Phytophthora cinnamomi as a Cause of Root Rot and Dieback of Cranberry in Massachusetts

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ABSTRACT

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Cranberry beds in Massachusetts commonly exhibit areas of decline and dieback associated with low spots that accumulate excessive water. Affected plants typically are stunted, unproductive, off-color, and have poorly developed root systems; severely affected plants often die. When portions of beds with symptomatic vines are replanted with new vines, the replacement vines also usually die. *Phytophthora cinnamomi* was isolated from necrotic roots and underground runners of symptomatic plants in 215 different beds. When rooted cuttings of the cranberry cultivar Early Black were transplanted into potting mix artificially infested with individual isolates of *P. cinnamomi*, fresh weights of shoots and roots were reduced by 48–88% and 83–96%, respectively, compared with uninoculated controls. In similar tests involving a single isolate of *P. cinnamomi*, there were no significant differences in root or shoot fresh weights among inoculated and control plants of the cultivars Bergman, Franklin, and Stevens. In contrast, root and/or shoot weights were significantly lower for inoculated plants of the cultivars Crowley, Early Black, Howes, and Pilgrim, and plant mortality was particularly high for the cultivar Pilgrim. This is the first study documenting a species of *Phytophthora* as a pathogen of cranberry and the first to report the occurrence of *P. cinnamomi* in Massachusetts.

Numerous cranberry (*Vaccinium macrocarpon* Aiton) beds in Massachusetts commonly exhibit areas of dieback or decline. Affected plants in these locations traditionally have been diagnosed as suffering from anaerobiosis caused by prolonged water accumulation in the soil, as symptoms usually appear to be associated with low-lying sites in the bed, and no evidence of insect feeding, winter injury, or other deleterious factors have been consistently present. Following an abnormally wet spring in 1986, plants showing dieback appeared in beds of previously healthy looking plants. Because the symptoms and occurrence of dieback were similar to that on other fruit crops affected by Phytophthora root rot, investigations were initiated in 1986 to determine whether species of *Phytophthora* were involved. Portions of this study have been published previously (2,3).

MATERIALS AND METHODS

Observations of symptoms in the field and isolation of a *Phytophthora* species. Eighteen cranberry beds throughout southeastern Massachusetts with plants showing typical symptoms were visited during October and November 1986. Vine samples were also taken from other beds in April 1987. Samples were taken from the periphery of an area of dead plants and consisted of plants that were unthrifty, stunted, off-color, and possessed poor root systems. One handful of vines was uprooted from five to 10 locations per bed and placed into a plastic bag with a small quantity of soil; composite vine samples were refrigerated until they were processed. When different cultivars were sampled within the same bed, each was collected and stored separately.

Roots and underground runners were washed in running tap water. Discolored organs were selected, and the outer periderm was stripped off. Segments of discolored woody roots and/or runners (1–2 cm in length) were excised with a razor blade, surface-sterilized in 0.5% of sodium hypochlorite plus Tween 20 (two drops per 100 ml) for 15 min or in 70% ethanol for 2–3 sec, and blotted dry on sterile filter paper. Thirty to 40 segments from each location were plated onto acidified cornmeal agar (CMA) (11) or a modified selective medium containing pimaricin, ampicillin, rifampicin, and hymexazol, such as P_5ARH (7). Plates were incubated in the dark at 24 C and examined daily for 1 wk. Colonies resembling *Phytophthora* spp. were transferred to CMA and stored at 15 C until they could be identified.

Identification of *Phytophthora* species. Isolates were identified on the basis of mycelium characteristics, cardinal temperatures for vegetative growth, morphology and dimensions of sporangia, and production of sex organs. For studies of mycelium characteristics and cardinal temperatures, individual 4-mm-diameter plugs cut from the advancing margin of a colony growing on CMA were transferred to plates containing fresh CMA poured to a standard thickness (20 ml of medium per 90-mm-diameter petri dish); plates were then incubated at 3-degree increments from 4 to 36 C for 4 days in the dark. Radial growth was measured daily for four replicate plates of each of 15 different isolates at each temperature. Sporangia were produced by cutting 4-mm-diameter disks from the advancing margin of a colony growing on V-8 juice agar (11) and floating these disks in plates containing 10 ml of 1.5% nonsterile soil extract (8) for 48 hr at 19 C under fluorescent light. Sporangia were then fixed and stained with acid fuchsin, and the dimensions of 40 sporangia for each of 11 representative isolates were measured.

