Pathogenicity and Histopathology of Phytophthora cinnamomi on Highbush and Rabbiteye Blueberry

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Journal Series Paper No. 4538 of the North Carolina Agricultural Experiment Station, Raleigh.
Accepted for publication 24 February 1975.

ABSTRACT

All highbush and rabbiteye blueberry cultivars tested were susceptible to infection by *Phytophthora cinnamomi*. Isolates from blueberry, azalea, and cedar differed in pathogenicity, and rabbiteye blueberry cultivars were consistently more resistant to root-rot than highbush cultivars. Rabbiteye tolerance was partially correlated negatively with zoospore attraction and accumulation on young rootlets. Although the fungus infected the epidermis, cortex, and vascular tissue of both blueberry species, colonization of the vascular tissue by the fungus in rabbiteye roots was less extensive. No anatomical differences were observed between highbush and rabbiteye root systems.

Phytopathology 65:789-793

Additional key words: Vaccinium corymbosum, V. ashei, root-rot, histology, susceptibility.

Two *Vaccinium* spp. are grown commercially in North Carolina; the highbush blueberry (*Vaccinium corymbosum* L.) and the rabbiteye blueberry (*V. ashei*). In general, the rabbiteye cultivars are more resistant to the predominant fungal pathogens than the highbush. From previous greenhouse inoculations (10) and field observations, this appears to be true for the root-rot disease caused by *Phytophthora cinnamomi* Rands.

Phytophthora cinnamomi is pathogenic on highbush blueberry (2, 10, 12). In 1967, Milholland and Galletta (10) found *P. cinnamomi* widespread in North Carolina blueberry plantings, and noted that all cultivars tested were susceptible to the fungus but that rabbiteye cultivars were more tolerant to damage by *P. cinnamomi*. Clones of *Vaccinium atrococcum* growing in naturally infested soil (3) and in greenhouse tests (4) were resistant to *P. cinnamomi*.

The present studies were conducted with highbush and rabbiteye cultivars to (i) determine variation in pathogenicity of *P. cinnamomi* isolates, (ii) determine the relative susceptibility of blueberry cultivars to infection by zoospores of *P. cinnamomi*, and (iii) study the histological effects of the pathogen on various root tissues.

MATERIALS AND METHODS.—Cultures of *P. cinnamomi* used in these studies were isolated from *V. ashei* (isolate P-1), *V. corymbosum* (isolate P-2), *Rhododendron* spp. (isolate P-3), and *Cedrus deodora* (isolate P-4). Cultures were grown on V-8 juice agar (V-8A) at 25 C under continuous light at 2,000 lx. Growth rate, habit, and sporangial production on V-8A were similar for all four isolates.

Inoculations to determine pathogenicity and cultivar susceptibility were made by removing 5-mm disks of mycelium from 7-day-old cultures grown on V-8A and inserting the disks into each of five holes 2.5 cm deep in the soil and 2.5 cm from each plant. Plants were grown in a peat:sand (1:1, v/v) mixture and forced from well-rooted cuttings in the greenhouse prior to inoculation. Two highbush cultivars Croatan and Wolcott, and two rabbiteye cultivars Garden Blue and Tifblue were used. Five plants of each cultivar were inoculated with each of the four isolates. Five plants of each cultivar were included as noninoculated control plants. Disease was evaluated 90 days after inoculation and rated according to the severity of symptoms in stems, leaves and roots. Top growth of plants was rated 0-5: 0 = healthy; 1 = slight chlorosis, growth good; 2 = moderate chlorosis, growth

fair; 3 = severe chlorosis, reddening of lower leaves, growth fair to poor; 4 = reddening of leaves, defoliation, growth poor; 5 = plants dead. Root necrosis was rated as follows: 0 = healthy; 1 = 1-5% root necrosis; 2 = 6-25% root necrosis; 3 = 26-50% root necrosis; 4 = 51-75% root necrosis; 5 = 76-100% root necrosis.

Isolations from inoculated and noninoculated plants were made using the selective antibiotic medium developed by Eckert and Tsao (5).

