# Cellular and Histological Changes Induced by *Phytophthora cinnamomi* in a Group of Plant Species Ranging from Fully Susceptible to Fully Resistant

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## ABSTRACT

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The pattern of invasion and the histological changes in primary root tissues after infection by *Phytophthora cinnamomi* are described from the following species: *Xanthorrhoea australis, X. resinosa, Themeda australis, Eucalyptus marginata, E. sieberi*, and *Acacia melanoxylon* (susceptible); *A. pulchella, E. calophylla, E. maculata, Gahnia radula, Juncus bufonius, Zea mays*, and *Triticum aestivum* var. *cappelle* (resistant). Zoospores germinated on and penetrated the roots of all species, and lesions formed within 8–16 hr after invasion at 20–24 C. Root growth ceased within 24 hr of inoculation but resumed within 48 hr in resistant species, usually from a lateral branch. In susceptible species, progressive symptom development included water soaking of tissues, lesion extension through the root to the hypocotyl, and root death. This was accompanied by wilting and chlorosis of the leaves, die-back of shoots, and plant death. Sporulation occurred between 24 and 72 hr after inoculation on all but the most resistant species. In resistant species, lesions were contained well before they extended to the hypocotyl. Deposition of phenolic materials, granulation of the cytoplasm, shrinkage of the protoplast, and cell wall distortion and disruption accompanied infection in all species. Lignification of cell walls, deposition of phenolics, and the formation of callosic papillae were more commonly observed in the resistant species but occurred in some susceptible species. No specific anatomical feature or histological change was consistently associated with resistance. Although the species examined were classed as either resistant or susceptible on the basis of their field response, examination of the anatomical and histological changes that followed infection showed a gradient in which the fully susceptible and most resistant types represent the extremes.

*Additional keywords*: histopathology, hyphal penetration resistance, tissue colonization.

*Phytophthora cinnamomi* Rands is a particularly destructive plant pathogen with an exceptionally wide host range, invading almost 1,000 plant species (33). Penetration and infection by *P. cinnamomi* have been demonstrated for all plant species tested (14). Recognition between host and pathogen leading to a hypersensitive reaction, such as occurs with host-specific pathogens, is therefore unlikely in a pathogen with such a broad host range, crossing angiosperm-gymnosperm barriers. Resistance develops after the pathogen becomes established within the host root and after it has induced the primary symptoms of lesion formation and cessation of root growth (21). Resistance is then expressed as limited extension of colonization and necrosis, although the pathogen remains alive in host tissue and may sporulate. This type of reaction is quite distinct from hypersensitive resistance, and the anatomical, histological, and physiological changes associated with it are only poorly understood.

This article describes the anatomical, histological, and cellular responses observed in primary root tissues of different species following inoculation with *P. cinnamomi*. The plants studied range in their field response from fully susceptible through various levels of resistance. Sedges and cereals, which have now been shown to be infected by *P. cinnamomi* are not normally regarded as hosts. Some of the changes have been reported incidentally during previous investigations (4–6,29–31) but are recorded here with greater detail to allow comparison with the studies on all of the species.

The source of the pathogen, conditions of its maintenance, and the method of zoospore production were those described by Phillips et al (21). Axenic suspensions of zoospores were produced, essentially as described by Byrt and Grant (3), except that the mycelial mat was incubated at room temperature. Zoospore concentrations were determined by counting in a hemacytometer, and the suspension was adjusted to a concentration of 20 zoospores per 10 μl for inoculation. Root inoculations were made using six replicate seedling roots of each host species. A 10-μl drop of suspension was placed immediately behind the undamaged root tip as described previously (5). Control roots were sham-inoculated with water. In one series of experiments with *Eucalyptus marginata* and *E. calophylla*, inoculation was made using 2-mm cubes of mycelium on agar, and this has been indicated where applicable in the presentation of results and discussion. The mycelial inoculations were made as part of a series to compare the effects of the two types of inoculations. One set was performed at 24 C and a second at 14 C.

Where fungi were reisolated from infected roots, the infected root material was surface-sterilized and plated as described in Grant and Byrt (11).

Plant material was obtained from the following sources: *Eucalyptus calophylla*, seed lot 8855; *E. maculata*, seed lot 6168; *E. marginata*, seed lot 9899; and *E. sieberi*, seed lot 12129, all from CSIRO Division of Forest Research, Canberra; *Acacia pulchella* and *A. melanoxylon* from Nindethana Seed Service, Western Australia; and *Juncus bufonius* from J. Warcup, Waite Agricultural Research Institute, South Australia. *Gahnia radula, Xanthorrhoea australis, X. resinosa*, and *Themeda australis* were from field collections at various sites within Victoria. *Zea mays* and *Triticum aestivum* var. *cappelle* Desprez were purchased from Gippsland and Northern Seed Co., Melbourne.

Seed were surface-sterilized, germinated, and grown axenically as described previously (6,31). After 1–2 wk, *Eucalyptus* seedlings were transferred into polyvinylchloride root-observation boxes (11) and maintained in a controlled growth chamber at 25 ± 1 C with a 14/10-hr day/night period. Light intensity varied between 700 and 900 $\mu E\ m^{-2}\ sec^{-1}$ of photosynthetically active radiation. Humidity levels fluctuated between 60 and 90%. Plants were watered to field capacity on alternate days with nutrient solution suitable for *Eucalyptus* spp. (17) and flushed every 2 wk with water to reduce mineral accumulation. Roots of *Eucalyptus* spp. were pruned where necessary to ensure a comparable state of develop-

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ment, then allowed to recover, and at 2 mo were inoculated. All other species were inoculated as young seedlings 2–4 wk old.