To study the production of sex organs, isolates were grown for 4 wk at 22 C in the dark on an agar medium containing clarified V-8 juice, β-sitosterol, tryptophan, $CaCl_2$, and thiamine (13). Each isolate was grown on three separate plates, either paired with a known A_1 isolate of *P. cryptogea* Pethybr. & Lafferty (University of California, Riverside, Collection P1088), paired with a known A_2 isolate of *P. drechsleri* Tucker (University of California, Riverside, Collection P1087), or grown alone.

Pathogenicity tests. Previously described methods (8,14) were used to prepare inocula. Individual isolates were grown in sterile vermiculite moistened with V-8 juice broth in 1-L flasks for 5 wk at 22 C. The inoculum was then rinsed with sterile water to remove excess nutrients and added to a 1:1 (v/v) mixture of peat and sand at the rate of 50 cm^3 of inoculum per 1,000 cm^3 of peat:sand mixture. Control pots received the peat:sand mixture combined with uninfested vermiculite:V-8 broth mixture at the same rate.

Cranberry plants for these experiments were propagated by rooting sections of aerial shoots about 8 cm in length in a propagation bench, after which they were transplanted to 7.5-cm-diameter plastic pots containing the pasteurized peat:sand potting medium. When plants were approximately 2 mo old, they were

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evenly distributed into groups according to shoot length and then transplanted into individual 12.5-cm-diameter plastic pots containing the peat:sand:inoculum mixture. Plants were randomly distributed on the shelves of a growth chamber maintained at 22 C during the daytime and 16 C during the nighttime with a 14-hr photoperiod. Two weeks after transplanting and at 2-wk intervals thereafter, plants were flooded for a 48-hr period by transferring pots of each treatment group to a plastic container within the growth chamber and adding water until free water collected on the soil surface.

One set of controls consisted of uninoculated plants that also were flooded at 2-wk intervals, whereas in some experiments a second group of uninoculated plants was left unflooded in order to determine the effect of periodic waterlogging in the absence of the pathogen. All plants were routinely fertilized every 2 wk with full-strength Hoagland's solution (5) following each flooding episode.

In initial experiments to fulfill Koch's postulates, we examined 13 isolates identified as *P. cinnamomi* Rands, each of which was recovered from a different bed. The experiment was conducted twice and utilized the cultivar Early Black, with six replicate plants per treatment. In subsequent experiments to determine relative susceptibility of the cultivars Bergman, Crowley, Early Black, Franklin, Howes, Pilgrim, and Stevens, only the most virulent isolate, PC-12B, was used as inoculum. This experiment also was conducted twice and used 15 replicate plants per treatment.

RESULTS

Field symptoms and isolation of *Phytophthora* species. The disease typically occurred in low-lying spots in beds where water accumulated after rainfall or application for crop management purposes (for example, frost control, harvest operations, removal of organic debris). Individual areas of plants exhibiting dieback symptoms sometimes were as small as 1 m^2 but often were much larger, occasionally coalescing to cover an area of 10 ha or more (Fig. 1A). Cranberry plants at the centers of disease foci generally were dead, whereas remaining plants showed progressively diminishing symptoms of decline with increased distance from zones of plant mortality. Declining plants appeared pale green to reddish in color, often developed premature autumn coloration, and sometimes suffered significant defoliation during the growing season.

Symptomatic plants had brittle shoots and were easy to pull from the soil, revealing poorly developed root systems with few or no fine fibrous roots (Fig. 1B). When the periderm was removed, diseased roots or runners usually appeared dark (olive green to dark brown), in contrast with healthy roots which typically were light (white to yellow) (Fig. 2A and B). However, root discoloration was sometimes absent on plants from which the pathogen was isolated and, therefore, was unreliable as a sole diagnostic criterion.

By April 1989, the disease had been found throughout all cranberry-growing regions of Massachusetts and was documented in 215 individual beds, comprising about 25% of producing beds within the state. Symptoms were observed on cvs. Aviator, Black Veil, Centennial, Crowley, Early Black, Howes, McFarlin, and Stevens, which are planted on 95% of the commercial acreage in Massachusetts. When standard commercial practices have been used to replant these cultivars back into areas where dieback has occurred, the replants also have died.

