Root Deterioration Associated with Verticillium Wilt of Horseradish

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ABSTRACT

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A root-fragmentation plating technique was used to analyze populations of microorganisms in root samples taken at regular intervals from naturally infested soil. No organisms were detected by this method in the root tissue at planting. Within 1 mo of planting, total internal bacterial populations rose to 1.5×10^4 colony-forming units per gram of tissue without noticeable alteration in appearance of the tissue. By the sixth month, bacteria were present at or above 1×10^6 colony-forming units per gram. The major component of these populations with pectolytic activity was similar to *Pseudomonas marginalis*. *Verticillium dahliae* was first detected in the root tissue 2 mo after planting. Several *Fusarium* species were isolated during the fifth month of disease development. *V. dahliae* is believed to be primarily responsible for initiation of disease. Bacteria apparently do not incite disease in the absence of *V. dahliae*. The fungal and bacterial infections apparently weaken the root tissue, allowing ingress of other pathogens such as *Fusarium* sp., resulting in rapid and complete breakdown of root tissue.

Verticillium wilt of horseradish (*Armoracia rusticana* Gaertn., Mey. & Scherb.) and the root deterioration associated with it have been known since at least the middle of the 19th century in Europe. In 1899, Sorauer (17) described the syndrome in detail but did not identify its cause. Potschke (16) was the first to show that *Verticillium* spp. were involved in the disease complex. Poole (15) attributed the soft rot phase of the disease to an unidentified bacterium and reported that it caused "very heavy losses" in storage pits. Klebahn (9) implicated bacteria, *Verticillium* spp., and an unidentified fungus as causal agents.

Verticillium wilt of horseradish, attributed to *V. albo-atrum*, was first reported in the United States in Michigan in 1931, where it was said to have caused a 20% loss in yield (2). In 1937, Heald et al (6) reported *V. dahliae* on horseradish in the western part of Washington state. No other outbreaks of the disease were reported in the United States until 1973, when a disease involving wilt and root deterioration resulted in substantial losses in Wisconsin and became a recurrent cause of reductions in yield and root quality.

The first indications of the disease are foliar symptoms typical of Verticillium wilt: temporary wilting of foliage at midday and chlorosis and necrosis proceeding inward from the leaf margins and often on only one-half of the leaf. Root infection is unique and is first evident as a black flecking in the vascular ring, which increases in density until the entire ring is involved. The tissue adjacent to the cambial ring becomes water-soaked with a yellow-brown or reddish cast. Root deterioration occurs rapidly during September. When plants with severe foliar symptoms are harvested, the only evidence of the set root is a black fibrous mass.

Horseradish is propagated commercially by planting root sections approximately 25 cm long and 1 cm in diameter. These are called "set" roots and normally enlarge in diameter twofold to fivefold to become the "process" roots that are used for preparing condiments. Shoots and secondary roots develop from the set root. The shoots develop one or more fleshy crowns from which leaves and new roots develop. Secondary roots range in diameter from 10 to 15 mm down to less than 1 mm, with fine feeder roots. The larger ones are saved for sets and the remainder, designated "trim," are processed. The most severe effect of the disease is on the process root upon which the value of the crop depends.

The objectives of this work were to isolate and identify the principal incitants of the root deterioration, to determine the sequence of microbial succession, and to determine the most feasible control method.

MATERIALS AND METHODS

Initial isolations were made from roots, crowns, and petioles of diseased plants obtained from the field at various times during the growing season. Small pieces of tissue were removed from affected areas, surface-sterilized, and pressed into an agar medium. Media used included water agar, potato-dextrose agar (PDA), and Nash-Snyder medium (12), each with 100 ppm of streptomycin sulfate and 100 ppm of choramphenicol, nutrient-dextrose agar (NDA), and nutrient agar (NA). Two or three small tissue pieces were ground aseptically with a glass rod in a tube with 3 ml of sterile distilled water, and a portion of the suspension was streaked onto NDA or NA plates. Bacteria from isolated colonies were streaked onto NDA or NA plates. Fungal colonies were transferred to PDA containing antibiotics, freed of contamination, and then maintained on slants of plates of PDA.