Whole mounts were cleared and stained using alcoholic lactophenol cotton blue (2). Thin sections of inoculated root tips were fixed in Karnovsky's fixative (16) for 24 hr, dehydrated, embedded in JB-4 resin (Polysciences), and then sectioned at 4 $\mu$m using an ultramicrotome. General histological examinations were made using tissue stained in toluidine blue O (pH 4). Lignin was detected with phloroglucinol-HCl, starch with potassium iodide/iodine, and suberin with Sudan black B in ethanol (19). Lignin was detected in fresh material by gently squashing the intact root in saturated phloroglucinol in ethanol for 10 min and then immersing it in fuming HCl. Lignin was demonstrated by an immediate development of a purplish red color. Callose was detected by fluorescence induced by staining with 0.05% decolorized aniline blue in 0.1 M phosphate buffer, pH 8.4 (9). Stained sections were examined using an Olympus microscope equipped with Nomarski optics and an M35 camera system. Fluorescence was observed on a Zeiss photomicroscope using a mercury arc source (HB100) and a Zeiss incident illuminator equipped with type 1 (BG12) exciter filters and barrier filters adjusted to exclude light below 500 nm.

Complete anatomical descriptions of normal roots of each species described here, accompanied by micrographs, have been recorded (4).

A minimum of six separate inoculated and sham-inoculated roots were examined for each species.

TABLE 1. Sequential sporulation and production of exit hyphae following inoculation of roots from six replicate seedlings with zoospores of *Phytophthora cinnamomi*[a]

| | | Hours after inoculation before production of | | |
|---|---|---|---|---|
| Species | Field reaction[b] | Sporangia | Chlamydospores[c] | Exit hyphae |
| *Eucalyptus calophylla*[d] | R | 24–48 | 48–72 | Absent |
| *E. maculata* | R | 24–48 | 48–72 | 72–96 |
| *Acacia pulchella* | R | Absent | Absent | Absent |
| *Gahnia radula* | R | 72–96 | 24–48 | 72–96 |
| *Juncus bufonius* | R | 72–96 | 24–48 | 48–72 |
| *Zea mays* | R | Absent | Absent | Absent |
| *Triticum aestivum* | R | Absent | Absent | Absent |
| *Eucalyptus marginata* | S | 16–24 | 48–72 | 24–48 |
| *E. sieberi* | S | 24–48 | 24–48 | 24–48 |
| *Acacia melanoxylon* | S | 16–24 | 48–72 | 48–72 |
| *Themeda australis* | S | 16–24 | 24–48 | 24–48 |
| *Xanthorrhoea australis* | S | 16–24 | 48–72 | 24–48 |
| *X. resinosa* | S | 24–48 | 24–48 | 24–48 |

[a] Experiment was repeated six times, each with three replicate tissue samples.
[b] S = susceptible, R = resistant.
[c] Includes some data from five species previously published (31) and used here for comparison.
[d] Authorities for plant names as cited are given by Forbes et al (10).

## RESULTS AND DISCUSSION

Zoospores of *P. cinnamomi* encysted and germinated on all species within 30–60 min of inoculation, and 24–48 hr later sporangia were produced on the root surface (Table 1). The pathogen did not sporulate on *A. pulchella, Z. mays*, or *T. aestivum*, each of which is described as resistant. However, sporangia formed some days later on other resistant species such as *J. bufonius, G. radula, E. calophylla*, and *E. maculata*. Chlamydospores and exit hyphae were produced on the same hosts as sporangia, but their formation required more time.

In all species, a progression of symptoms was observed, from cessation of root growth, to water soaking and discoloration, to tissue necrosis. In some species, such as *Themeda australis*, foliar chlorosis occurred, and in the more susceptible species, such as *Xanthorrhoea* spp., foliar chlorosis was followed by collapse and death of the whole plant (Table 2). Species could be grouped into two categories according to whether invasion by the pathogen was restricted (resistant) or unrestricted (susceptible). These categories corresponded to the field response observed in the presence of *P. cinnamomi*.

The roots of all species ceased growth within 24–48 hr after inoculation. Those of the resistant species *E. calophylla, J. bufonius, Z. mays*, and *T. aestivum* resumed growth from lateral branches 48 hr later. In *G. radula*, new root tips grew out of infected and necrotic root tissue as recorded for another sedge, *Lepidosperma laterale* (21). In contrast, growth of inoculated roots of the susceptible species *E. sieberi, E. marginata*, and the two *Xanthorrhoea* spp. ceased within 24 hr and did not resume.

The roots of most species appeared water-soaked at both the lesion site and for several millimeters around the lesion between 24 and 72 hr after inoculation. Water-soaking of tissue was not evident in species that did not develop large lesions, such as *A. pulchella, Z. mays*, and *T. aestivum*. The leaves as well as the roots of *X. australis* became water-soaked after 48 hr.

Discoloration at the site of penetration was observed 2–8 hr after inoculation of *Themeda australis, E. calophylla*, and *T. aestivum*, and 24 hr after inoculation in the case of *Z. mays* (Table 2). In most species, root discoloration and lesion formation occurred between 8 and 16 hr after inoculation, the exception being *E. marginata* inoculated at 14 C, as described later. The color of the lesion varied with species but was usually brown. Lesion length 24 hr after inoculation ranged from 5 to 20 mm. Small lesions (5 mm) consisting of isolated patches of necrotic cells surrounding the penetration site were produced on roots of *A. pulchella, J.*

TABLE 2. Sequential appearance of symptoms, following inoculation of roots from six replicate seedlings with zoospores of *Phytophthora cinnamomi*[a]

| | | | Lesions | | | | |
|---|---|---|---|---|---|---|---|
| Species | Field[b] reaction | Lesion length at 24 hr (mm) | Color | At time (hr) | Water-soaked tissue (hr) | Leaf wilt (hr) | Chlorosis (hr) |
| *Eucalyptus calophylla* | R | 10 | Brown-black | 8 | 48–72 | Absent | Absent |
| *E. maculata* | R | 10 | Dark brown | 16 | 48–72 | Absent | ... |
| *Acacia pulchella* | R | <5 | Scattered small brown flecks | 8–16 | Absent | Absent | ... |
| *Gahnia radula* | R | 5 | Brown | 16 | 48–72 | Absent | ... |
| *Juncus bufonius* | R | <5 | Light brown flecks | 8–16 | 48–72, roots | Absent | Absent |
| *Zea mays* | R | <5 | Yellow | 24 | Absent | Absent | ... |
| *Triticum aestivum* | R | 5 | Orange-brown | 8 | Absent | Absent | ... |
| *Eucalyptus marginata* | S | 15–20 | Brown | 8 | 24–48 | Absent | ... |
| *E. sieberi* | S | 15–20 | Brown | 8 | <24 | Absent | ... |
| *Acacia melanoxylon* | S | 15 | Roots yellow-brown | 16 | 24–48, roots | 48–72 | Absent |
| *Themeda australis* | S | 8 | Bright yellow | 8 | 48–72, roots | 24–48 | 48–72 |
| *Xanthorrhoea australis* | S | 15–20 | Roots brown | 15 | 48, roots and leaves | 48–72 | 48–72 |
| *X. resinosa* | S | 10 | Roots brown | 16 | 48–72, roots | 48–72 | Absent |

[a] Includes some data obtained previously (6,31).
[b] S = susceptible, R = resistant.