Identification of the pathogen. Morphologically similar isolates resembling a *Phytophthora* species were recovered consistently from roots and underground runners of symptomatic cranberry plants. Each of 15 representative isolates was identified as *P. cinnamomi* Rands on the basis of coralloid mycelium; broadly ellipsoidal, nondeciduous,

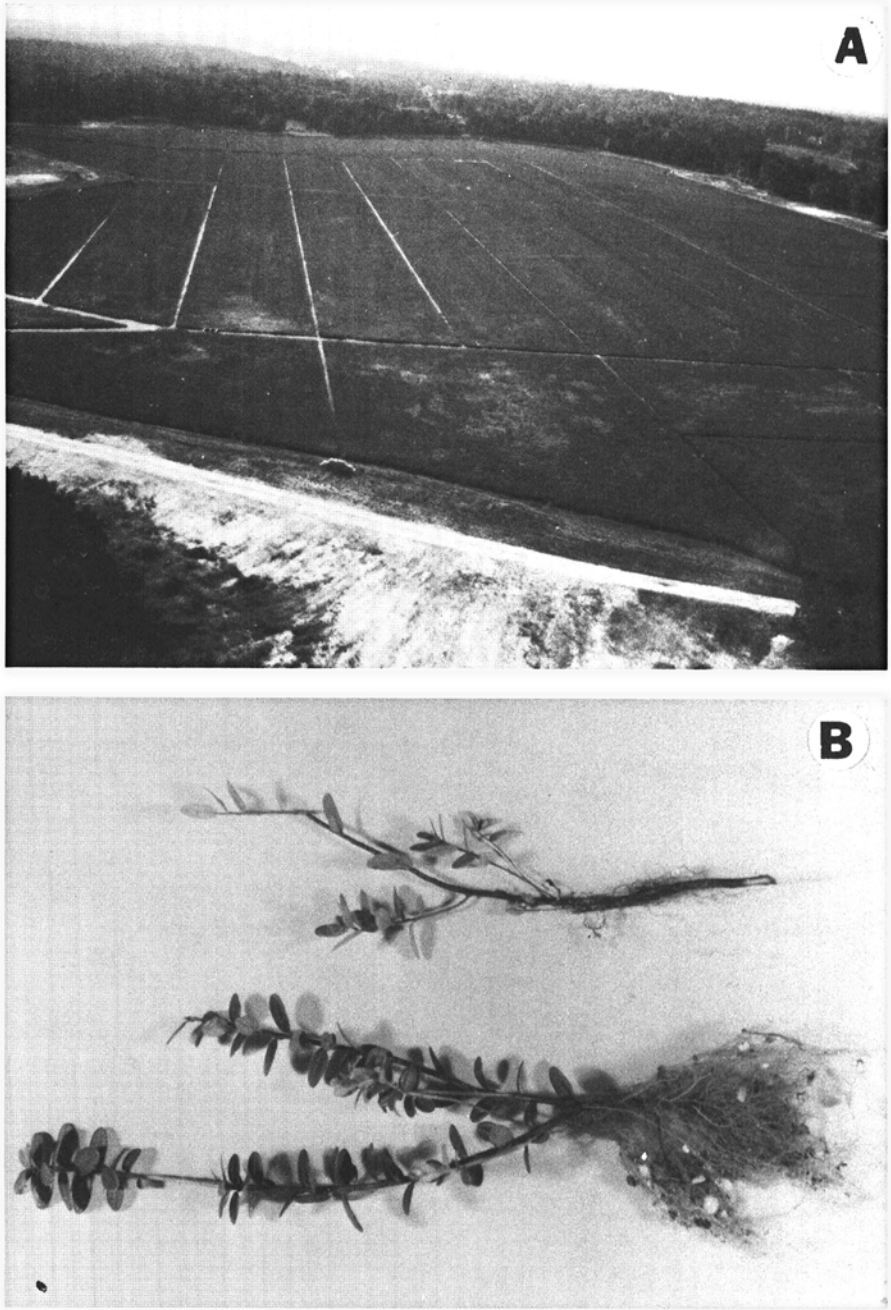

Fig. 1. Root rot and dieback of cranberry caused by *Phytophthora cinnamomi*. **(A)** Areas of dieback in a 12-ha cranberry bed in Wareham, Massachusetts, where *P. cinnamomi* was isolated and identified from symptomatic plants. Damage reportedly has been evident in this bed for 10 yr. **(B)** Infected (top) and healthy (bottom) cranberry plants. Diseased plants have poorly developed root systems with few feeder roots.

