Verticillium dahliae: A Causal Agent of Root Discoloration of Horseradish in Illinois

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ABSTRACT

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In a survey of horseradish roots taken from commercial production fields in southwestern Illinois over 3 yr, *Verticillium dahliae* was found to be the primary pathogen associated with symptoms of root discoloration. The pathogen was isolated from 75% of roots showing symptoms of vascular discoloration, and from 55 and 56% of roots with symptoms of localized internal necrosis and rotting, respectively. *V. dahliae* was isolated from only one of the 54 asymptomatic roots that were assayed. Other species of bacteria and fungi, including *Fusarium solani,* also were isolated from horseradish roots, but none were consistently associated with specific symptoms. The inoculation of greenhouse-grown horseradish plants with *V. dahliae* resulted in vascular discoloration symptoms similar to those observed on field-grown plants.

"Schwarzwerden," or black discoloration, was used to describe a condition of horseradish (*Armoracia rusticana* P. Gaertn., B. Mey. & Scherb.) in Germany as early as 1895, and was further discussed by Sorauer in 1899 (11). Sorauer found that a fungus was associated with the disease, but it was not until 1923 that a species of *Verticillium* Nees was reported as a causal agent of the disease (10). Shortly thereafter, the fungus was identified as *Verticillium dahliae* Kleb. (2,5–7) from both Europe and the United States. Mueller et al (8) found that *V. dahliae* was the primary agent responsible for initiating root deterioration in horseradish grown in Wisconsin, but Percich and Johnson (9) later reported that three organisms, *Fusarium roseum* Link:Fr. emend. Snyder & Hansen 'Acuminatum', *V. dahliae,* and *Pseudomonas fluorescens* Migula were involved in the root rot complex.

Approximately half of the total commercial U.S. horseradish crop is grown in the Mississippi River Valley near East Saint Louis, Illinois. Over the past 10–15 yr, the horseradish growers in this area have experienced significant reductions in marketable yield as a result of discoloration and rotting of roots. Some of the most productive fields have since become unusable for horseradish production because of the discoloration problem. The objective of this study was to survey horseradish production fields in southwestern Illinois to determine the distribution and the primary cause of internal root discoloration of horseradish grown in this area.

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MATERIALS AND METHODS

Sampling and isolation. Symptomatic and asymptomatic horseradish roots were collected from commercial production fields and packing sheds during the harvest periods of 1989 (only symptomatic roots), 1990, and 1991. A total of 42 roots representing four varieties were taken from eight locations in 1989, 151 roots representing five varieties from 10 locations in 1990, and 125 roots representing five varieties from five locations in 1991. Each root was washed, sectioned, and evaluated for symptoms. Following the terminology used in the horseradish industry, roots were categorized as "clean" for asymptomatic roots; "pepper" for roots with symptoms of vascular discoloration; "peg" for roots with localized, internal pockets of necrotic tissue; and "rot" for roots with internal areas that had begun to decompose (Fig. 1). Roots exhibiting more than one symptom were recorded in each of the appropriate categories. Small pieces of diseased tissue were taken from the roots, soaked in 0.5% sodium hypochlorite for 30–90 sec, rinsed in sterile distilled water, and placed on the surface of acidified potato-dextrose agar (APDA). Fungi were identified either from the original isolation colonies or from colonies transferred onto potato-dextrose agar (PDA). Representative samples from different bacterial colony types were streaked on nutrient agar to obtain single-colony isolates, and then identified using the Biolog, Inc. (Hayward, CA) bacterial identification system.

Pathogenicity. The pathogenicity of four selected cultures, isolated from horseradish and identified as *V. dahliae* (3,4), were tested on horseradish plants grown in the greenhouse using two different inoculation methods. In the first method, 200 g of oat seed was combined with 200 ml of distilled water, autoclaved at 121 C for 60 min, and after 24 hr autoclaved a second time for 60 min. These sterilized oat seeds were inoculated with 2- to 3-wk-old cultures of *V. dahliae* and allowed to incubate at room temperature (22 to 24 C) for 3 wk with occasional mixing. Colonized oats were then air-dried and used to infest a soil:peat:perlite mix (1:1:1) at the rate of 10 g of inoculum per liter of potting soil. Segments of horseradish root cv. 647 (approximately 2 cm diameter by 10 cm long) were washed in tap water, dipped in 70% ethanol for 15 sec, soaked in 0.5% NaOCl for 5 min, and rinsed in tap water. Root segments were then planted in the infested soil in 15-cm clay pots. The pots were placed in the greenhouse at 25–30 C, under a combination of 1,000-W high-pressure sodium- and mercury-vapor lamps, with a 12-hr/day photoperiod. Controls were planted in noninfested potting soil with and without a noncolonized oat-seed amendment.