Inoculum of *V. dahliae* for pathogenicity tests was prepared by comminuting a 1-mo-old PDA culture in 300 ml of distilled water for 2 min in a Waring Blendor at high speed. The suspension was then passed through 300-, 150-, and 75-μm sieves without rubbing, thoroughly rinsed in cold running tap water, and used as inoculum. This inoculum consisted of 500–900 microsclerotia per milliliter, with a few hyphal fragments but no conidia. The concentration was determined by counting the number of microsclerotia in 0.01-ml droplets on glass slides at ×63.

Inoculum of *Fusarium* was grown on PDA plates for 2–3 wk. Two plate

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cultures of an isolate were suspended in 300 ml of distilled water by mixing for 2 min in a Waring Blendor at high speed. The suspension was then brought to a volume of 500 ml with distilled water. The inoculum, consisting of hyphal fragments, was quantified by plating dilutions of the inoculum on Nash-Snyder medium and counting colonies 4 days later. There were approximately 105 viable propagules per milliliter of inoculum.

Test plants were grown from horseradish set root sections, 3–5 cm long, which were dipped in 70% ethanol, soaked for 10 min in 0.6% sodium hypochlorite, rinsed in tap water, and planted into trays of sterile silica sand. They were grown in a greenhouse with the thermostat set at 24 C for 3–4 wk until shoots with roots formed. Plants were then gently removed from the sand and inoculated by dipping the feeder roots and set roots in the inoculum suspensions for approximately 15 sec. Control plants were dipped in distilled water. Plants were then grown in steamed muck soil in sterilized 10-cm plastic pots or in 10-cm waxed cups (475 ml) in a greenhouse at 24 C.

The presence or absence of infection was determined 60 days after inoculation by observing cross sections through the center of the set roots that were originally planted and longitudinal sections through the crown emerging from them.

Experiments were designed to simulate the root deterioration in field-grown plants, which only develops on relatively mature roots late in the season. The relationship between the development of microbial populations and of disease symptoms was determined on plants grown in a greenhouse at 20–24 C in naturally infested muck soil over 5–6 mo. Three 90 × 90 × 20 cm compartments on a greenhouse bench were filled with the soil that had been mixed thoroughly and screened to remove large pieces of crop debris. Approximately 60 root sections (25 cm long) were planted horizontally, 10 cm apart, and covered with about 10 cm of soil.

Six plants were selected at random at monthly intervals for 6 mo, and two sections from each set root were fragmented. All operations were done under a laminar flow hood. After thorough washing, the periderm was removed, and 2 cm of tissue at each end of the set root was discarded. A 1-cm section of the root was cut from the proximal and distal end of the root with a sterile razor blade. Each section was weighed, rated for disease severity, and cut into four to six sections with a sterile scalpel. The sections were dipped in 70% ethanol and soaked for 30 sec in 0.6% sodium hypochlorite solution. A Sorvall Omnimixer, fitted with a 70-ml stainless steel cup, was used to fragment the root tissue. The cup and rotor were sterilized with 70% ethanol and rinsed with sterile distilled water. The surface-sterilized root piece sections were transferred aseptically to the cup containing 40 ml of sterile 0.1 M phosphate buffer, pH 7.2, and the root was fragmented for approximately 1 min.

Aliquots of the resulting suspension or dilutions were plated on Nash-Snyder medium (for *Verticillium* and *Fusarium*) and NA (for total bacterial populations). A sterile, bent glass rod was used to spread the suspension over the surface. The plates were incubated at 20–22 C, and colonies were counted after 7 and 14 days and the colony-forming units (CFU) in the tissue calculated. This experiment was repeated during the winter of 1979–1980.

