurred, especially in young plantations. Foliage wilts, become chlorotic, and reddens. Deaths in the first year after planting can be as high as 170 per hectare per year, but mortality rates decrease rapidly to about two trees per hectare per year at 5 yr. Mortalities of 18 trees per hectare per year have occurred after culling and pruning in a 6-yr-old stand (5).

When *P. cinnamomi* infects pine seedlings, it causes a root and collar rot (12); however, Newhook (15) suggested that death of mature pine results from fine-root necrosis. When investigating mortality of pine shelterbelts, he did not find cankers on major roots and at the root collar but recovered *Phytophthora* spp.

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from both soil and fine roots. Stukely et al (24), however, concluded that collar infections do occur, because they isolated *P. cinnamomi* and *P. cryptogea* from bark and wood at the root collar and in major roots of dying 5- to 9-yr-old plantation-grown trees. In this study, we used a time course to follow axial and radial invasion of *P. cinnamomi* in woody stems and roots of jarrah and pine to determine when xylem is invaded in relation to phloem invasion; whether this fungus can persist in xylem; how xylem responds to invasion; and whether conduction is reduced adjacent to necrotic lesions.

MATERIALS AND METHODS

Fungal isolates and production of inoculum. Two A_2 isolates of *P. cinnamomi* were used in different experiments. DCE 60 was isolated from *Hibbertia subvaginata* in 1965, and DCE 230 (DAR 53101) was isolated from *E. marginata* in 1981. Inoculum was produced in two ways. A 5-mm disk cut from the margin of a 12-day-old culture on half-strength potato-dextrose agar (1/2 PDA) (19.5 g of PDA, 7.5 g of agar, 1 L of distilled water) was used in experiments 4 and 7. A Miracloth (Chicopee Mills, NY) disk inoculum was used in the other experiments. Sterile 6-mm-diameter Miracloth disks were placed on pea-agar (200 g of macerated frozen peas, 15 g of agar, 1 L of distilled water) cultures adjacent to the margin of a developing colony. Plates were incubated at 25 C for 7 days, and the colonized disks were removed aseptically and stored in sterile distilled water for no more than 1 day before use. A 5-mm disk of 1/2 PDA (experiment 1) or a noncolonized, sterile Miracloth disk served as the controls.

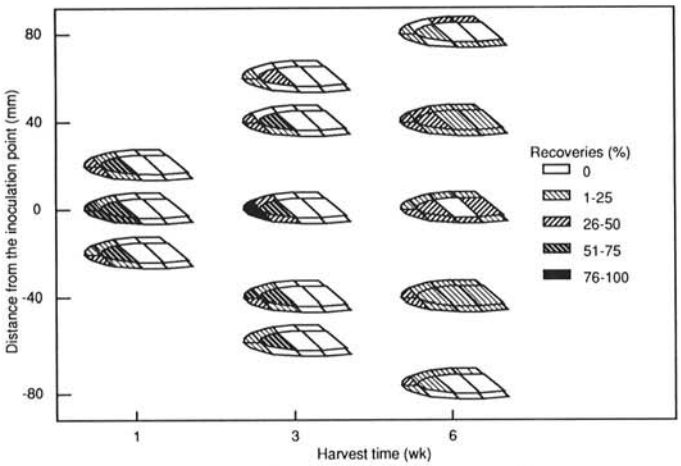

Fig. 1. Recovery of *Phytophthora cinnamomi* from *Eucalyptus marginata* roots inoculated during spring (experiment 1). Disks were cut from roots at fixed distances from the inoculation point. Each disk was cut into 5-mm-wide strips, and each strip was separated into bark and wood. There were eight replicates at each harvest.