*bufonius, Z. mays*, and *T. aestivum*. Susceptible species *X. australis, E. marginata*, and *E. sieberi* developed lesions that had extended 20 mm or more at this stage.

In resistant species, lesions stopped growing within 48–72 hr and therefore remained limited in size. However, the pathogen remained alive and could be isolated; for example, from *Z. mays* from the initial lesion site 10 days after inoculation, after root growth had resumed. Survival of *P. cinnamomi* in tissues of resistant plants that showed no visible symptoms of infection has been demonstrated in wheat inoculated at germination. The pathogen was isolated from these plants at harvest (30). This response was in total contrast to the response of the susceptible species, where lesions continued to extend as the pathogen grew along the root and eventually entered the hypocotyl or collar region of the seedlings.

Delayed symptom expression occurred in one set of conditions in a susceptible species. When plants of *E. marginata* were inoculated with hyphae rather than zoospores, at 14 C rather than 24 C, enlarged cortical cells formed at the inoculation site, resulting in macroscopically visible hypertrophies. The cortical cells were modified and intercellular spaces penetrated by hyphae within the hypertrophy. Papillae and callose deposits were frequently observed, predominantly in the epidermis and cortex, rarely in the vascular parenchyma. Papillae have previously been reported in other resistant species (15,31), but it is clear that they can also form in susceptible species under conditions where growth of the pathogen is temporarily restricted. Hypertrophies formed in 19 of the 31 hyphal inoculations made at this temperature. They were observed in only a single root (of 20) of *E. marginata* inoculated at 24 C, and never when zoospore inocula were used at either temperature. They were not observed in *E. calophylla* under any conditions. When hypertrophies formed, the roots continued to extend and lesion formation was delayed, for at least 48 hr and for as long as a week. After this extended period without symptoms, root growth ceased and a lesion appeared and extended rapidly, reaching the same extent as in roots that had developed normally. This difference in behavior may be due to a difference in the early recognition response at lower temperatures when hyphae rather than zoospores provided the inocula. The experiments were not designed to determine the extent to which the presence of the nutrient agar was also responsible for the reaction, but the low frequency of the reaction at higher temperatures and its absence in resistant species suggests that the contribution of the agar is unlikely to be large. Hypertrophies were also observed in the wheat roots in which no symptoms developed following mycelial inoculation (30).

Cellular and histological changes in resistant species were confined to the lesion. In *E. calophylla*, a brown lesion developed on the inoculated primary root and extended upward 40–50 mm. The root reacted by branching above the lesion to form new healthy roots that continued growth and did not become infected. The contents of cells adjacent to hyphae stained more densely with toluidine blue. In fresh unstained hand sections, there was a concentration of brown phenolic/tanninlike material within the stele that disappeared during fixation. Hyphae grew inter- and intracellularly in the cortex and stele, predominantly within phloem tissue (Fig. 1).

Primary roots of *Acacia pulchella* exhibited a striking reaction to infection by *P. cinnamomi*, with rapid necrosis of individual cells and a sharp demarcation between dead and apparently healthy cells (Fig. 2), similar to those described previously in this species (25). As the hyphae penetrated through the epidermis and cortex, the cell cytoplasm became granular in appearance and then contracted around the inner cell wall and stained blue-black with toluidine blue. This host reaction did not, however, stop the fungus

