Phytophthora Root Rot of *Banksia*: Host Range and Chemical Control

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ABSTRACT

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Phytophthora cinnamomi was isolated from blackened roots of 2-yr-old *Banksia speciosa* plants grown on the island of Maui, Hawaii. Infected plants wilted rapidly, and one side or all of the plant died; other symptoms were collapse of the bark and dark brown discoloration of xylem tissue. *B. prionotes* and *B. occidentalis* were also affected. Inoculation of 20 1-yr-old *B. speciosa* plants with a mycelial homogenate confirmed pathogenicity. Inoculation studies with 0, 1×10^1, 1×10^2, 1×10^3, or 1×10^4 zoospores per plant indicated that *B. occidentalis, B. prionotes, B. burdettii,* and *Protea cynaroides* were highly susceptible; *B. menziesii* was moderately susceptible; *B. caleyi* was moderately tolerant; and *B. integrifolia* and *B. collina* were highly resistant to *P. cinnamomi.* Susceptibility among species differed at inoculum levels of 1×10^2 and 1×10^3 zoospores per plant. Soil drenches of metalaxyl protected plants against *P. cinnamomi* better than did ethazole and fenaminosulf.

The genus *Banksia* belongs to the Proteaceae, which includes a large number of species concentrated in the southern hemisphere and has its greatest development in Australia (6). Because of their large, spectacular inflorescences, several *Banksia* spp. are being grown commercially in California and Hawaii for cut flowers.

Several diseases have been identified on members of the Proteaceae in Hawaii. Among these, the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood (4) and shoot blight caused by *Botrytis cinerea* Pers. ex. Fr. (3) have been especially damaging. In 1976, plants of *Banksia* spp. were dead or dying in several commercial fields on the island of Maui. The disease was characterized by rapid wilting and death of part or all of the plant and by collapse of the bark and dark brown discoloration of the xylem tissue. *Phytophthora cinnamomi* Rands was isolated from roots and crowns of affected plants.

This paper reports results of studies of pathogenicity of *P. cinnamomi* to various members of the Proteaceae and its control by a fungicide drench.

MATERIALS AND METHODS

Isolation and cultivation. Isolations were made from the root and crown tissues of dead and dying *Banksia* plants in several commercial farms on Maui. Diseased materials were collected, washed free of soil, surface sterilized in 0.5% sodium hypochlorite for 10 min, rinsed in sterile distilled water, placed on water agar and selective agar medium (13), and incubated at 24 C. Subcultures were made on vegetable juice agar (20% Campbell's V-8 juice, 0.2% $CaCO_3$, and 2% agar) and incubated under constant illumination at 24 C.

Pathogenicity tests. An isolate of *P. cinnamomi* obtained from a naturally infected *Banksia speciosa* R. Br. was used in all pathogenicity studies. Inoculum was prepared from a homogenate (20 ml of agar culture in 10 ml of sterile distilled water) of a 1-wk-old *P. cinnamomi* culture grown on vegetable juice agar.

Twenty 1-yr-old *B. speciosa* seedlings grown in 3-gal pots were each inoculated by placing 10 ml of the fungal homogenate 5–10 mm below the soil surface adjacent to each seedling crown. Similarly, 20 control plants were inoculated with 10 ml of autoclaved vegetable juice agar homogenate. Plants were removed from pots, washed, and examined for symptoms 2 mo after inoculation. Reisolations were made on vegetable juice agar.

Banksia occidentalis R. Br. seedlings were used as a susceptible control in subsequent tests because seed was readily available and previous studies indicated it to be comparable to *B. speciosa* in susceptibility to *P. cinnamomi.*

Seven *Banksia* spp., *B. burdettii* E. G. Bak., *B. caleyi* R. Br., *B. collina* R. Br., *B. integrifolia* L. f., *B. menziesii* R. Br., *B. occidentalis,* and *B. prionotes* Lindl., were compared for relative susceptibility to *P. cinnamomi. Protea cynaroides* L., which is tolerant to *P. cinnamomi* (22,23), was included as a resistant control.