nonpapillate sporangia (av. 57.0 × 38.2 μm, range 49.4–62.4 μm × 32.0–40.8 μm); cardinal growth temperatures (minimum 4–7 C, optimum 28 C, maximum 33–36 C); and the production of chlamydospores but no sex organs in single culture (12). All isolates studied formed oospores only when paired with an A_1 mating type isolate of *P. cryptogea*.

Pathogenicity tests. All 13 isolates of *P. cinnamomi* were pathogenic to Early Black cranberry plants under experimental growth chamber conditions (Table 1). Forty-eight-hour flooding periods had no apparent effect on the growth of plants in uninfested potting mix. *P. cinnamomi* caused reductions in shoot and root weights of 48–88% and 83–96%, respectively, compared with the flooded control plants. Plant mortality was caused by only four of the isolates.

In tests examining relative disease susceptibility among cultivars (Table 2), root and shoot weights of the cvs. Bergman, Franklin, and Stevens were not significantly different from those of the controls. In contrast, root and/or shoot fresh weights of inoculated plants were significantly less than those of controls for the cvs. Crowley, Early Black, Howes, and Pilgrim. The greatest incidence of plant mortality occurred in cv. Pilgrim, followed by the cvs. Crowley and Howes, with eight, three, and one plant killed, respectively, of the 15 replicates used for each cultivar. Isolate PC-12B did not significantly reduce shoot growth of Early Black plants as it did in the experiments summarized in Table 1. However, cuttings of Early Black plants used in these two separate sets of experiments came from different cranberry beds, suggesting that certain strains or biotypes of Early Black may exist that are differentially susceptible to the pathogen.