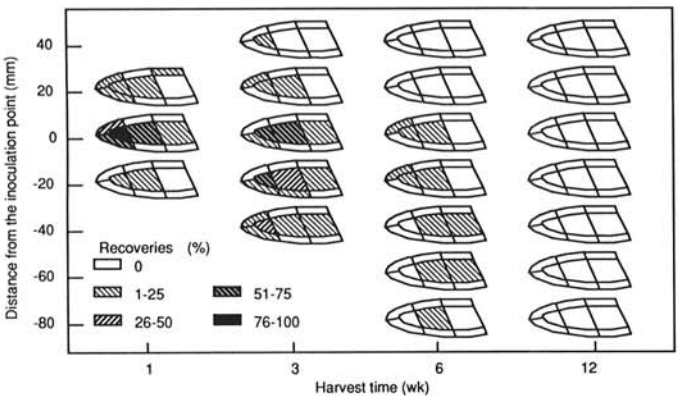

Fig. 2. Recovery of *Phytophthora cinnamomi* from *Pinus radiata* stems inoculated during spring (experiment 4). Disks were cut from stems at fixed intervals above and below the inoculation point. Each disk was cut into 5-mm-wide strips, and each strip was separated into bark and wood. There were no recoveries from disks cut above the inoculation point at the 6- and 12-wk harvests. There were 16 replicates at each harvest.

Plant material and inoculation procedure. Roots of jarrah saplings growing in a native forest at Chandler Block, Jarrahdale, were inoculated during the spring (experiment 1) or stems and roots of similar trees at the same site were inoculated during the autumn (experiments 2 and 3). Inoculated roots and stems were secondarily thickened and were at least 15 mm in diameter. Stems of 2- and 3-yr-old clonal pines growing at Nannup Nursery were inoculated during spring (clones 118 and 162, experiment 4) and autumn (clone 162, experiment 5), and roots of self-sown pines in Folly Plantation, Nannup, were inoculated during autumn (experiment 6).

Roots for inoculation were exposed by removing surface soil. Two inoculation techniques were used. In experiment 1, a hole was drilled through the bark to the cambium of the root with a sterile, 6-mm drill bit. A 5-mm-diameter colonized or noncolonized agar inoculum disk was placed in the hole, sealed with petroleum jelly, and covered with plastic flagging tape. One *P. cinnamomi* and one control inoculation were made on the same root; the *P. cinnamomi* inoculation was always at least 15 cm distal to the control. Inoculated roots were covered with polythene sheeting and buried to a depth of 10 cm. Roots were harvested after 1, 3, or 6 wk; eight roots were excavated at each harvest time. In experiments 2–6, a sterile scalpel was used to cut a flap about 8 mm wide and 8 mm long through the outer bark into live phloem. A colonized or noncolonized Miracloth disk was inserted under the flap, the cut sealed with petroleum jelly, and covered with plastic flagging tape. In experiment 4, there was a single inoculation (either *P. cinnamomi* or control) on each stem, in all other experiments one control and one *P. cinnamomi* inoculation were made on each stem or root and treated as described above. Eight stems and eight roots were harvested after 1, 3, 6, and 12 wk, except in experiment 6 when only seven roots were harvested at 6 wk.

Soil and air temperatures were measured for experiments 2, 3, 5, and 6 with thermistors buried at 10 cm or fixed at 1 m above ground. In experiments 5 and 6, radial growth of DCE 230 at either a depth of 10 cm or at 1 m above the ground was measured by inoculating 9 cm of Difco (Difco Laboratories, Detroit, MI) cornmeal agar plates on one side with a 5-mm agar plug and marking the colony margin on the base of the plate after 1 and 3 wk.

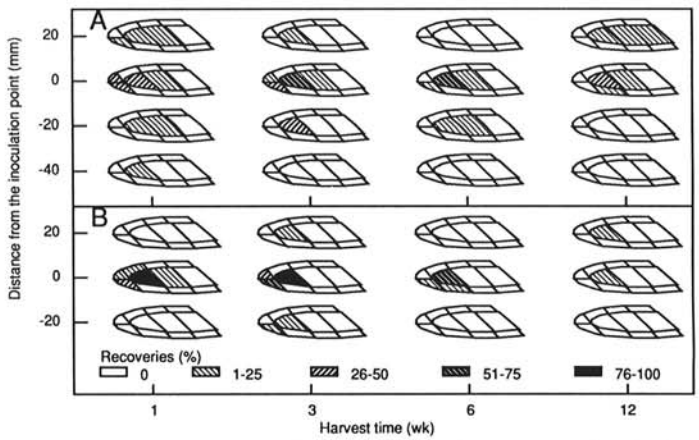

Fig. 3. Recovery of *Phytophthora cinnamomi* from *Pinus radiata* in **A,** stems (experiment 5) and **B,** roots (experiment 6) inoculated during autumn. Disks were cut from stems and roots at fixed intervals above and below the inoculation point. Each disk was cut into 5-mm-wide strips, and each strip was separated into bark and wood. There were no recoveries from disks cut above or below those illustrated. There were eight replicates at the 1-, 3-, and 12-wk harvests, but only seven replicates at the 6-wk harvest.