Ten 2-wk-old seedlings of each species were potted in 5-cm plastic pots that contained a medium grade vermiculite. Plants were irrigated every other day. Zoospores for inocula were produced by a modification (7) of Chen and Zentmyer's technique (2). Zoospore concentration was determined by a modified microsyringe procedure (12), in which five 1-μl drops separated from one another were placed on the bottom of a 16 × 100 mm plastic petri dish and the number of motile zoospores was estimated microscopically at ×60 magnification.

Inoculations were made at the soil surface adjacent to each seedling hypocotyl with inoculum levels of 0, 1×10^1, 1×10^2, 1×10^3, or 1×10^4 zoospores per plant. Sterile distilled water was used for the untreated controls. The time between harvesting of zoospores and agitation of inoculum suspensions was kept at a minimum to minimize zoospore encystment.

Relative susceptibility was based on the percentage of diseased plants after 3-mo growth. Infection was confirmed by reisolation of the fungus from the crown or root tissues. Identification of *P. cinnamomi* was determined microscopically and was based on the presence of grapelike chlamydospores and characteristic mycelium.

Susceptibility tests were conducted twice.

Fungicide treatments. *B. occidentalis* and *B. prionotes*, determined to be highly susceptible to *P. cinnamomi*, were used in these studies. Test plants were 1-mo-old seedlings grown in 5-cm pots containing a medium grade vermiculite.

The following fungicide treatments were compared: metalaxyl (Ridomil 5W) at 9.3, 18.6, 37.2, and 74.4 a.i. μg/ml; ethazole (Truban 30 W) at 111 a.i. μg/ml; and fenaminosulf (Lesan 35W) at 209.7 a.i. μg/ml. All plants were inoculated with 1×10^3 zoospores and drenched immediately with 25 ml of fungicide. Each test comprised three replications of nine *B. occidentalis* and 10 *B. prionotes* plants each.

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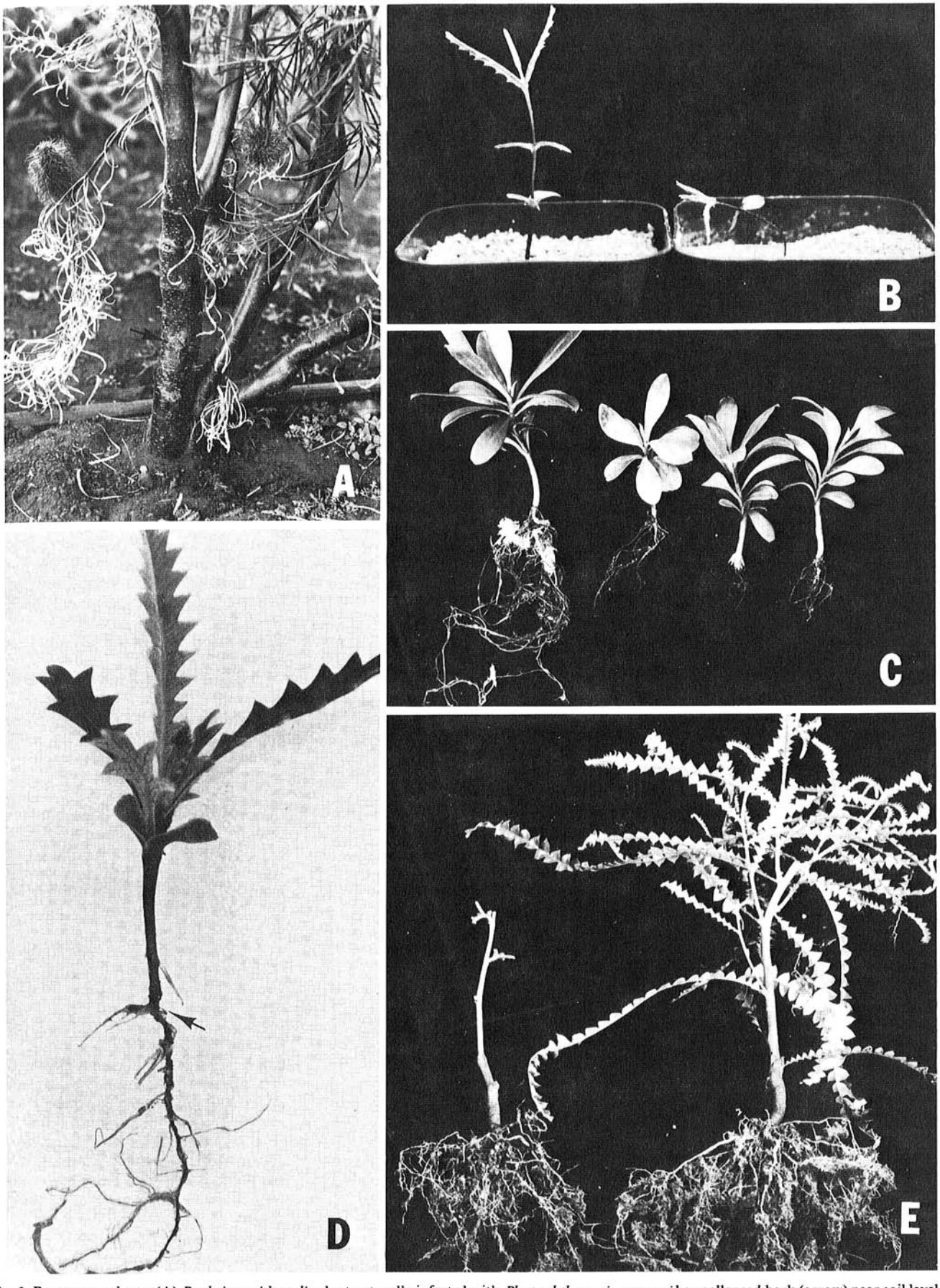