To determine whether *Verticillium* alone would cause the set root disintegration, we filled sixteen 20-L containers with soil treated with aerated steam at 60 C for 30 min. Half of this was infested with microsclerotia of *V. dahliae* isolated from diseased horseradish plants. Set roots were planted in May and dug and examined on 10 October. Isolations were made on crystal violet-pectate medium (3) and Menzies-Griebel medium, which favors *Verticillium* (11), from the set root or crown of 18 plants that showed evidence of root deterioration.

The extent of infection of the roots by *V. dahliae* was estimated visually by using a rating system consisting of six classes. Cross sections of roots were viewed, and vascular cylinder discoloration was rated on a 0–5 scale shown in Table 1. Generally associated with the black discoloration was brown water-soaking that extended from the vascular cylinder into the surrounding pith and cortex. The extent of this brown discoloration in the root cross section was expressed by a scale analogous to the one used for *V. dahliae* infection (Table 1). Each root was assigned a separate severity rating for *V. dahliae* infection and for severity of the browning symptom. Roots that had severity ratings of 2 or less for *V. dahliae* infection and/or brown water-soaking would be usable by the horseradish processor.

Pectolytic activity of bacterial isolates was tested by streaking each isolate on plates of crystal violet-pectate medium and by inoculating sterile horseradish root slices and potato tuber slices with bacterial suspensions.

To determine whether infection of horseradish plants by *Verticillium* could occur when the only contact between the developing plant and the inoculum was via uninjured feeder roots several centimeters from the set root, we used a modification of a previously described planting method (1). *V. dahliae* microsclerotia inoculum was added to a steamed mixture of muck soil and coarse sand (4:1) to achieve a final inoculum concentration of 36 microsclerotia per gram of soil. A 3-cm lower layer of infested soil and a 5-cm upper layer of noninfested, steamed soil were placed in each of ten 10-cm plastic pots. Horseradish sprouts, prepared from surface-sterilized, 6-cm-long set root sections grown in sterile silica sand for 2 wk, were gently floated out of the silica sand and rinsed in tap water. These were planted on the layer of noninfested soil, one per pot, and more noninfested soil was added. Horseradish sprouts were planted into 10 additional pots filled completely with noninfested soil and 10 filled completely with infested soil.

The plants were grown in a greenhouse at 24 C for 70 days and harvested, and the set roots, crown, petioles, and leaves were inspected for occurrence and extent of symptoms of *V. dahliae* infection.

RESULTS

When isolations were made from diseased field-grown plants, *V. dahliae* was invariably isolated from blackened areas of the root, crown, petiole, and leaf midrib. Several types of bacteria were isolated from the brown, water-soaked root tissue. The most common component with pectolytic activity appeared to be *Pseudomonas marginalis* (Brown) Stevens. Isolations from brown flecks in the center and cortex of set roots yielded the same bacteria, as well as *Fusarium roseum* (Link) Sn. & H. (var. *acuminatum*) and occasionally *Fusarium solani* ((Mart.) App. & Woll.) Sn. & H.

In the pathogenicity tests, the symptoms of infection by *V. dahliae* were unequivocal and easy to detect. Infected roots had black flecks that were restricted to the vascular cylinder. Vascular traces in the crowns and petioles were also blackened. *V. dahliae* was reisolated from diseased root, crown, and petiole tissue.

A high percentage of plants developed typical symptoms of Verticillium wilt when grown in soils infested individually

Table 1. Rating scale for severity of *Verticillium dahliae* infection and browning reaction in horseradish root cross sections

***Verticillium* severity (Black discoloration of vascular ring)**	**Severity value**	**Root browning severity (Brown water-soaked discoloration of vascular ring)**
None	0	None
Trace, a few flecks	1	Trace, few vascular traces
1/4 to 3/4 of ring	2	1/4 to 3/4 of ring
Entire ring flecked	3	Entire vascular ring
Entire ring blackened	4	Entire root cross section
Root decomposed	5	Entire root decomposed

with all isolates of *V. dahliae* obtained from horseradish. An isolate obtained from and pathogenic to potato (*Solanum tuberosum* L.) infected only one of 10 horseradish plants exposed to it, and an isolate from and pathogenic to velvetleaf (*Abutilon theophrastii* Medic) was not pathogenic to horseradish.