For the second inoculation method, segments of horseradish root were surface disinfested as described above, planted in flats containing potting soil, and placed in the greenhouse. After the set roots had produced lateral roots and a few leaves (approximately 4 wk), the plants were removed from the flats, most of the soil was washed from the roots, and the lateral root systems were trimmed to 6 cm. PDA slant cultures of *V. dahliae* were macerated in small amounts of sterile distilled water, spread onto the surface of PDA in 90 × 15 mm petri dishes, and allowed to incubate at room temperature (22–24 C) for 3–4 wk. These cultures were then blended with distilled water at high speed for 2 min. The resulting suspension was passed through a series of 250-, 180-, and 44-μm sieves. The material (mostly microsclerotia) on the 44-μm sieve was rinsed with tap water, collected from the sieve, and resuspended in water to obtain a final concentration of 500 microsclerotia per milliliter. The trimmed root systems were dipped in the suspension of microsclerotia for 15 sec and then planted in 15-cm clay pots. The pots were placed in the greenhouse and incubated under the conditions described above. Control plants with both trimmed and nontrimmed roots were dipped in sterile water prior to planting.

With both inoculation methods, the main set roots were recovered, sectioned, and evaluated for symptoms 4 mo after inoculation. Representative samples of

symptomatic tissue were surface disinfested and placed on APDA to reisolate the pathogen. Four isolates of *V. dahliae* were used in each method, with five replicates (each replicate was one plant in one pot) per isolate per method. The experiment was repeated once using only the oat-seed inoculum method, a mixed-isolate inoculum, and 10 replicates (plants) per treatment.

RESULTS

In 1989, only symptomatic roots were assayed, and in 1991 no roots showing the rot symptom were detected. *V. dahliae* was isolated from 75.0% of roots with vascular discoloration (pepper) over a 3-yr period (Fig. 2). *Fusarium solani* (Mart.) Sacc. emend Snyder & Hansen was isolated from 7.7% of the same roots. *V. dahliae* also was isolated from roots showing the peg symptoms and from roots with internal rot at frequencies of 55.4 and 55.9%, respectively. *F. solani* was isolated from 14.9% of the roots showing peg symptoms and from 14.7% of roots with internal rot. *V. dahliae* was isolated from one of the 54 asymptomatic (clean) roots, while *F. solani* was isolated from 13.0% of the asymptomatic roots.

Several other fungi, subsequently identified as species of *Aspergillus* P. Mich. ex Link:Fr., *Gliocladium* Corda, *Mucor* P. Mich.:Fr., *Penicillium* Link:Fr., *Phialophora* Medlar, and *Rhizoctonia* DC., also were observed growing on the isolation medium from both symptomatic and asymptomatic root tissues. None of these were consistently associated with particular symptoms. Several different colony types of bacteria were also observed growing from both symptomatic and asymptomatic root tissues on APDA. Isolates of the most prevalent colony types were identified using the Biolog system. Several isolates were identified as *P. fluorescens,* and one isolate was identified as *Erwinia carotovora* var. *carotovora* (Jones) Bergey et al, but the attributes of many of the other isolates did not match well with entries in the Biolog database and were not identified. The strain of *E. carotovora* isolated from horseradish caused a soft rot of both potato and horseradish when streaked onto the cut surfaces of tubers and roots, respectively (R. J. Chang and D. M. Eastburn, *unpublished*).

Systemic vascular discoloration symptoms were consistently reproduced on inoculated plants using both the oat-seed and root-dip methods with each of the four isolates of *V. dahliae* tested (Table 1). Some vascular discoloration also was observed on a few of the noninoculated roots. However, this was usually a brown discoloration, as compared to the gray-black discoloration that was normally associated with infection by *V. dahliae,* and the discoloration was often restricted to those areas near the cut ends of the root segments. *V. dahliae* was reisolated from an average of 43% of all the inoculated root segments (combining results from each of the isolates and methods of inoculation) in the first trial, all but one of which had symptoms of vascular discoloration (Table 1), and was not reisolated from any of the noninoculated roots. In the second trial, 90% of the inoculated roots were discolored, while none of the noninoculated roots had symptoms of internal discoloration. *V. dahliae* was isolated from 40% of the inoculated roots but was not reisolated from any of the noninoculated roots.

DISCUSSION

The high frequency of isolation of *V. dahliae* from field-grown roots with vascular discoloration (pepper) and the less frequent but consistent isolation from roots with localized internal necrosis (peg) and internal deterioration (rot) suggests that *V. dahliae* is the primary causal agent of the root discoloration problem