Fig. 1. Proteaceae plants: **(A)** *Banksia occidentalis* plant naturally infected with *Phytophthora cinnamomi* has collapsed bark (arrow) near soil level. **(B)** Healthy (left) 2-mo-old *B. occidentalis* seedling and (right) collapsed seedling inoculated with *P. cinnamomi*. **(C)** Healthy 4-mo-old *Protea cynaroides* seedlings (far left) and inoculated seedlings (right) with discolored and dead or dying roots. Note adventitious roots (third plant from the left). **(D)** Four-month-old *B. caleyi* seedling with constriction at the root-hypocotyl zone (arrow). Note adventitious root in the hypocotyl region. **(E)** One-year-old *B. speciosa* seedlings. Inoculated plant (left) has chlorotic leaves and wilted shoots.

The effectiveness of each treatment was evaluated on the basis of percent mortality 3 mo after inoculations. All data were analyzed by analysis of variance and Duncan's Bayesian least significant difference test between means.

Table 1. Effect of inoculum level on the incidence of infection of seven *Banksia* spp. and *Protea cynaroides* by *Phytophthora cinnamomi* 3 mo after inoculation

Plant	Percentage diseased plants[y]				
	0	10^1	10^2	10^3	10^4
Banksia burdettii	0	0	25	90	nt[z]
caleyi	0	0	30	55	65
collina	0	0	0	0	0
integrifolia	0	0	10	5	nt
menziesii	0	5	45	75	nt
occidentalis	0	5	90	95	nt
prionotes	0	10	60	90	nt
Protea cynaroides	0	10	90	100	100

[y] Average of 10 plants, repeated twice.
[z] nt = not tested.

RESULTS

Field isolations. *P. cinnamomi* was consistently isolated from dead or dying plants of *B. speciosa, B. prionotes,* and *B. occidentalis.* Infected plants had slight to severe chlorosis of the leaves of one side or of the entire upper portion of the plant. Inspection of the growth of these plants showed collapse of the bark and dark brown discoloration of the xylem tissue (Fig. 1A).

Pathogenicity tests. Of the 20 *B. speciosa* plants inoculated with a mycelial homogenate of *P. cinnamomi,* 13 showed symptoms of infection, which was confirmed by isolation. None of the 20 control plants showed symptoms. Infected plants became chlorotic; chlorosis began on the lower, older leaves and progressed to the younger, fully expanded leaves. Premature leaf drop and wilting of the young branches were also noted. Most of the roots of plants that had severe aboveground symptoms were blackened and dead (Fig. 1E).