Infection by *Fusarium* spp. was evidenced by scattered brown flecks in the pith and cortex of the root and by red discoloration of the vascular system in the crown. In some cases, the original root piece had deteriorated to a dry, fibrous mass enclosed in what was originally the periderm. *F. roseum var. acuminatum* was isolated from the flecked roots and crown tissue of 42% of the plants. The one isolate of *F. solani* tested caused similar symptoms in 62% of the plants and was isolated from five of the six discolored crowns plated.

The effect of temperature on infection by three isolates of *F. roseum* var. *acuminatum* and *V. dahliae* on horseradish was determined; 100% infection by *F. roseum* var. *acuminatum* and *V. dahliae* occurred at 24 and 20 C, respectively. Infection by *Fusarium* was greatest between 24 and 28 C, and the *V. dahliae* was most aggressive between 16 and 20 C with 80 and 100% infection, respectively. At 32 C, growth and development of horseradish was poor and only 10% of the plants were infected.

Optimum mycelial growth of three selected isolates of *F. roseum* var. *acuminatum* and *V. dahliae* on potato-dextrose broth occurred at 24 and 20 C, respectively. Higher temperatures resulted in diminished growth.

Corn, the principal crop grown in rotation with horseradish, was a host for *F. roseum*. No disease symptom expression was visible in corn seedlings grown in muck soil artificially infested with *V. dahliae*, regardless of incubation temperature. Root rot occurred in corn seedlings grown in soil infested with *F. roseum* var. *acuminatum*, and infection increased with soil temperature. No disease was evident at 16 C, but 16, 24, 64, and 88% of the plants were infected at 20, 24, 28, and 32 C, respectively.

The sequence at which the various pathogens develop in the root tissue provides evidence of their relative importance in causation. The rate of development of populations of *V. dahliae*, bacteria, and *Fusarium* spp. in the root tissue of plants grown in naturally infested soil are illustrated in Figure 1. When the monthly samples of roots of plants growing in infested soil were plated, roots from the same lot of sets that had been stored at 4 C were also plated. Bacteria and fungi were never isolated from these roots.

V. dahliae, the first fungus detected in the root tissue, was initially recovered from roots assayed 2 mo after planting. At this time, there was an average of 7.1 CFU/g even though the roots showed no symptoms indicating the presence of the pathogen. By the third month, when Verticillium and root browning ratings averaged 2.3 and 2.4, respectively, *V. dahliae* populations averaged 2.2 CFU/g. By the fourth and fifth months, *V. dahliae* populations had increased to averages of 132 and 170 CFU/g, respectively. *V. dahliae* infection severity and root browning ratings averaged 2.3 and 2.1 for the fourth month and 2.5 and 2.8 for the fifth month, respectively.

Fusarium was not detected in root tissue until disease development was substantial (fifth month) and then only from roots that had deteriorated tissue with red to brown discoloration. Average populations were then 16 CFU/g.

Bacterial populations of about 1×10^4 CFU/g were present 1 mo after planting in infested soil, even though the roots showed no symptoms of infection. The average bacterial population doubled by the fourth and fifth months. By the sixth month, bacterial populations exceeded 4×10^6 CFU/g. The sudden rise in bacterial population coincided with an increase in severity ratings to 4.4 (*Verticillium* infection) and 4.5 (root browning). Although the root fragmentation assay did not detect bacteria in the set root tissue before planting, they have often been isolated from apparently healthy root tissue by the standard technique of plating whole tissue pieces (J. A. Percich, *unpublished*). They may have been present at levels below those detectable with the fragmentation technique.

To determine whether the problem could be controlled by means of resistant cultivars, we obtained set roots of 46 horseradish selections from A. M. Rhodes, University of Illinois, Urbana, in 1974. Six more selections were obtained in 1975. The selections originated from seed obtained by crossing horseradish varieties commonly used by commercial growers near East St. Louis, IL.