TABLE 1. Linear regressions of lesion length (millimeters) against time (days) for stems and roots of *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*

Experiment	Inoculation site	Inoculum	*n*	Intercept	Slope	*t*	*P* (slope ≠ 0)	r^2
1 (spring)	Root	*P. cinnamomi*	24	18.90	3.09	5.11	0.000	0.54
		Control	23	3.86	0.30	3.53	0.002	0.37
2 (autumn)	Stem	*P. cinnamomi*	32	21.48	0.08	0.39	0.696	0.01
		Control	32	1.07	0.32	4.27	0.000	0.38
3 (autumn)	Root	*P. cinnamomi*	32	15.91	0.33	2.16	0.039	0.13
		Control	32	7.02	0.11	2.03	0.051	0.12

Harvesting. Harvested organs were cut off proximal to the inoculation point and were returned to the laboratory. Dead outer bark around the inoculation point was removed, and the length and breadth of discolored tissue was measured. In experiments with the Miracloth disk, the disk was removed and plated onto $P_{10}VP$ agar containing 50 mg of hymexazol/L ($P_{10}VPH$) (25). A vertical line was drawn on the stem or root through the inoculation point, so disks cut from the organ could be oriented in relation to one another. Using a band saw, the organ was cut into disks 2–3 mm thick at the inoculation point and at set distances above and below the inoculation point. Each disk was cut into 5-mm-wide tangential strips, starting from the inoculation point or the corresponding side of disks above or below the inoculation point. Live inner bark and wood from each strip were separated and plated onto $P_{10}VPH$ agar without surface-sterilization. The exact arrangement of these strips is shown in Figures 1–3. Plates were incubated at 20 C for up to 7 days, at which time each strip was examined for the distinctive coralloid hyphae of *P. cinnamomi*. The presence or absence of *P. cinnamomi* was recorded for each piece of tissue.

Effect of inoculation on water movement through stems. When sapwood is damaged or invaded by a pathogen, host responses include the formation of nonconducting wood around the damaged or invaded area (7,22). Dye movement in stems is one way to demonstrate nonconducting zones in wood (14,16).

During autumn, 16 stems each of jarrah and pine (clone 162) were inoculated with Miracloth colonized or noncolonized with *P. cinnamomi*. Four inoculated and four control stems were cut after 1, 3, 6, and 12 wk, placed immediately in a bucket of 0.01% phloxine, and left for at least 1 day. After this time, stems were cut transversely at the inoculation point and at 2-cm intervals above and below the inoculation point, and any unstained xylem was recorded. Preliminary experiments with eucalypts showed that dilute phloxine was taken up by the xylem and confined to that tissue.

Histology. A block of tissue either immediately above or below the inoculation point was fixed and stored in formal-acetic-alcohol. Radial longitudinal hand sections were cut from this material in experiments 1 and 4. A sledge microtome was used to cut 40-μm-thick transverse sections of tissue close to the inoculation point in the other experiments. Transverse sections were stained with Melzer's iodine (1).

Statistical analysis. SYSTAT was used to calculate linear regressions of lesion lengths and colony diameters against time; slopes were compared by the homogeneity of slopes test (29).

RESULTS

Visible lesions in jarrah stems and roots. Jarrah stems and roots inoculated with *P. cinnamomi* developed necrotic lesions in the phloem within 1 wk. No symptoms were observed in the wood at any harvest. A fresh kino vein was present at the cambium of at least half the roots inoculated during spring (experiment 1) and the stems inoculated during autumn (experiment 2) within 6 wk. A kino vein had formed in one control inoculation (12-wk harvest, experiment 2). In the spring inoculation (experiment 1), lesion length increased with time, and the rate of increase was significantly greater than it was in the control (Tables 1 and 2). In autumn inoculations, root lesions, but not stem lesions, increased in length with time (Table 1). They did not differ from the control (Tables 1 and 2).