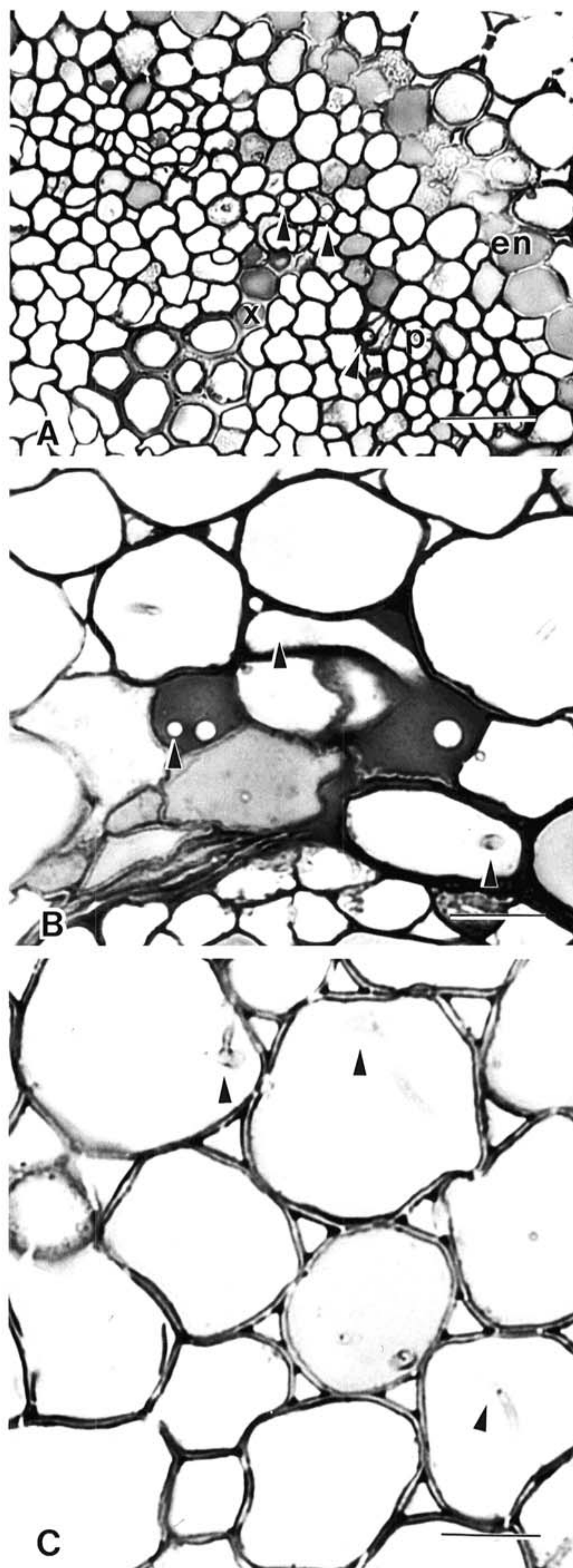



**Fig. 1.** *Eucalyptus calophylla*: infected roots 24 hr postinoculation (toluidine blue staining). **A,** Detail of vascular tissue. Hyphae (arrows) can be seen in both phloem (p) and xylem tissue. The endodermis (en) and some cells of the xylem, phloem, and vascular parenchyma are darkly stained. x = protoxylem, bar = 20 $\mu$m. **B,** Hyphae (arrows) within intercellular spaces of the inner cortex in conjunction with dark-staining phenolics. Bar = 20 $\mu$m. **C,** Intracellular hyphae (arrows) within cells of the central cortex. Bar = 20 $\mu$m.

from penetrating the stele, and distinct necrotic areas were observed in this tissue. Both protoxylem and phloem tissues were invaded. Hyphae within the cortex often appeared lysed, but sufficient hyphae remained viable to colonize the whole root, including the stele, much of which became necrotic. Sections of infected roots inoculated with only 20 zoospores showed more extreme tissue destruction than might be expected from an effective hypersensitive reaction (Fig. 2).

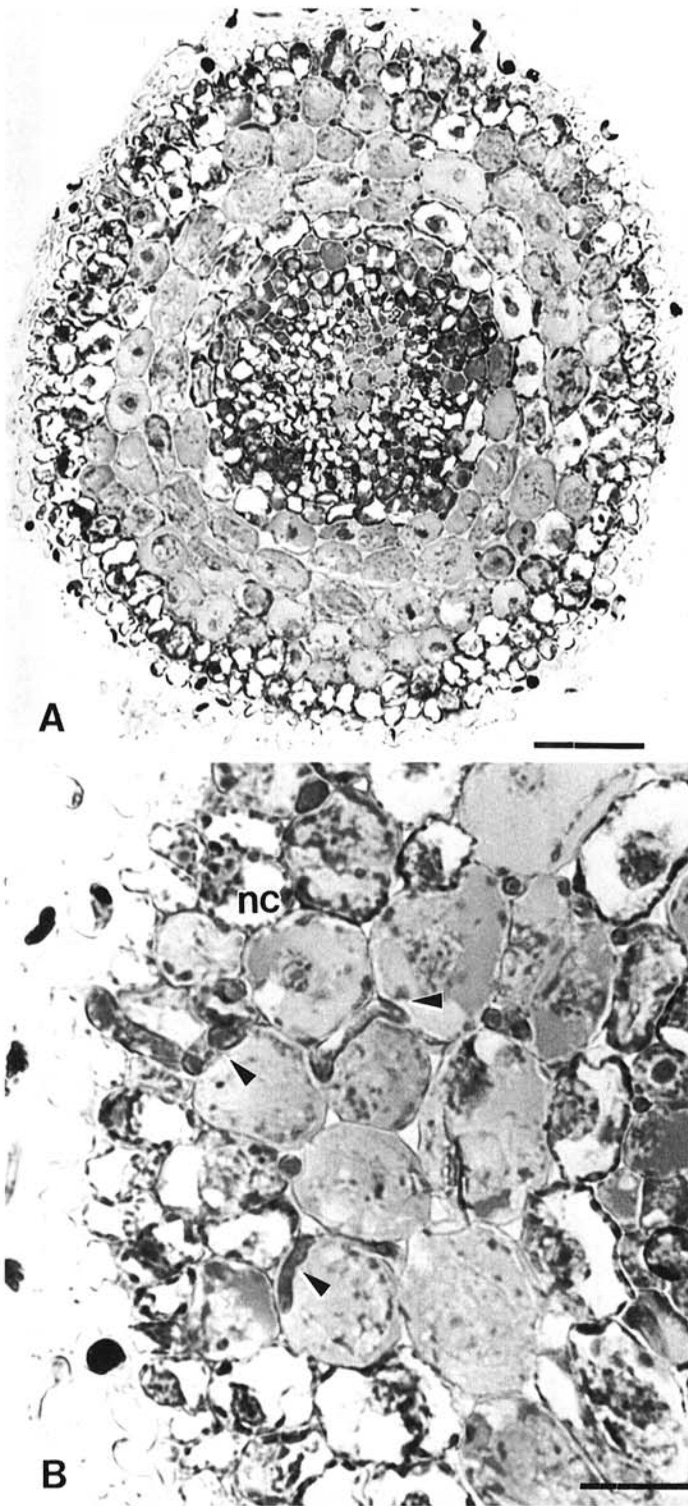



**Fig. 2.** *Acacia pulchella*: infected roots (toluidine blue staining). **A,** Transverse section of infected root. Cellular disorganization and necrosis evident. Bar = 100 μm. **B,** Root cortex. Hyphae have penetrated along the middle lamella through cell walls (arrows). Localized necrotic cells (nc) are found in both the epidermis and cortex, but some cells appear to be healthy. Bar = 10 μm.

**Fig. 3.** *Gahnia radula*: infected roots 48–72 hr postinoculation (toluidine blue staining). **A,** Transverse section of mature vascular cylinder. The endodermis (en) has U-shaped cells. Sclerenchyma (sc) fibers occur in the inner cortex, and there are many large metaxylem (mx) elements. Some protoxylem elements have hyphae in them (arrows). p = phloem, bar = 100 μm. **B,** Longitudinal section in provascular tissue, showing cell damage and hyphae (arrows) within the cortex and in the differentiating xylem (x) and phloem tissues. Secondary wall thickening of the xylem is evident. Bar = 20 μm.