Zoospore inoculum used in attraction studies was obtained by floating V-8A disks containing mycelia from 6-day-old cultures in nonsterile soil leachate (14). Four 20-mm root sections from each cultivar were placed in each of four petri dishes. All root sections contained the regions of meristematic activity, elongation, and maturation. The root sections in each dish were flooded with a suspension of approximately 5×10^4 zoospores/ml and incubated at 25 C. Roots in each dish were examined microscopically for accumulation of zoospores within 1 hour. Following accumulation, the root sections were placed at 25 C and examined microscopically for germ tube penetration after 4 and 24 hours. All four isolates were tested and the experiment was repeated five times.

Fungal penetration and disease development was determined 90 days after inoculation. Roots were cut into 10-mm sections, cleared and fixed in formalin acetic-alcohol (FAA), dehydrated in tertiary butyl alcohol, and embedded in paraffin. Sections 10 μm thick were mounted on slides with Haupt's adhesive and stained with safranin and fast green (7). Root sections were also removed from inoculated plants and naturally infected plants to determine the presence of chlamydospores and oospores. Roots were cleared using the method described by Bevege (1).

RESULTS.—*Pathogenicity.*—Characteristic symptoms of blueberry root-rot included stunting of plants, yellowing and reddening of leaves with some necrosis of leaf margin, small terminal leaves, defoliation and root necrosis. Differences in virulence of the four isolates occurred within a given cultivar and among the four blueberry cultivars tested (Table 1 and 2). The blueberry isolate (P-1) was more virulent than the azalea (P-3) or cedar (P-4) isolates, with isolate P-3 being the least virulent.

The highbush cultivar Wolcott was highly susceptible to all four isolates, whereas the rabbiteye cultivar Garden Blue was resistant to most isolates tested. Three of the five Wolcott plants inoculated with isolate P-1 were dead 6 weeks after inoculation. The amount of root necrosis was normally correlated with aboveground symptoms on the highbush cultivars. In most cases, symptoms of the aboveground portions of rabbiteye plants were not severe even though the roots were often severely necrotic. None of the rabbiteye plants showed a reddening of the leaves or defoliation 3 months after inoculation as was evident with the highbush plants. Noninoculated control plants remained healthy during the test.

Isolation attempts were made from 20 root sections of

TABLE 1. Effect of four *Phytophthora cinnamomi* isolates on above ground symptoms of blueberry cultivars 3 months after inoculation

Cultivars	Isolates and mean disease ratings[x]					Cultivar means[y]
	P-1	P-2	P-3	P-4	Control	
Wolcott	4.6 a	4.2 a	2.2 a	3.8 a	0.0 a	2.95 a
Croatan	4.6 a	3.0 b	1.4 b	2.4 b	0.0 a	2.25 b
Tifblue	2.6 b	0.4 c	0.2 c	0.4 c	0.0 a	0.72 c
Garden Blue	0.4 c	0.6 c	0.0 c	0.0 c	0.2 a	0.24 c
Isolate means[z]	3.05 a	2.05 b	0.95 c	1.65 b	0.05 d	

[x]Disease ratings: 0 = plants healthy; 1 = slight chlorosis, growth good; 2 = moderate chlorosis, growth fair; 3 = severe chlorosis, reddening of lower leaves, growth fair to good; 4 = reddening of leaves, defoliation, growth poor; 5 = plants dead, Average of five replications. Means in a single column followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[y]Cultivar means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[z]Isolate means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 2. Effect of four *Phytophthora cinnamomi* isolates on roots of blueberry cultivars 3 months after inoculation

Cultivars	Isolates and mean disease ratings[x]					Cultivar means[y]
	P-1	P-2	P-3	P-4	Control	
Wolcott	5.0 a	5.0 a	4.4 a	5.0 a	0.2 a	3.92 a
Croatan	4.8 a	4.4 b	3.0 b	3.4 b	0.6 a	3.24 b
Tifblue	5.0 a	2.6 c	1.6 c	2.6 c	0.2 a	2.45 c
Garden Blue	2.4 b	0.2 d	0.0 d	0.0 d	0.2 a	0.56 d
Isolate means[z]	4.30 a	3.05 b	2.28 c	2.75 bc	0.30 d	