TABLE 2. Implied contrasts between lesion lengths for stems and roots of *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*

Experiment	Contrasts	df	*F*	*P*
1	*P. cinnamomi* vs. control	1	20.47	0.000
2	*P. cinnamomi* stem vs. control stem	1	1.25	0.268
3	*P. cinnamomi* root vs. control root	1	1.91	0.173
2 and 3	*P. cinnamomi* stem vs. *P. cinnamomi* root	1	0.96	0.330
	Control stem vs. control root	1	5.50	0.022

Reisolation of *P. cinnamomi* from jarrah. *P. cinnamomi* persisted in inoculum disks for at least 3 wk in stems (experiment 2) and for at least 1 wk in roots (experiment 3). At 1 wk, the pathogen was recovered from all inoculated stems (experiment 2) and from 13 of 16 inoculated roots (experiments 1 and 3). Combining harvests at 1 and 3 wk for autumn inoculations, *P. cinnamomi* was recovered from the inoculum disk but not from adjacent tissue in four of 32 samples.

P. cinnamomi was isolated from both bark and wood in both spring and autumn inoculations. Although it was isolated from many pieces of wood (Fig. 1), growth on agar was sparse and came only from a narrow strip adjacent to the cambium. It was isolated from the wood of 64% of the samples in which a kino vein had formed in the cambium. Tissue invasion was less extensive in autumn inoculations than in spring inoculations.

In spring (experiment 1), *P. cinnamomi* was recovered from symptomless tissue at least 10 mm in front of the lesion margin. Combining data from all spring harvests, it was isolated from 58% of the samples (bark and/or wood) taken in advance of the necrotic phloem lesion. In autumn (experiments 2 and 3), although lesions were smaller, the pathogen was reisolated from in front of the lesion margin in 22 and 32% of stems and roots, respectively.

Effect of infection on water movement in jarrah stems. Jarrah xylem stained uniformly with phloxine. There were no areas of unstained xylem adjacent to the inoculation point at any harvest. A fresh kino vein was present in the cambium of all stems inoculated with *P. cinnamomi* after 6 and 12 wk.

Histology of infected jarrah xylem. Hyphae were rarely seen in inoculated stems and roots. They were seen in the cambium, adjacent ray parenchyma and xylem vessels in roots (experiment 1), in ray cells and xylem vessels close to the cambium in one stem (experiment 2), and in one xylem vessel close to the cambium in one root (experiment 3).

Transverse sections of all stems 1 wk after inoculation (experiment 2) showed that ray cells had dark contents and very little starch. At this time, the cambium was active, indicated by adjacent thin-walled cells, in at least half the stems from the two treatments. After 3, 6, and 12 wk, a portion of the cambium was brown and had collapsed in eight of 21 inoculated stems, whereas this occurred in only one of 22 controls. A barrier zone with associated kino vein formed after 6 wk in some stems inoculated with *P. cinnamomi*; where a barrier zone formed in control stems, it was not as extensive.

After 1 wk, axial parenchyma cells were inconspicuous because they had neither dense contents nor starch. After 6 and 12 wk

TABLE 3. Linear regressions of lesion length (millimeters) against time (days) for stems and roots of *Pinus radiata* inoculated with *Phytophthora cinnamomi* and for *P. cinnamomi* colony diameter (millimeters) against time (days) on agar plates maintained under conditions similar to inoculated stems and roots