In mature roots of *G. radula* inoculated with *P. cinnamomi*, hyphae were observed within the phloem and occasionally within protoxylem elements (Fig. 3), but there was no indication of cellular disruption or accumulation of phenolic material or tannins. Hyphae were never observed in the spaces of the cortex. Sections taken closer to the root tip in the provascular region showed damage to cells, and hyphae were present within the primary vascular tissues in the area inoculated.

In susceptible species, tissue and cellular damage spread as the lesion extended throughout the roots and into the junction of root and stem. Inoculation of roots of *E. marginata* resulted in the deposition of phenolic materials in the cortex, particularly the inner cortex (Fig. 4). These deposits stained brilliant green with toluidine blue and autofluoresced brightly. The deposits often totally filled a cell, were sometimes granular in appearance, and frequently occurred as globules that were appressed to the cell wall and protruded into the cell lumen (Fig. 4A). They occurred only in root tissue in which *P. cinnamomi* was present and were observed in regions beyond the lesion front. This type of inclusion has also been reported in secondary roots of *E. marginata* and *E. obliqua* (23,27) and was more conspicuous in the primary roots of *E. marginata* studied in these experiments.

The pathogen penetrated all tissues, including phloem and protoxylem, and hyphae were observed intra- and intercellularly. Both cortex and stele were extensively damaged. Similar changes have been recorded in other eucalypt species (26). The protophloem was particularly susceptible, and the protoxylem was also often damaged extensively as invasion extended.

Callose deposits, both as papillae and as layers adjacent to hyphae or injury, were observed when *E. marginata* was inoculated at temperatures below 15 C (Fig. 4C). The figure also shows penetration of a papilla by the hyphae that presumably induced its formation.

Roots of *A. melanoxylon* were invaded rapidly, resulting in granulation and disruption of cytoplasm (Fig. 5). Hyphae penetrated the stele and were often observed within metaxylem elements. The rapid cellular necrosis, which was characteristic of the interaction with *A. pulchella*, was not observed in this species.

In response to invasion by *P. cinnamomi*, cortical cell walls of *Themeda australis* rapidly lignified (Fig. 6C). The radial walls lignified first, and 72 hr after inoculation both radial and tangential walls were coated with lignin. However the lignified areas were bypassed and hyphae grew up through the stele and emerged beyond the lignin barrier, causing gross disintegration and cell lysis in the phloem and cortex of the upper root and collar zone. In some regions, the cytoplasm of the cortex and endodermis became granulated and protoplasts were shrunken and darkly stained (Fig. 6B). Hyphae penetrated into the stele and totally filled some phloem elements (Fig. 6C). Hyphae and tyloses were frequently observed in metaxylem vessels (Fig. 6A).

Inoculation of young roots of *X. australis* resulted in rapid penetration and ramification of the pathogen throughout the root tissue. The epidermis was penetrated intercellularly down the middle lamella, but growth through cortical tissues was both intra- and intercellular (Fig. 7A and B). There was shrinkage and granulation of cell protoplasts, and cell walls became distorted. Hyphae were observed in both phloem and xylem tissue. The less susceptible species, *X. resinosa*, was affected similarly when inoculated with *P. cinnamomi*.

The susceptible group showed a consistent pattern of hyphal penetration into the root tissues of each species. Intercellular penetration of the epidermis usually occurred through radial cell walls, although occasionally hyphae directly penetrated the outer tangential wall. Within the cortex, hyphae grew both inter- and intracellularly, and within cells there were often hyphal branches

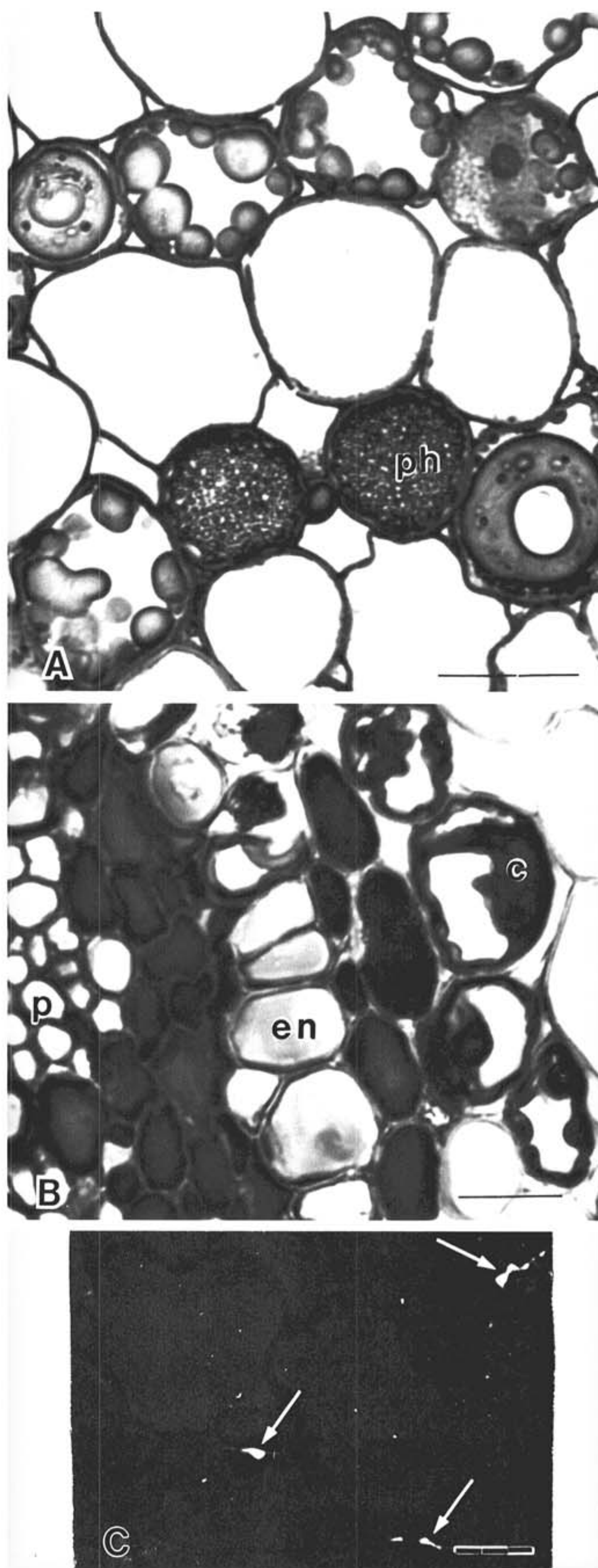
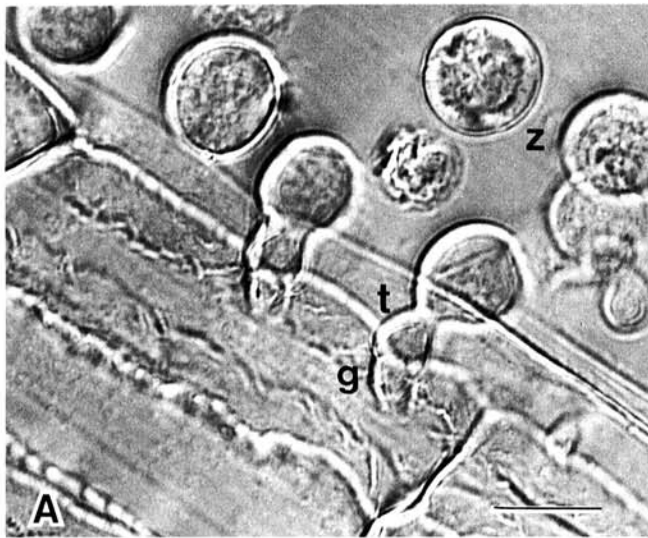
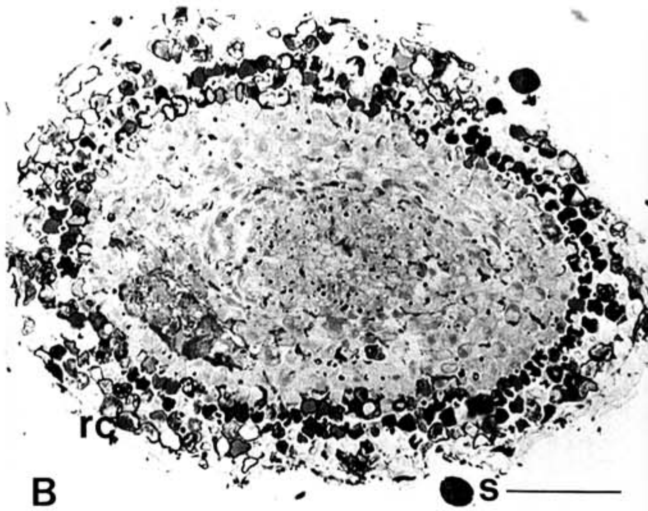