[x]Disease ratings: 0 = roots healthy; 1 = 1-5% root necrosis; 2 = 6-25% root necrosis; 3 = 26-50% root necrosis; 4 = 51-75% root necrosis; 5 = 76-100% root necrosis. Average of five replications. Means in a single column followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[y]Cultivar means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

[z]Isolate means followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

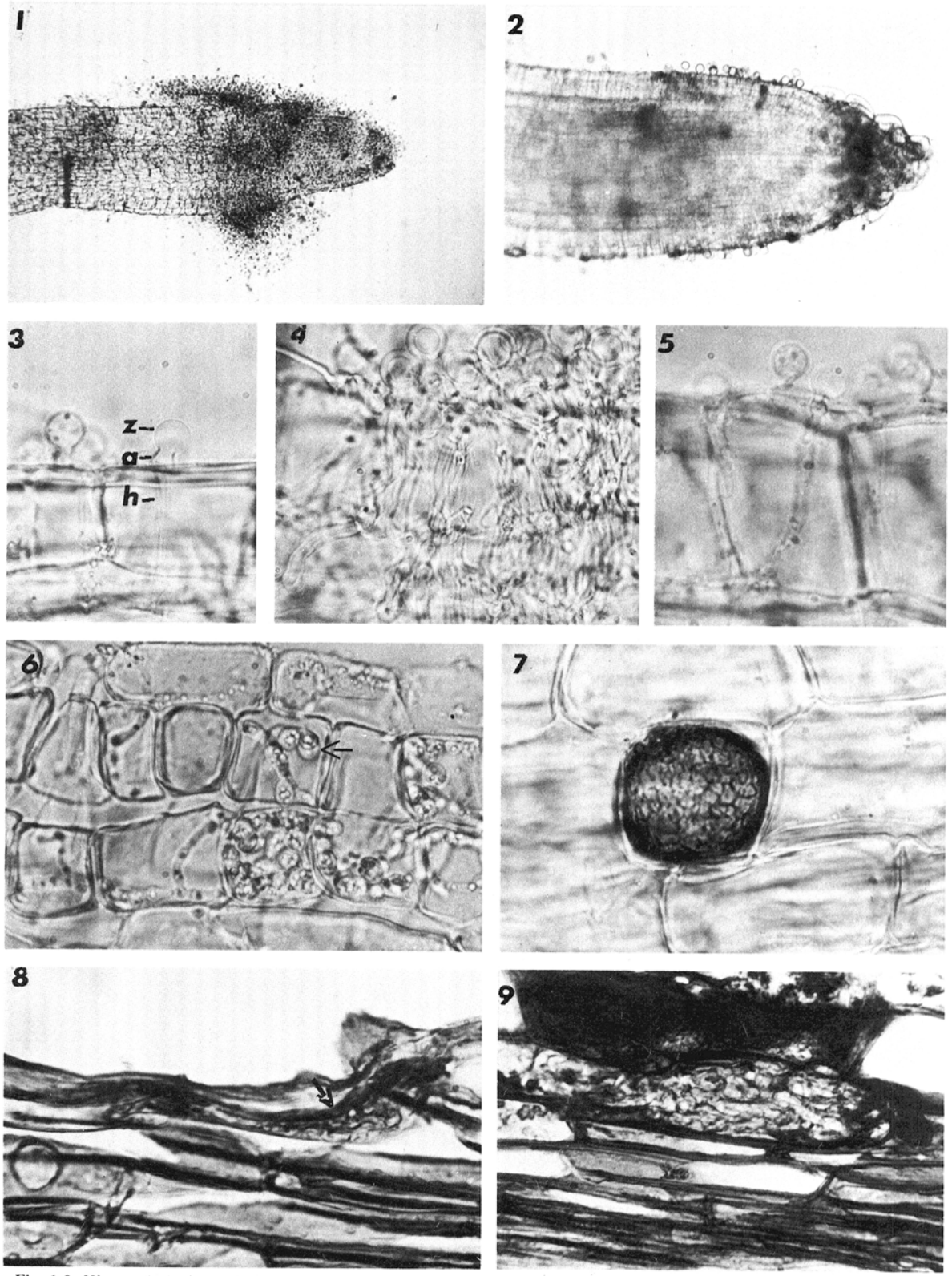

Fig. 1-9. Histopathological relationships of highbush and rabbiteye blueberry and *Phytophthora cinnamomi*. **1)** Zoospore accumulation on highbush blueberry root 1 hour after inoculation (× 7.3). **2)** Zoospore accumulation on rabbiteye blueberry root 1 hour after inoculation (× 20.9). **3)** Zoospore germination and penetration 4 hours after inoculation: a = appressoria; h = penetration hyphae; z = zoospore (× 83.6). **4)** Zoospore infection and colonization of highbush blueberry root 24 hours after inoculation (× 83.6). **5)**. Zoospore infection and penetration of rabbiteye blueberry root 24 hours after inoculation (× 83.6). **6)** Intracellular hyphae and vesicles (arrow) of *P. cinnamomi* in epidermis and cortex of necrotic highbush blueberry root (× 83.6). **7)** Chlamydospore of *P. cinnamomi* in epidermis of highbush blueberry root (× 83.6). **8)** Collapsed epidermal cell from mycelial infection (arrow) (× 83.6). **9)** Enlargement and rupture of parenchyma cell from mycelial infection (× 83.6).

each cultivar inoculated with each of the four isolates. The percent isolation of *P. cinnamomi* isolates P-1, P-2, P-3, and P-4 from roots of the highly susceptible cultivar Wolcott was 50, 30, 15 and 20, respectively. The percent isolation from the resistant cultivar Garden Blue was 10, 10, 0, and 15, respectively. The fungus was not recovered from noninoculated plants.