Experiment	Inoculation site	Inoculum	n	Intercept	Slope	t	P (slope $\neq 0$)	r^2
4 (spring)	Stem, clone 118	*P. cinnamomi*	32	−2.43	0.77	8.92	0.000	0.73
		Control	32	0.06	0.23	6.22	0.000	0.56
	Stem, clone 162	*P. cinnamomi*	32	3.61	0.74	7.05	0.000	0.62
		Control	32	2.61	0.21	8.55	0.000	0.71
	Stem, clones 118 and 162	*P. cinnamomi*	64	0.59	0.76	11.15	0.000	0.67
		Control	64	1.33	0.22	9.91	0.000	0.61
5 (autumn)	Stem, clone 163	*P. cinnamomi*	32	9.41	0.03	1.09	0.285	0.04
		Control	32	6.77	0.05	3.97	0.000	0.34
	Agar plate	*P. cinnamomi*	12	5.50	2.87	10.65	0.000	0.88
6 (autumn)	Root	*P. cinnamomi*	31	4.89	0.31	2.71	0.011	0.20
		Control	32	9.06	−0.02	−0.81	0.422	0.02
	Agar plate	*P. cinnamomi*	12	4.86	3.46	21.66	0.000	0.97

TABLE 4. Implied contrasts between lesion lengths for stems and roots of *Pinus radiata* inoculated with *Phytophthora cinnamomi* and colony diameter on agar plates maintained under conditions similar to those for inoculated stems and roots

Experiment	Contrasts	df	F	P
4	*P. cinnamomi* clone 118 vs. *P. cinnamomi* clone 162	1	0.04	0.838
	Control clone 118 vs. control clone 162	1	0.11	0.743
	P. cinnamomi clones 118 and 162 vs. control clones 118 and 162	1	56.99	0.000
5	*P. cinnamomi* stem vs. control stem	1	0.10	0.754
	P. cinnamomi stem vs. agar	1	198.45	0.000
6	*P. cinnamomi* root vs. control root	1	8.10	0.006
	P. cinnamomi root vs. agar	1	55.03	0.000
5 and 6	*P. cinnamomi* stem vs. *P. cinnamomi* root	1	5.40	0.024
	Control stem vs. control root	1	4.60	0.036

in inoculated stems, some had dark contents. In both inoculated and control stems, there were some vessels close to the cambium with either tyloses or brown contents. These appeared to be more abundant in inoculated than control stems at 3, 6, and 12 wk. Transverse sections of inoculated roots (experiment 3) showed similar changes to those that occurred in stems, except that axial parenchyma cells were more obvious and more abundant in root xylem than in stem xylem.

Visible lesions in pine stems and roots. Lesion lengths increased at a similar rate on clones 118 and 162 (experiment 4) (Table 3); therefore, results from these two clones were combined for analysis and presentation. Pine stems and roots inoculated with *P. cinnamomi* developed watersoaked lesions in the phloem of some replicates within 1 wk and brown, necrotic lesions within 3 wk. A segment of resin-soaked or dry wood had formed internal to the inoculation point in eight of 32 samples within 1 wk and in 16 of 32 inoculations within 3 wk. Resin-soaked or dry wood was formed in two controls.

In spring inoculations (experiment 4), the length of necrotic lesions increased with time, but in autumn inoculations, this either did not occur or was less pronounced (Tables 3 and 4). The extension of stem lesions was significantly greater than in controls in spring (experiment 4) but not in autumn (experiment 5), whereas extension of root lesions was greater than the control (experiment 6) (Tables 3 and 4). In the autumn inoculations, root lesions increased in length more rapidly than did stem lesions (Tables 3 and 4).

Necrotic lesions formed in autumn were smaller than colony diameters on agar (Tables 3 and 4). In autumn, mean daily soil and air temperatures during the course of experiments 2 and 3 ranged from 11.3 to 17.7 C and 6.1 to 23.1 C, respectively.

Reisolation of *P. cinnamomi* from pine stems and roots. *P. cinnamomi* persisted in inoculum disks for at least 1 wk in stems (experiments 4 and 5) and for at least 3 wk in roots (experiment 6). It was recovered from 26 of 32 inoculated stems and roots after 1 wk, and in four additional stems and roots it was reisolated from the inoculum disk but not from adjacent tissue.