**Fig. 4.** *Eucalyptus marginata*: infected roots 16 hr postinoculation (toluidine blue staining). **A,** Detail of cortical cell inclusions that are probably phenolic materials (ph). Bar = 30 $\mu$m. **B,** Cells of the inner cortex (c), pericycle, some phloem (p) cells, and vascular parenchyma are darkly stained. In this photomicrograph, the endodermis (en) is largely unstained. Bar = 20 $\mu$m. **C,** Callose formation in infected roots stained with aniline blue and viewed under epifluorescence. Section of the cortex 10 mm from the tip taken through hypertrophy. Intracellular hyphae are visible together with callose surrounds where the hyphae have penetrated the cells (arrows). Bar = 20 $\mu$m.



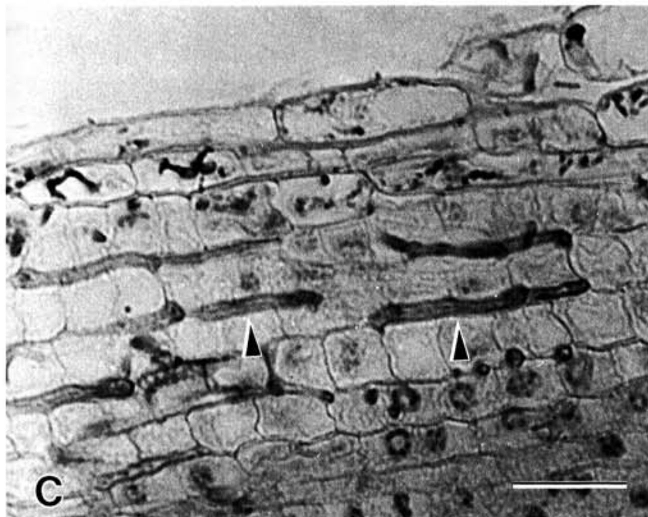
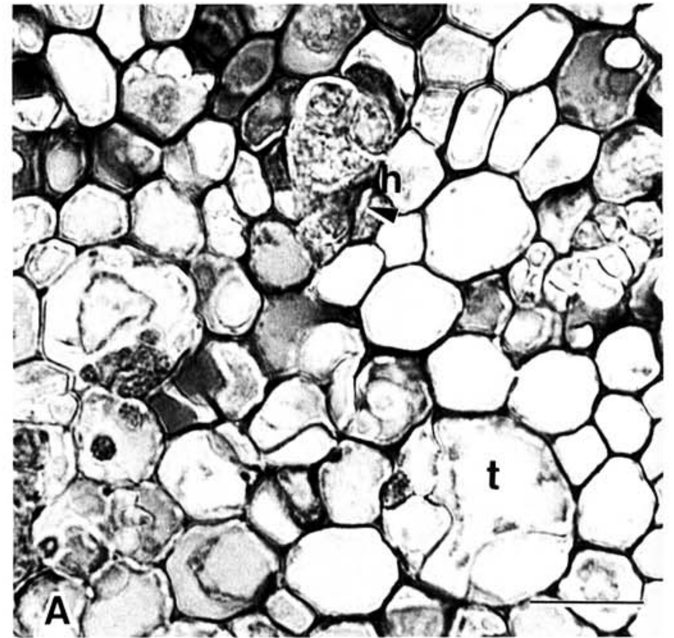
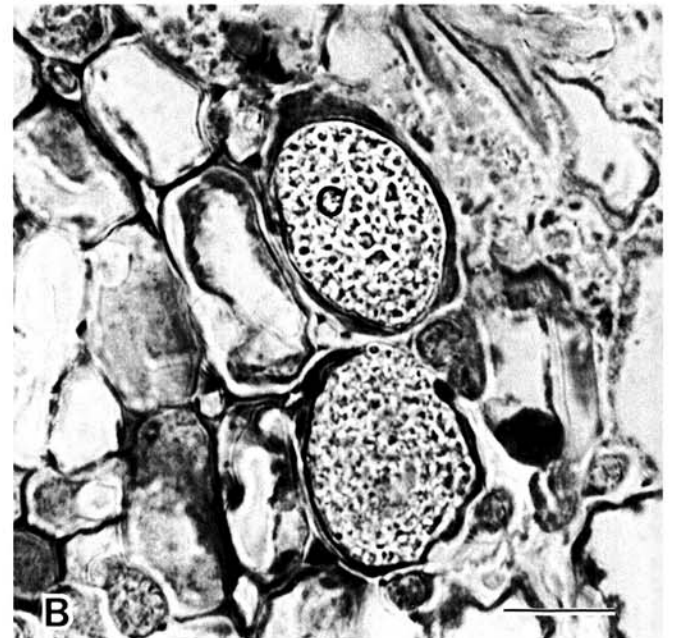
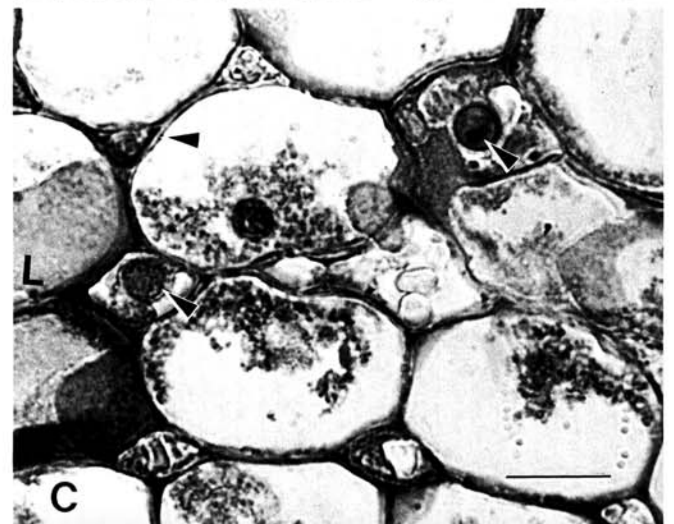