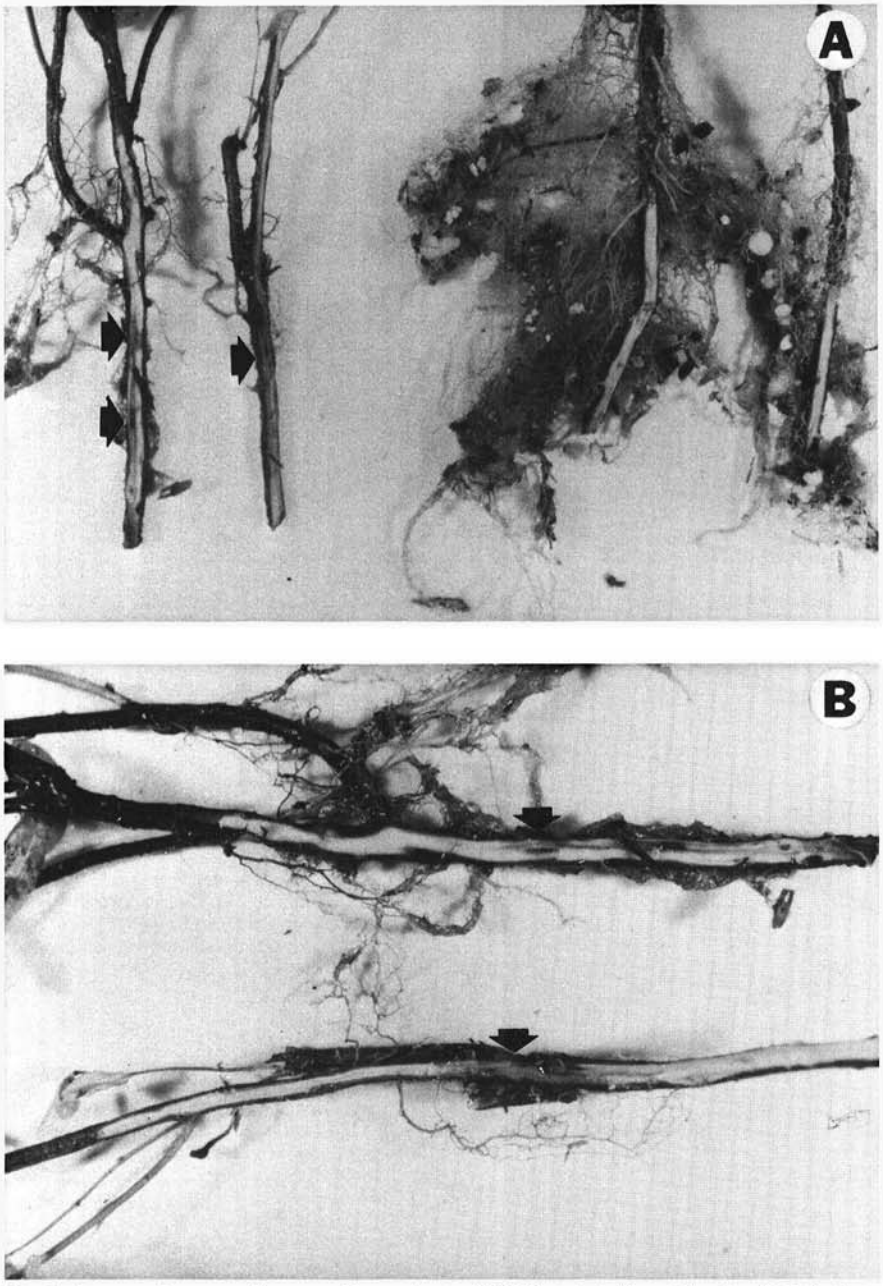

Fig. 2. Roots and runners from cranberry plants. **(A)** Infected (left) and healthy (right) plants with the periderm removed. Note the light coloration of the healthy runner tissue and the areas of discoloration in the infected runners (arrows). **(B)** Closeup of infected runners showing the dark discoloration (arrows).

DISCUSSION

This is the first study to document a *Phytophthora* species as a pathogen of cranberry, although *P. cinnamomi* is a well-documented pathogen of other ericaceous plants, including *Rhododendron* spp., highbush blueberry (*Vaccinium corymbosum* L.), rabbit's-eye blueberry (*V. ashei* Reade), and Lawson cypress (*Chamaecyparis lawsoniana* [Andr. Murray] Parl.) (15). Additionally, this is the first documented occurrence of *P. cinnamomi* on any host in Massachusetts, although the pathogen has been reported in nearby Rhode Island (15). *P. cinnamomi* also has been isolated recently from declining cranberry plants in six beds in New Jersey (A. Stretch, *personal communication*) but has not been detected in cranberry beds in Washington or Oregon (P. Bristow, *personal communication*) or in Wisconsin (S. N. Jeffers, *personal communication*). However, *P. megasperma* Drechsler and an unidentified species of *Phytophthora* recently have been associated with declining cranberry plants in Wisconsin, although their pathogenicity has yet to be determined (6).

It is possible that *P. cinnamomi* has long been an unrecognized cause of decline in Massachusetts cranberry beds, as growers report that some affected beds from which the pathogen was isolated have exhibited these symptoms since the 1940s. Such perennation in a climate like that of Massachusetts, where temperatures may remain significantly below freezing throughout much of the winter, would appear to be inconsistent with several laboratory and field studies suggesting that *P. cinnamomi* does not readily survive prolonged exposure to subfreezing temperatures (1,15).

However, it has long been standard practice in Massachusetts to flood cranberry beds to a depth of approximately 50–150 cm in mid-December to encase the vines in a layer of ice that prevents their desiccation when temperatures fall below freezing during the winter. This layer similarly insulates the soil from subfreezing air temperatures and therefore might provide an environment suitable for overwintering survival of *P. cinnamomi*. Cardinal temperatures for growth of our isolates of *P. cinnamomi* are typical of those reported for this species (12,15), suggesting that these isolates do not represent a unique low-temperature variant peculiarly adapted

Table 1. Pathogenicity and relative virulence of 13 isolates of *Phytophthora cinnamomi* to cv. Early Black cranberry plants[w] grown for 12 wk in artificially infested potting mix