Zoospore accumulation and penetration.—Roots of the two blueberry species differed in their ability to attract zoospores. A heavy accumulation of zoospores was observed on all root sections of the highbush cultivar Wolcott 1 hour after inoculation, and was slight to heavy on Croatan roots. Zoospores accumulated behind the root tip in the region of elongation and at the cut ends of the roots (Fig. 1). Many of the zoospores had encysted, germinated and penetrated the root tissue within 1 hour. No heavy accumulation of zoospores of any isolate occurred on root sections of Tifblue and Garden Blue, except at the cut ends. Zoospores were mostly scattered over the entire root sections. A slight to moderate accumulation of zoospores was observed at the region of elongation on Tifblue roots but none in the region of maturation (Fig. 2). Zoospore accumulation was absent or very slight on Garden Blue roots. No marked differences existed among isolates of *P. cinnamomi* in the amount of zoospore accumulation to roots of a given cultivar.

Germination, appressoria formation, and penetration were complete 4 hours after inoculation (Fig. 3). Appressoria measured 7-8 μm in diameter and developed either immediately upon germination by the zoospore or after extensive growth (100-150 μm in length) by the germ tube. Extensive colonization of Wolcott epidermal cells by a large number of zoospores was observed 24 hours after inoculation (Fig. 4). Penetration into the vascular tissue was also observed. Hyphae measured 2-3 μm in diameter and grew intracellularly within the root. Hyphae usually enlarged prior to penetrating cell walls, with the penetrating hyphae becoming constricted and then enlarging as it entered the cell. Penetration into the young rootlets of rabbiteye plants was essentially the same as that of the highbush roots. The primary difference was the limited colonization of the root tissues due to the smaller number of zoospores accumulated in the region of elongation (Fig. 5).

Root sections from inoculated plants were examined for the presence of the fungus after 14 days. Extensive amounts of intracellular hyphae and vesicles were observed in the epidermal cells and cortex of infected roots (Fig. 6). Oospores and chlamydospores were not observed on the sections examined at this time. Although some of the infected cells appeared to be functional, no tests were made to determine if any or all of the infected or surrounding cells were dead or alive.