In 1975, the selections were screened for resistance to Verticillium wilt in four greenhouse experiments, and 16 selections

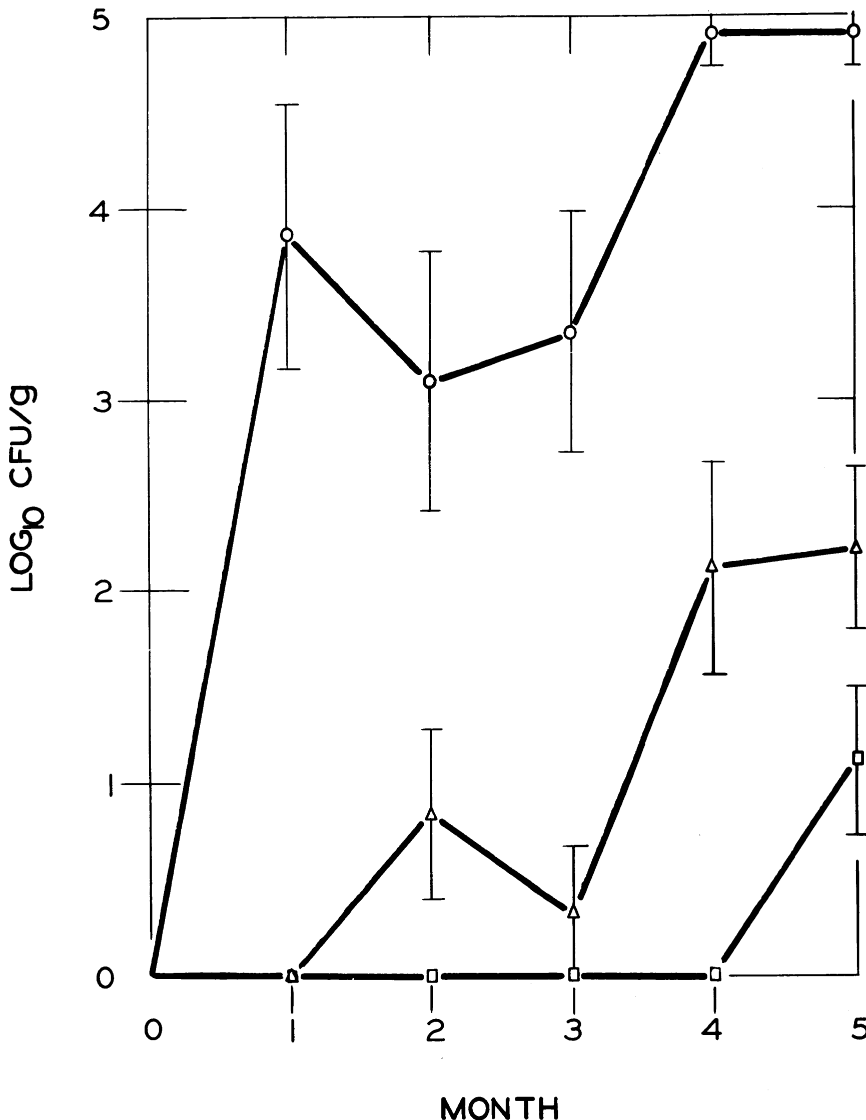

Fig. 1. Average colony-forming units of bacteria (O), *Verticillium* (△), and *Fusarium* (□) per gram of horseradish root tissue grown in naturally infested soil (plants grown at 20–24 C).

had markedly less disease than the very susceptible selections. Planting stock of these 16 selections was increased near Belle Glade, FL, during the 1975–1976 winter. In 1976, these selections were planted in a *V. dahliae*-infested muck field and a field of infested sandy loam soil in completely randomized blocks replicated four times at each location. The same 16 selections were retested in 1977 in the same two infested fields and also in a noninfested field in which horseradish had never been grown before.