Table 2. Effect of metalaxyl, ethazole, and fenaminosulf soil drenches on mortality of *Banksia occidentalis* and *B. prionotes* infected by *Phytophthora cinnamomi* 3 mo after inoculation

Fungicide	Rate[x] (μg/ml)	Percentage mortality[y]	
		B. occidentalis	*B. prionotes*
Metalaxyl	9.3	0 a[z]	0 a
	18.6	0 a	3.3 a
	37.2	0 a	6.7 a
	74.4	0 a	0 a
Fenaminosulf	209.7	7.3 b	16.7 b
Ethazole	111.0	3.7 ab	20.0 b
Untreated	0	92.7 c	93.3 c

[x] 25 ml applied per plant.
[y] Average of three replicates, each of nine or 10 plants for each species.
[z] Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's least significant difference (LSD) test for significance among means.

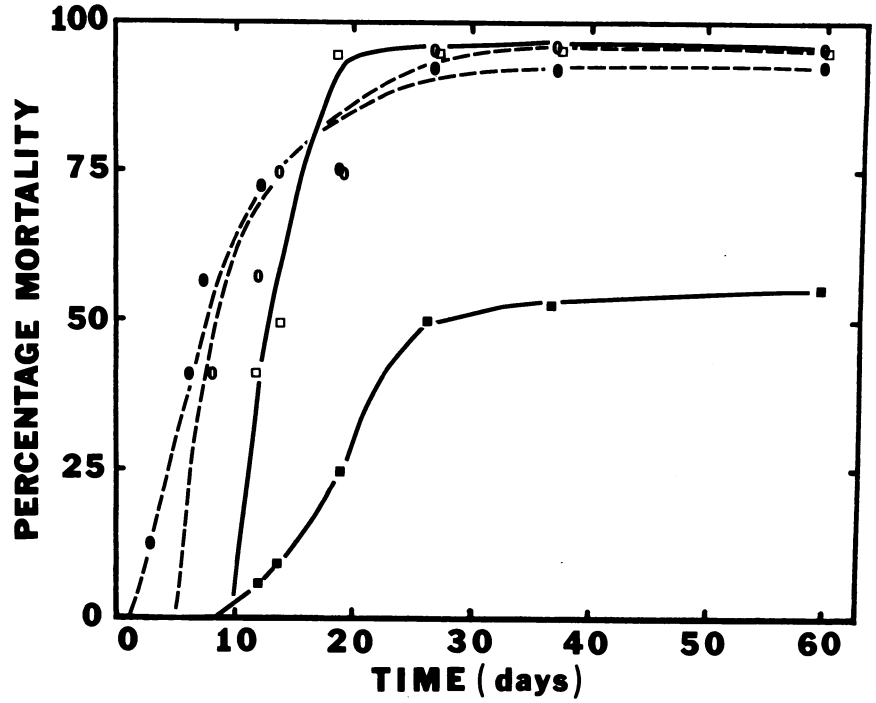

Fig. 2. Mortality of 1-mo-old *Banksia occidentalis* (o,●) and *B. prionotes* (□, ■) seedlings inoculated with 1×10^2 (●, ■) and 1×10^3 (o, □) zoospores of *Phytophthora cinnamomi* per plant.

The effects of increasing *P. cinnamomi* zoospore levels on the incidence of infected plants of seven *Banksia* spp. and *Protea cynaroides* are summarized in Table 1. Relative susceptibilities of the species could be distinguished at the 1×10^3 zoospore inoculum level; however, the highly susceptible species, *B. occidentalis* and *P. cynaroides,* could be differentiated at the 1×10^2 zoospore inoculum level. *B. collina* and *B. integrifolia* were highly resistant to *P. cinnamomi; B. caleyi* was moderately resistant; *B. menziesii* was moderately susceptible; and *B. burdettii, B. occidentalis, B. prionotes,* and *P. cynaroides* were highly susceptible. Plant mortality was observed 6 days after inoculation for the highly susceptible *B. occidentalis* (Fig. 1B) and approximately 10 days to 2 wk after inoculation for the other susceptible *Banksia* spp. (Fig. 2).

Aboveground symptoms were characterized by dark discoloration and water-soaking of the hypocotyl tissues beginning near the soil line and progressing into the cotyledons. As infection advanced, the older infected tissues collapsed and died, resulting in wilting and drying of the leaves and death of the plant. Infected primary and secondary roots were blackened within the vascular cylinder, with the outer tissue becoming soft and water-soaked. *P. cinnamomi* was readily isolated from the discolored tissues of the hypocotyl and roots.