To determine if any of the overwintering spores of *P. cinnamomi* (chlamydospores and oospores) were produced in the root tissue, root sections 10-20 mm in length were excised from all inoculated plants after 3 months and cleared in 1N KOH. Twenty-five root sections of each cultivar inoculated with each of the four isolates were examined microscopically. Oospores were not observed in any of the roots and only two chlamydospores were found in the 400 sections examined (Fig. 7). No oospores or chlamydospores were observed in roots of naturally infected Wolcott or Garden Blue cultivars. Numerous sporangia were observed on the root sections.

Histology.—Necrotic roots of the highbush cultivar Wolcott were permeated with mycelium of *P. cinnamomi* 3 months after inoculation. Hyphae measured 1-5 μm in diameter, and grew intracellularly in the epidermis, cortex, phloem, and xylem vessels. Growth of the hyphae inside the xylem vessels was primarily longitudinal with some branching occurring, resulting in invasion of adjacent tracheids. Growth from one cell into another was by direct penetration of the cell wall. In many instances where hyphae had penetrated the root, the epidermal cells had completely collapsed (Fig. 8). Hyphae were observed in parenchyma cells in large masses, often causing the cell to enlarge and eventually rupture (Fig. 9). Penetration and colonization of the epidermis and cortex of rabbiteye roots by hyphae of isolates P-1 and P-2 were extensive. In most instances, establishment of the fungus in the vascular tissue of Garden Blue and Tifblue was less extensive.

DISCUSSION.—Pathogenic variation among isolates of *P. cinnamomi* has been reported on several plant species (8, 9). The results reported herein provide further evidence for the existance of pathogenic variability among isolates of *P. cinnamomi*.

Differences in susceptibility between and within blueberry species were also noted. These results tend to substantiate findings from previous tests (10), where rabbiteye cultivars were more tolerant to disease expression than the highbush cultivars. Although results from previous susceptibility tests indicated little or no differences among the rabbiteye cultivars, differences in susceptibility were observed in the present studies between Garden Blue and Tifblue, particularly with the P-1 isolate. A possible explanation for this difference is that in the previous studies, only the P-2 isolate of *P. cinnamomi* was used. Although inoculations with the P-2 isolate in these studies showed similar results to the previous tests, Tifblue roots inoculated with the P-1 isolate were severely affected by the fungus. Above ground portion of these plants, however showed tolerance to the disease. Several of the inoculated highbush plants exhibited characteristic root-rot symptoms (reddening of leaves, and defoliation) within 4 weeks of inoculation; whereas, all rabbiteye plants appeared healthy.

An excellent review on zoospore attraction and accumulation of *Phytophthora* species is given by Hickman (6). Results of these tests indicate zoospores of *P. cinnamomi* are strongly attracted to the young rootlets of highbush blueberry plants and weakly attracted to rabbiteye roots. As was demonstrated with avocado roots (13), zoospores accumulated in the largest numbers at the region of elongation, with little or no accumulation at the region of maturation. Wounded sites were also areas for maximum zoospore accumulation for both highbush and rabbiteye roots. Although the young rabbiteye rootlets are apparently equally susceptible to penetration and subsequent infection as highbush rootlets, they do not attract the large masses of zoospores as do highbush rootlets. I believe this to be an important factor in the cultivar's ability to tolerate this disease, since no anatomical differences were found between the highbush

and rabbiteye root systems.

The infection process, zoospore germination, penetration, and establishment of *P. cinnamomi* in blueberry roots occurs quite rapidly. In highly susceptible highbush rootlets, the fungus penetrates the epidermis and invades the vascular tissue within 24 hours. Hyphae grow intracellularly in the epidermis, cortex, phloem, and xylem vessels and penetration from one cell into another is direct. Infection of highbush blueberry roots by large masses of hyphae results in the collapsing of epidermal cells followed by some disorganization of the cortex.

The fact that no oospores and only two chlamydospores were observed from the inoculated and naturally infected blueberry root sections indicates that these overwintering structures are not produced in great abundance in blueberry roots as was reported for avocado (11).

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