P. cinnamomi was isolated from both bark and wood 1, 3, and 6 wk after inoculation (Figs. 2 and 3). Within 1 wk in spring (experiment 4), *P. cinnamomi* had spread up to 15 mm radially across the wood (Fig. 2). It was recovered more frequently from wood than from bark after 1, 3, and 6 wk but was not recovered from either tissue at 12 wk (Fig. 2). In autumn inoculations (experiments 5 and 6), *P. cinnamomi* had invaded both bark and wood of stems and roots within 1 wk (Fig. 3A and B), but invasion in both tissues was less extensive than in spring. Radial invasion of wood was less in roots than in stems (Fig. 3A and B). The fungus was recovered from resin-soaked and dry wood as well as from symptomless wood.

In spring (experiment 4), *P. cinnamomi* was isolated from symptomless bark and wood at least 10 mm in front of the lesion margin. Invasion was initially in both tissues, but after 3 wk, in 11 of 16 stems, it was recovered only from wood. In stems in autumn (experiment 5), even though lesions were smaller and tissue was less extensively invaded, the fungus also was recovered in advance of the lesion front in 14% of all samples; recoveries were mainly from wood.

Effect of infection on water movement in pine stems. Pine xylem stained uniformly with phloxine. A wedge of unstained xylem internal to the inoculation point was formed in one inoculated stem after 1 wk and in half the inoculated stems in subsequent harvests. A wedge of unstained xylem formed in only one of the control inoculations.

Histology of infected pine xylem. Radial longitudinal hand sections from preliminary experiments showed hyphae growing radially in rays and axially in tracheids. Hyphae were infrequent. Transverse sections of stems 1 wk after inoculation (experiment 5) showed that the cambium was active, indicated by adjacent thin-walled cells with degenerating contents, and there was very little starch in ray and axial parenchyma (Fig. 4A). Starch was more abundant in these cells 3, 6, and 12 wk after inoculation than after 1 wk (Fig. 4B). It was noticeably absent from ray cells adjacent to resin-filled tracheids.

A barrier zone of starch-rich parenchyma cells had started to form in one inoculated and five control stems after 3 wk and was present in seven of eight inoculated and all control stems after 12 wk (Fig. 5A). The barrier zone in controls was narrower than that formed in inoculated stems (Fig. 5B). Resin was formed within 3 wk in tracheids in 11 of 24 inoculated stems but was not present in the control. These resin-filled tracheids often were associated with ray parenchyma, not resin ducts, and often were some distance internal to the point of inoculation.

Transverse sections of roots 1 wk after inoculation (experiment 6) showed that the cambium was inactive. In control roots, starch was present in ray and axial parenchyma, whereas in four inoculated roots resin was present in tracheids. Starch was noticeably absent from ray cells adjacent to resin-filled tracheids. Resin

formed earlier and more abundantly in roots than in stems, and as in stems, resin-filled tracheids often were associated with ray parenchyma and often were several millimeters internal to the inoculation point. Tracheids close to those containing resin had aspirated pits.

In many of the roots, the sections broke up at the cambium, so it was impossible to determine the timing and extent of barrier-zone formation. In sections where it could be recognized, the barrier zone was narrow, often consisting of an incomplete zone of resin ducts and associated starch-rich parenchyma.

DISCUSSION

Our results show that after wound inoculation of woody stems and roots of jarrah and pine with *P. cinnamomi*, the fungus can be isolated from symptomless phloem well in advance of the necrotic lesion. From macroscopic symptoms, infection is hemibiotrophic, because it is initially biotrophic but becomes necrotrophic with time (2). Recoveries from phloem were initially high but decreased over time, indicating that *P. cinnamomi* was either dying out or had become dormant. In addition to invading phloem, the fungus rapidly invaded adjacent xylem, in which it persisted longer and from which it was more frequently isolated. Invaded jarrah xylem in both stems and roots showed no symptoms of pathogenic invasion, whereas in some pine stems and roots invaded xylem became dry and/or resin-soaked.

In jarrah, the region of invaded xylem was limited. Radial spread was confined to a narrow band of sapwood immediately internal to the cambium, where *P. cinnamomi* persisted for at least 3 mo. A nonconducting zone was not formed in the wood adjacent to the necrotic lesion during this time. A barrier zone develops where the cambium is damaged. In eucalypts, polyphenols accumulate in these traumatic parenchyma. The cells collapse, and their released contents (kino) become a kino vein (23). In jarrah, kino veins are included in the sapwood (26), so if *P. cinnamomi* persists as a latent infection in stems and roots, it will be confined to a narrow band of invaded wood immediately internal to a barrier zone. Tippett et al (27) found that *P. cinnamomi* could persist in inoculated woody jarrah stems and roots for at least 1 yr; they assumed that it persisted in secondary phloem, not wood. In some instances, they observed a sudden increase in the size of phloem lesions, although they were unable to predict when or why this happened. Neither their observations nor our work indicates when extensive xylem invasion, as seen in recently dead trees, occurs (11,20,21).