**Fig. 5.** *Acacia melanoxylon*: infected roots 2–24 hr postinoculation (toluidine blue O staining). **A,** Thin unstained section of encysted zoospores (z) on a root of *Acacia melanoxylon* 2 hr postinoculation. Cysts have formed a germ tube (t) that appears swollen at the site of wall penetration. Note that the wall is indented at penetration sites. g = germ tube within middle lamella, bar = 10 $\mu$m. **B,** Transverse section through region of provascular development of an infected root 24 hr after inoculation; cortical cells are highly cytoplasmic and there is a distinct three- to four-layered root cap (rc), many cells of which stain darkly, indicating the presence of phenolics. s = sporangium on root surface, bar = 100 $\mu$m. **C,** Longitudinal section through the zone of differentiation. Hyphae (arrows) run longitudinally through cortical tissue. Some root cap cells contain dark staining material. Bar = 30 $\mu$m.



like haustoria, vesicles, or young chlamydospores, which showed dark staining of the fungal cytoplasm. Intracellular hyphae were constricted as they passed through host cell walls, and general disorganization of host cells was observed in areas both directly adjacent to fungal hyphae and in cells several cell layers from the nearest visible hypha. Cells of the cortex adjacent to hyphae, but not penetrated by them, often contained phenolic deposits.

Although lignification of plant tissue has been considered a potential disease-resistance mechanism (1,28), it was clearly ineffective in restricting extension of *P. cinnamomi* in some of the species investigated. In *Themeda*, rapid lignification of walls separated invaded tissue from healthy tissue in a manner similar to that reported for *Eucalyptus* and avocado (20,22). However, in the primary roots of *Themeda*, the lignified regions were bypassed and did not prevent fungal growth and lesion extension.

Despite suberization and lignification of walls, the pathogen invariably penetrated to the stele and colonized phloem and xylem in susceptible species but was restricted to the lesion zone in resistant species.

In primary roots, which are those frequently invaded by *P. cinnamomi*, access to the stele occurred most commonly through provascular tissue in the zone of elongation. Little or no resistance to penetration occurred in this zone, where the young epidermis is often thin walled, the exodermis is undifferentiated, and the endodermis is unthickened. In these tissues, the fungus grew along the stelar elements rather than penetrating through the more mature endodermal and pericycle tissues further back along the root.

The endodermis was usually penetrated through the middle lamella of the radial cell walls. Deposition of callose and the production of phenolic materials were frequent responses to invasion in all species. The pericycle was readily penetrated by hyphae in most species, although the sedge *Gahnia* was an exception. In this species, both endodermis and pericycle are composed of extremely thick-walled cells.

In the stele, *P. cinnamomi* was predominantly intracellular, and the major tissues invaded were the phloem vessels and phloem parenchyma (27). Hyphae were found within xylem parenchyma in most species but were observed only infrequently in metaxylem elements. In species such as *E. calophylla* and *A. pulchella*, where colonization of the xylem occurred, hyphae were observed more often in protoxylem rather than metaxylem.

Lesion containment has been shown to be temperature dependent in field-susceptible *E. marginata*, and the extent of lesion development was temperature dependent in the field-resistant *E. calophylla* (11). The time taken for lesion growth to stop in Duke 7 (resistant) avocado roots was found to be inversely proportional to temperature (D. Phillips, *personal communication*). Lesions induced by *P. cinnamomi* in *E. maculata* (7) and in a grass, *Poa sieberiana*, and two sedges, *Lepidosperma laterale* and *G. radula* (21), became restricted within days after inoculation. In the present study, finite lesions were also observed in *E. calophylla, E. maculata, G. radula, J. bufonius, Z. mays*, and *T. aestivum*. Examination of the roots of all of these species revealed no anatomical modification that could account for containment of the fungus. Similar results were found for *Z. mays* by others (13,15,32). This strongly suggests that the basis for resistance to *P. cinnamomi* is not anatomical but is regulated by physiological and biochemical mechanisms.