Three months after inoculations were made, all but one *P. cynaroides* plant inoculated with 1×10^3 zoospores appeared no different from the uninoculated controls and were thought to be healthy. Examination of the roots of these plants (Fig. 1C), however, showed that all primary and secondary roots were discolored, flaccid, or dead with little or no infection of the hypocotyl tissue. Adventitious roots had been initiated from the uninfected hypocotyl region in all plants; most of these roots were discolored and infected.

Infected plants of *B. caleyi* showed slight stunting, inward curling of the younger leaves, and slight chlorosis of the older leaves. Roots of these plants showed constriction of the hypocotyl-root border with little invasion of the adjacent tissue. No evidence of infection was observed in the primary or secondary roots. Adventitious roots had been initiated within the hypocotyl region (Fig. 1C). However, unlike *P. cynaroides* roots, none of these roots showed symptoms of infection. These observations were confirmed through reisolation of the fungus.

Fungicide tests. Results of the chemical control tests are summarized in Table 2. All treatments significantly reduced plant mortality compared with the untreated control. Compared with ethazole and fenaminosulf, drenches of *B. prionotes* at all metalaxyl concentrations significantly

reduced mortality. However, metalaxyl treatments of *B. occidentalis* were superior only to fenaminosulf. *P. cinnamomi* was isolated from all dead plants.

DISCUSSION

In western Australia and Victoria, *P. cinnamomi* is recognized as the cause of a disease known as jarrah dieback (*Eucalyptus marginata* Donn. ex Sm.); plants in 48 families and more than 100,000 ha of forest have been killed (15,18). Among these families, the Proteaceae (15–17,19,24) was highly susceptible.

In this study, several *Banksia* spp. were highly susceptible. The minimum number of zoospores required for infection of plants varied. Species that were highly susceptible (*B. menziesii, B. occidentalis, B. prionotes,* and *P. cynaroides*) were infected by 10 zoospores per plant, compared with 1×10^2 zoospores per plant necessary for the others. Use of 1×10^3 zoospores per plant produced near maximal mortality of highly susceptible plants. These results differ from those reported for *P. palmivora* on papaya (9,11,20), *P. parasitica* var. *nicotianae* on tobacco (5), and *P. cryptogea* on watercress (14), in which 10^3 zoospores per container allowed minimal infection compared with 10 zoospores per plant for susceptible Proteaceae plants. This difference may be attributed to differences in host susceptibility due to plant species or age (10), method of inoculation, and/or pathogen strain aggressiveness (8,21).

In contrast to reports on resistance of *P. cynaroides* to *P. cinnamomi* (22,23), young seedlings were unexpectedly found to be highly susceptible. On the other hand, *B. collina* and *B. integrifolia* were highly resistant. These resistant species made available suitable material for breeding programs and rootstocks for grafting. In preliminary tests, successful grafts have been made between species using *B. collina* and *B. caleyi* as rootstocks (*unpublished*). More importantly, several seedling clones of *B. collina* and *B. integrifolia* resistant to the root-knot nematode (*M. incognita*) have been identified and selected and can be easily propagated asexually by rooting. It is hoped that the major diseases limiting production of commercial banksias can be controlled through the use of such disease-resistant cultivars. Further investigations on graft compatibility and field performance of grafted combinations are being conducted to determine the usefulness of clonal materials.

As reported by Benson (1), metalaxyl drenches were more effective than fenaminosulf and ethazole in controlling *P. cinnamomi* infections. Furthermore, Benson reported that metalaxyl controlled *P. cinnamomi* infections on azalea 24–28 wk after a drench treatment, and postulated that this long-term effect may be related to the systemic nature of the fungicide. Although fenaminosulf and ethazole are effective when used properly, both have drawbacks; for example, monthly applications of diazoben were required to effectively control root rot of avocados (26), and ethazole has poor mobility in soil (1,25). Resistance and use of effective fungicides identified in this report presently offer the protea industry two means for control of *Phytophthora* root rot.

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