Isolate	Fresh wt (g)[x]		Plants dead[z]
	Shoots	Roots[y]	
PC-12B	0.11 a	0.04 a	0
PC-18B	0.21 ab	0.03 a	0
PC-16C	0.21 ab	0.04 a	0
PC-15C	0.21 ab	0.03 a	2
PC-11A	0.22 ab	0.04 a	3
PC-14C	0.23 ab	0.05 a	0
PC-6B	0.25 ab	0.15 a	0
PC-13C	0.28 ab	0.05 a	2
PC-7A	0.40 ab	0.11 a	0
PC-8C	0.41 ab	0.19 a	2
PC-10B	0.45 b	0.18 a	0
PC-17A	0.46 b	0.04 a	0
PC-9A	0.47 b	0.15 a	0
Uninoculated, flooded	0.90 c	0.90 b	0
Uninoculated, unflooded	0.95 c	0.94 b	0

[w] Plants were periodically flooded for 48 hr at 2-wk intervals to simulate field conditions commonly associated with disease incidence. Portions of this table were published previously (2).
[x] Means of six replicate plants per treatment. Values in each column followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. This experiment was repeated once with similar results.
[y] *Phytophthora cinnamomi* was isolated from roots of all symptomatic plants but not from uninoculated plants.
[z] Number of plants dead out of six plants per isolate.

Table 2. Fresh weights of roots and shoots of seven cranberry cultivars[w] grown for 12 wk in potting mix artificially infested or not infested with *Phytophthora cinnamomi* isolate PC-12B

Cultivar	Shoots (g)[x]		Roots (g)[x]		Plants dead[y]
	Uninfested	Infested	Uninfested	Infested	
Bergman	0.52 + 0.18	0.50 + 0.17	0.27 + 0.11	0.24 + 0.10	0
Crowley	0.56 + 0.16	0.20 + 0.14***[z]	0.36 + 0.19	0.15 + 0.10***	3
Early Black	0.52 + 0.18	0.41 + 0.27	0.35 + 0.14	0.20 + 0.10**	0
Franklin	0.46 + 0.14	0.40 + 0.11	0.30 + 0.10	0.24 + 0.10	0
Howes	0.61 + 0.19	0.43 + 0.15*	0.53 + 0.25	0.42 + 0.16	1
Pilgrim	0.40 + 0.13	0.13 + 0.10**	0.21 + 0.11	0.15 + 0.11	8
Stevens	0.39 + 0.14	0.37 + 0.13	0.30 + 0.12	0.23 + 0.10	0

[w] Plants were periodically flooded for 48 hr at 2-wk intervals to simulate field conditions commonly associated with disease incidence.
[x] Means of 15 replicate plants per treatment.
[y] Number of plants dead out of 15 plants grown in infested soil per treatment. There was no plant mortality among flooded control plants of any cultivar. This experiment was repeated once with similar results.
[z] Mean values for the infested plants significantly different from the uninfested plants using a Student's *t* test, $P = 0.05$(*), $P = 0.01$(**), or $P = 0.001$(***).

to the climate of the Northeast. Rather, we hypothesize that *P. cinnamomi* was introduced into Massachusetts in soil or plant material from a region more conducive to its survival and has become established in a particular niche associated with a unique agricultural practice. Thus, avoidance of winter flooding may be a possible means of eradicating *P. cinnamomi* from heavily infested beds, although this has not yet been investigated.

In addition to winter, flooding of beds during spring and fall is a common horticultural practice employed to provide insect control, facilitate harvest operations, and remove organic debris. Such flooding episodes not only provide ideal conditions for the development of root rots caused by *P. cinnamomi* (10), but also are likely to provide a means for dispersing *P. cinnamomi* within and among cranberry beds. Circumstantial evidence for the significance of water dispersal is provided by our isolation of *P. cinnamomi* from more than 25 different beds along the Weweantic River, where a common water system is used to flood and drain each of these individual beds. Previous studies have demonstrated that other *Phytophthora* spp. can be contaminants in surface waters (4,9).

Currently, the control of Phytophthora root rot of cranberry is being attempted through the integration of several pest management strategies, including 1) improving surface drainage and adding large volumes of sand (7–14 cm in depth) to low-lying areas, thus reducing the incidence and duration of water-saturated episodes in the soil surrounding underground runners and roots, 2) increasing fertilization of declining plants at peripheral areas of decline, and 3) applying metalaxyl in spring and early summer and/or postharvest in the fall. Work continues on the evaluation of more than 60 cranberry cultivars for the relative resistance to root rot under field conditions and on techniques for improved management of water employed in horticultural operations.

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