Each of the species examined showed individually distinct anatomical, histological, and cellular responses to infection with *P. cinnamomi*. Similar changes have been recorded by others (12,18,23,24), but none of these changes correlated with resistance. Although interactions ranged from susceptibility through various levels of resistance, no species was resistant to initial penetration and colonization. In each species, root growth ceased and an initial lesion formed. Colonization and necrosis extended throughout roots and stem bases in susceptible species and caused secondary

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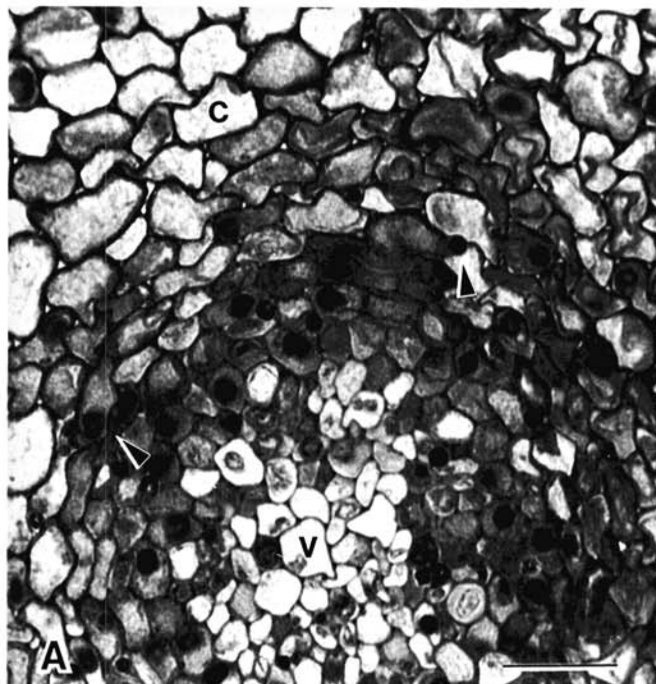
**Fig. 6.** *Themeda australis*: infected roots 48 hr postinoculation (toluidine blue staining). **A,** High power photomicrograph of vascular tissue 72 hr postinoculation, showing hyphae within both xylem and phloem. The lumen of the large metaxylem element contains tyloses (t). h = hyphae within phloem, bar = 20 $\mu$m. **B,** Chlamydospores (c) within the cortex 48 hr postinoculation. Bar = 20 $\mu$m. **C,** Granulation of cytoplasm and protoplast shrinkage in cortical cells within darkly stained lignified walls (L). Arrows = hyphae, bar = 20 $\mu$m.



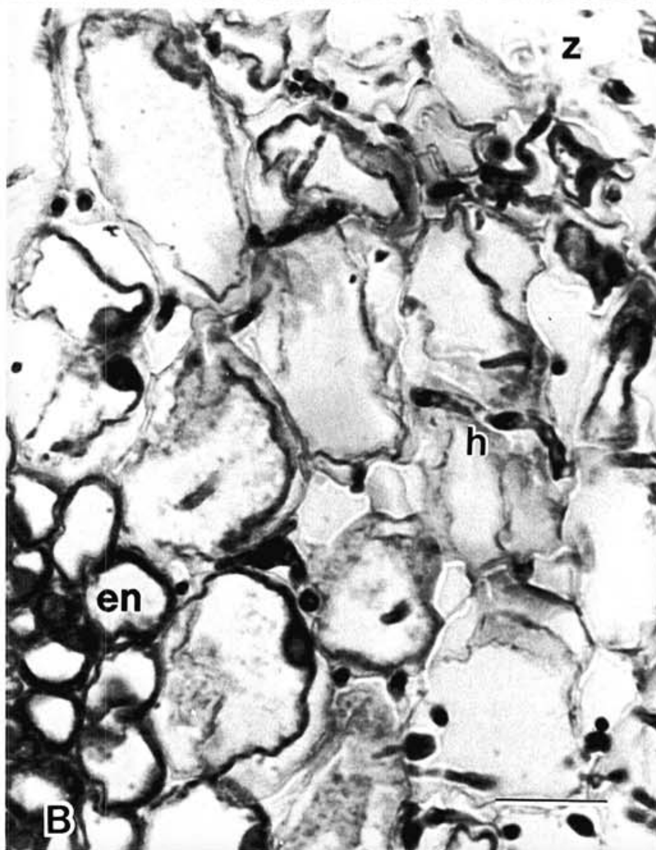




**Fig. 7.** *Xanthorrhoea australis*: infected roots 24 hr postinoculation (toluidine blue staining). **A,** Provascular tissue with inter- and intracellular hyphal penetration. v = developing vascular tissue, c = cortex, arrows = transverse and longitudinal sections through hyphae, bar = 10 $\mu$m. **B,** Hyphae predominantly colonize the cortical cell walls. Cells are distorted and cytoplasm has been disrupted. en = endodermis, h = intercellular hyphae, z = empty zoospore cyst, bar = 30 $\mu$m.

symptoms, but these were restricted in resistant species and no secondary symptoms appeared. However, the pathogen remained viable and in most cases reproduced. Although cereals and sedges are not recognized as hosts for *P. cinnamomi*, these experiments demonstrated invasion of the fungus and its survival for protracted periods in apparently healthy tissue.

The results support the conclusions based on earlier work (30), which demonstrated that invasive pathogenesis in wheat, accompanied by a 47% reduction in grain yield, is possible. We have no evidence about the nature of the factors that restricted fungal colonization, and, except for those results recorded from roots of oats (8) and certain avocado species (33), none have been reported. We suggest that this work, taken in conjunction with the previous anatomical studies cited, shows that the terms *susceptibility* and *resistance*, host and nonhost, have little significance at the level of the cell where this pathogen is concerned. Until interactions between *P. cinnamomi* and invaded plant species are adequately explained at the molecular level, this situation will remain. These interactions appear to be in total contrast to those controlled by one or a few genes for resistance, which are expressed as incompatibility reactions in certain cultivars.

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