In pine, radial invasion of xylem was greater than in jarrah (Figs. 2 and 3) but did not continue to extend after 3 wk. In some samples, host responses included the formation of nonconducting wood, indicated by dye movement; cavitation of tracheids, indicated by the formation of aspirated pits; and dry zones and resin-soaked wood. *P. cinnamomi* could be isolated from symptomless as well as symptomatic wood. It may not persist in pine wood for more than 3 mo.

The sites on which jarrah and pine grow may affect invasion. When woody plants are subjected to environmental stress, there is considerable evidence that host responses, such as initiation of periderm, are delayed (13), resin production in pines is reduced (28), and tissues that would normally be resistant to invasion can become vigorously colonized (6). Sites where pines die are seasonally wet, trees are shallowly rooted, and windthrow occurs. Similarly, sites where jarrahs die are poorly drained or water-gaining. Deaths occur either after prolonged, heavy winter rainfall (e.g., Dwellingup, Western Australia, rainfall June–August 1964

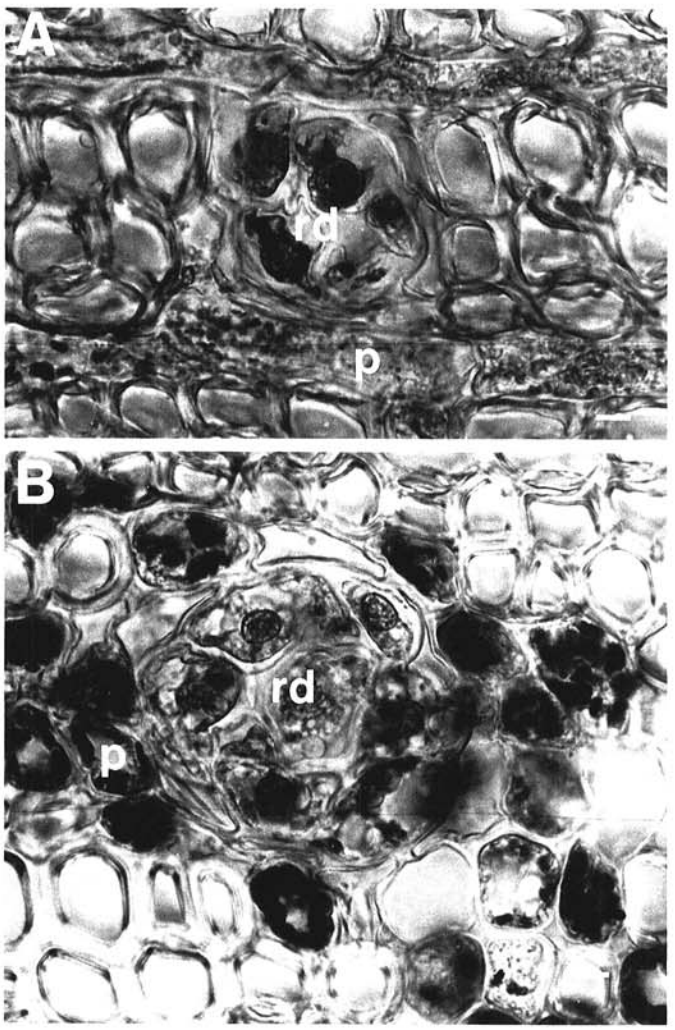

Fig. 4. Transverse sections of *Phytophthora cinnamomi*-inoculated *Pinus radiata* stems stained with iodine (experiment 5) **A,** after 1 wk and **B,** after 6 wk. Scale line represents 10 μm. Abbreviations: rd = resin duct, p = parenchyma cells.