The productivity of each selection in the infested and noninfested plots was evaluated in terms of average total yield per plant, the crown and process root yield per plant, and the set and trim yield per plant. Additional criteria of disease severity in plants from infested plots were severity of *V. dahliae* infection in the crown and process roots and in the proximal ends of the set roots and the extent of browning in the interior of the crowns and process roots and in the proximal ends of set roots. The disease severity systems were also used to rate these criteria.

Overall performance of each selection was determined by ranking the selections for each of the production and disease severity criteria. All the values for each selection were then summed, and this value was compared with those for all other selections.

Disease was almost always more severe in plants grown in the infested muck soil than in plants grown in the infested loam soil. At both locations, disease severity in the crowns was generally less than in the set, and disease in the proximal ends of the sets was more severe than in the distal ends. The field trials clearly demonstrated the potential of several horseradish selections for growth. The overall rankings for tissue productivity and disease severity of plants showed that when grown on infested soil all but one of the 16 selections were superior to the selections currently used by growers. Five selections were particularly outstanding in this respect. The value of some selections was apparent only on infested soil, because their productivity was below average in noninfested soil. One selection was very productive on noninfested and infested soils but, unfortunately, still had an appreciable amount of infection when grown on infested soil.

The original set root deteriorated completely in the plants of most selections grown in infested fields. These plants consisted of multiple crowns and lateral roots, with no main process root remaining. In contrast, most of the plants grown in the noninfested soil had intact set roots. It was evident that none of the selections had sufficient resistance to grow in soil infested with *Verticillium*.

The efficacy of chemical control was explored by using the soil fumigant Vorlex (20% methyl isothiocyanate, 80% chlorinated C_3 hydrocarbons including dichloropropenes, dichloropropane, and related chlorinated hydrocarbons), and benomyl. Both are known to be active against *Verticillium*. Vorlex was applied at 234, 468, and 701 L/ha (25, 50, and 75 gal/acre) on muck soils and at 168, 355, and 496 L/ha (18, 38, and 53 gal/acre) on loam soils to plots four rows wide and 30 m long. Each treatment was replicated four times at each site. Benomyl (50% WP) was applied by dipping sets for 10 min before planting in a suspension containing 1% benomyl at pH 2.0 or by drenching each set after planting with 500 ml of a suspension containing 1.6 g of benomyl and 0.25 g of Tergitol-NPX. Evidence of disease was never reduced significantly by chemical treatment, and there was no indication that this approach offers any hope of a practical means of control.

Avoidance of the problem by using noninfected planting material seemed to be the only practicable approach. We therefore explored the possibility of developing a reliable system to detect and eliminate infected sets on the basis of visible disease symptoms. Ten sets were taken from each of 36 boxes (500 sets per box). Each set was sliced and the proximal, middle, and distal end of the set visually rated as infected or healthy.

Roots that had distinct black flecks in the vascular ring, varying from one fleck to complete ringing of the vascular cylinder, were counted as infected. These flecks were sometimes accompanied by yellow discoloration around the flecked area.

The reliability of the visual rating system was compared with that of isolation of the pathogen from tissue. Segments of 268 roots were surface-sterilized and plated on water agar plus antibiotics, PDA plus antibiotics, and NDA. The results are summarized in Table 2. Symptoms were evident in the proximal end of 99% of the roots that had visual evidence of infection. When a subsample of the roots (268 roots) was plated, *V. dahliae* was isolated from 2.4% of the roots that were visually judged to be healthy and from 61% of the roots visually judged to be infected.

Infection generally first appeared at the proximal end of the set. If an infected proximal end is encountered, there is, on the average, an 84% chance that the middle of the set is infected and a 75% chance that the distal end is also infected. On the other hand, if the proximal end of the set appears healthy, there is, on the average, only a 0.6% chance that the middle or distal end is infected.

DISCUSSION

The isolations from diseased tissue made it apparent that several organisms may be involved in decomposition of horseradish root. The root fragmentation assay provided information on the relative levels of these organisms at various stages of disease development and made it possible to interpret the significance of the progression of organisms.

At planting, low levels of bacteria were present in many of the set roots. When the root tissue became active, these bacteria proliferated but did not incite disease. Infection by *V. dahliae* occurred during very early stages of plant development as the first feeder roots encountered inoculum or via wounds caused by emerging crowns and feeder roots. Additional bacteria may also enter by these routes. As *V. dahliae* proliferated systemically, the vascular tissues of the plant became progressively more disorganized. Resident bacterial populations increased and further weakened the root tissue. As the vascular tissue deteriorated, adjacent tissue became weakened and water-soaked, and other pathogens, notably *Fusarium* spp. and pectolytic bacteria, normally present in the cortex were able to proliferate and accelerate the deterioration of the root.

Effective control of horseradish root rot must be based on control of *V. dahliae*, which plays the major role in initiating disease development and appears to be responsible for allowing proliferation of other pathogens involved in later stages of root deterioration.

Control measures must be effective enough to reduce the amount of inoculum in the soil to very low levels. In view of the work with benomyl and Vorlex reported here and elsewhere, chemical control procedures cannot reduce *V. dahliae* inoculum levels sufficiently to result in adequate disease control. Rotation is an impractical means of control because *V. dahliae* survives for very long periods in the absence of the host crop (5,7,8,10,11,13,14,18).

One of the most important reservoirs of inoculum in infested fields is the "volunteer" horseradish plants that persist for many years after this crop is grown. Many small roots are left in the ground at harvest, and each of these can

Table 2. Patterns of symptom development in two samples of horseradish set roots infected with *Verticillium dahliae*

Infection pattern[a]	Percent of roots with pattern		
	Sample		
	1	2	Average
H-H-H	55.6	56.2	55.8
I-H-H	8.6	5.3	7.1
I-I-H	4.9	2.1	3.5
I-I-I	30.3	35.6	32.9
H-I-I	0.3	···	0.1
H-H-I	···	0.6	0.3
H-I-H	···	0.3	0.1
Total	100	100	100

[a] Three letters refer to proximal end, middle, and distal end of sets, respectively. H = healthy, I = infected (ie, symptoms present).

give rise to new plants that can harbor the pathogen and increase its population. Only by persistent use of herbicides or meticulous cultivation can these plants be eliminated.

None of the horseradish selections tested showed evidence of significant resistance. Although genes for resistance may exist, no material has been seen that offers encouragement. Breeding horseradish is a relatively difficult and time-consuming process that may not be warranted for this highly specialized crop.

The origin of the infestations with the strain of *V. dahliae* pathogenic to horseradish is not known. Intense culture of horseradish may have applied selection pressure on a native *Verticillium* population in favor of strains capable of parasitizing that plant. If this happened, it is surprising that it did not occur sooner and in other horseradish-growing areas. Alternatively, the pathogen may have been introduced in infected planting stock obtained from other areas. Strains pathogenic to horseradish may have been introduced on potato seed tubers, because potatoes are grown by the horseradish growers. Both the microsclerotial and the dark mycelial forms of *Verticillium* are carried by certified seed potatoes from Canada and 10 states, including Wisconsin (4). In limited tests, however, cross infection by strains from these two crops has not been found to occur.

Planting infected sets on noninfested land introduces *Verticillium* in the form that most favors its successful establishment. Once introduced, the infected plant material serves as a source of *Verticillium* propagules, which can be disseminated in a variety of ways.

A central element in any strategy for control of this problem must be prevention of spread of the pathogen. Strict sanitation measures must be used in all aspects of horseradish production. Growers should require "zero tolerance" for *Verticillium*-infected sets. All horseradish fields should be surveyed frequently to locate all areas of infestation. Soil samples should be tested for the pathogen before horseradish is planted. The costs of implementing these sanitation and avoidance measures would be substantial, but they are absolutely essential whenever infested land is under cultivation.

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