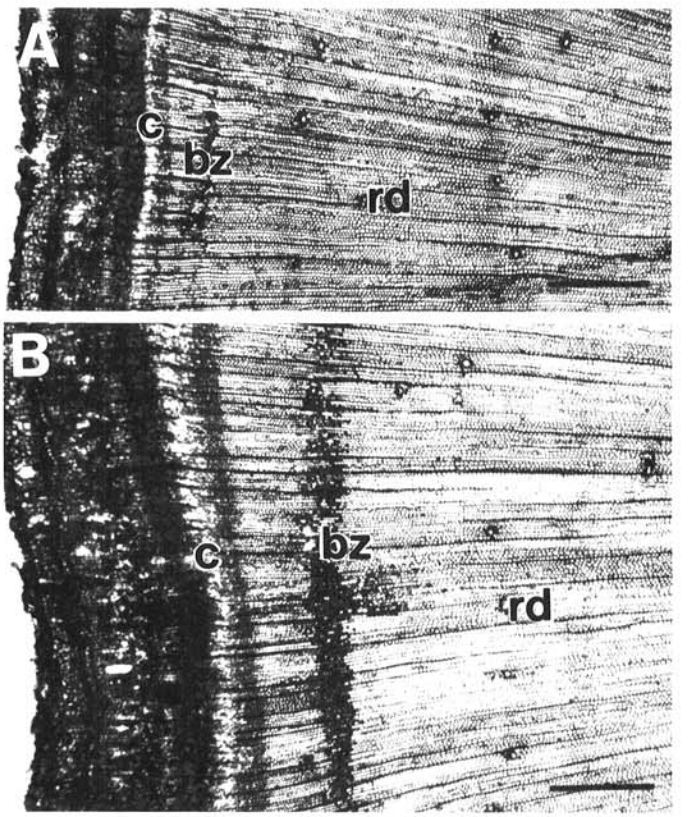

Fig. 5. Transverse sections of either **A,** control or **B,** *Phytophthora cinnamomi*-inoculated *Pinus radiata* stems stained with iodine (experiment 5). Scale line represents 1 mm. Abbreviations: bz = barrier zone; c = cambium; rd = resin duct and associated axial parenchyma.

was 1,397 mm, the average is 713 mm) or after cyclonic summer rainfall (e.g., Dwellingup, rainfall from 21 to 23 January 1982 was 228 mm, the January average is 17 mm). These sites remain wetter longer because much of the understorey vegetation is killed due to diseases caused by *P. cinnamomi*. Short-term waterlogging by itself resulted in sufficient xylem dysfunction in glasshouse-grown jarrah seedlings to cause wilting and death (10). Waterlogging also affects height and survival of glasshouse-grown pine (3). Thus, waterlogging may play an important role in field sites by suppressing the normal host responses to infection, so xylem invasion by *P. cinnamomi* may become more extensive.

In jarrah trees growing on infested sites, field sampling of roots has shown that root and root-collar lesions caused by *P. cinnamomi* are infrequent (8,19), indeed they were not found in early investigations (Harding, J. H. 1949. Pathogenic aspects of dieback in the jarrah forest of Western Australia. Aust. For. Conf. CALM Library, Perth, W.A.; and Stahl, W., and Greaves, R. 1959. Report on a field trip to Dwellingup, Western Australia, from 7.9.59 to 20.9.59, to investigate die-back in jarrah. CALM Library, Perth, W.A.). In attempts to reconcile how tree death can result from very limited infections, physiological explanations have been advanced by several workers. Cahill et al (4) found that the concentrations of zeatin- and isopentenyladenine-type cytokinins were reduced in xylem sap of jarrah seedlings after root infection by *P. cinnamomi*, and they proposed that this resulted in reduced water transport, wilting, and death. Woodward et al (30) suggested that wilting and tree death may be caused by a toxin, because they found that *P. cinnamomi* produced a β-glucan in culture that induced wilting in *E. sieberi* seedlings. Neither of these findings has been tested in the field.

Any explanation of how these trees die must address questions of effects on water movement, how and when extensive invasion of xylem occurs, and which site and weather conditions predispose both jarrah and pine to invasion by *P. cinnamomi*.

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