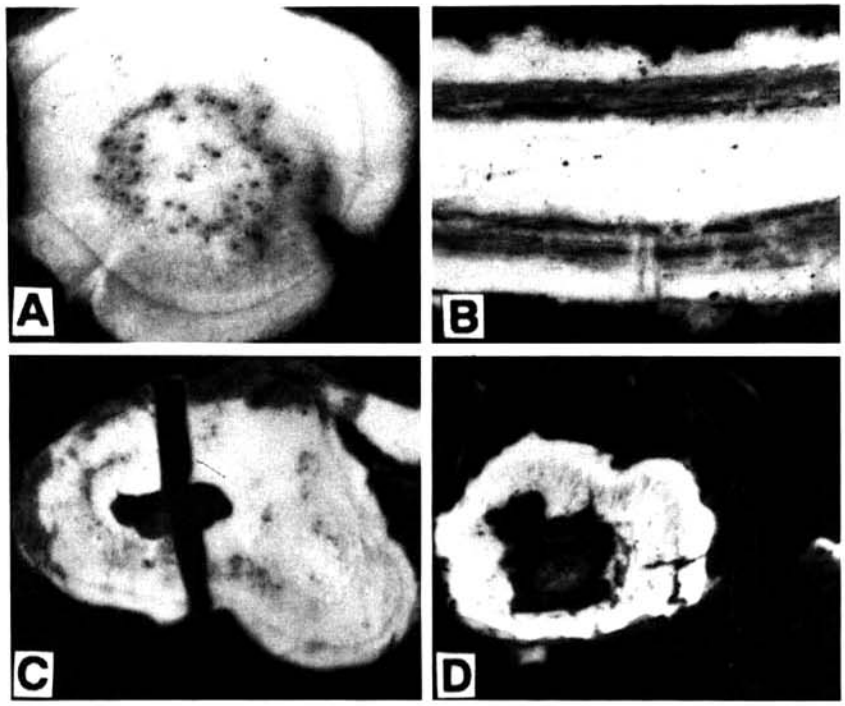

Fig. 1. The range of symptoms associated with internal root discoloration of horseradish: **(A)** cross section of a root showing vascular discoloration (pepper), **(B)** longitudinal section of a root showing vascular discoloration, **(C)** localized necrotic tissue (peg), and **(D)** internal rotting of root tissue.

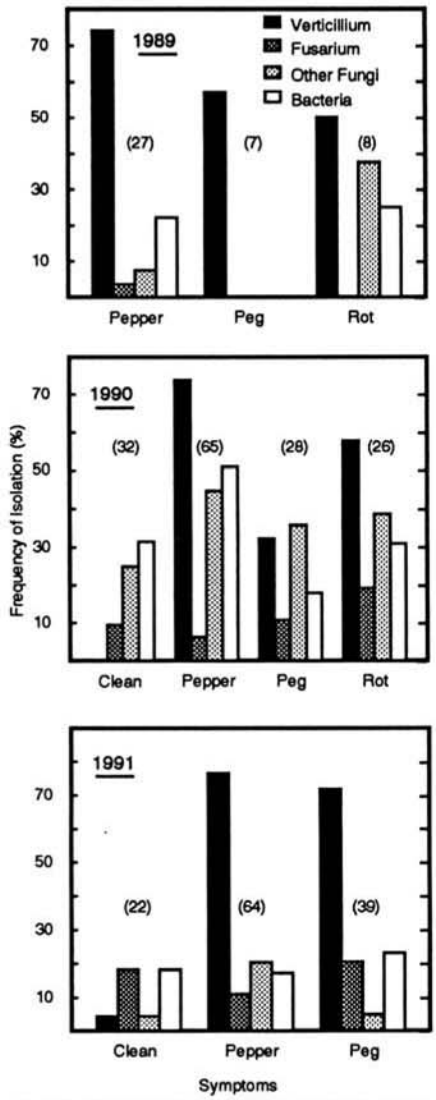

Fig. 2. Frequency of isolation of *Verticillium dahliae, Fusarium* spp., and various other fungi and bacteria from horseradish roots sampled from commercial production fields in southwest Illinois over 3 yr. The number of roots assayed in each symptom category for each year are indicated in parentheses. Clean = asymptomatic roots; pepper = roots with symptoms of vascular discoloration; peg = roots with localized, internal pockets of necrotic tissue; and rot = roots with internal areas that have begun to decompose.

Table 1. Frequency of root discoloration and reisolation of *Verticillium dahliae* from horseradish roots artificially inoculated by the oat-seed or root-dip method

Isolate	No. of roots	Root discoloration (%)		Pathogen reisolation (%)	
		Oat	Dip	Oat	Dip
HR0001	5	100	80	20	20
HR0004	5	100	100	20	80
HR0006	5	100	100	20	100
HR0015	5	100	100	0	80
Control - oat trim[a]	5	0	0	0	0
Control - no oat/no trim[b]	5	40[c]	0	0	0

[a] Noninoculated treatments using either noncolonized oats (oat-seed method) or trimmed but not inoculated roots (root-dip method).
[b] Noninoculated treatments using no oat seed (oat-seed method) or nontrimmed roots (root-dip method).
[c] Discoloration symptoms distinct from those observed on roots inoculated with *V. dahliae*.

in the commercial horseradish-growing region of Illinois, and that it may be an important component of a complex of organisms which cause root deterioration. Mueller et al (8) found that *V. dahliae* also was the primary pathogen associated with root deterioration of horseradish in Wisconsin but later proposed that *V. dahliae, F. roseum* cv. Acuminatum, and *P. fluorescens* were all associated with the root rot complex (9). However, Percich and Johnson (9) stated that Acuminatum could have been a secondary invader, following *V. dahliae* in the disease complex. Acuminatum was never isolated from horseradish roots in the present study. Although *F. solani* was isolated from field grown horseradish roots, the frequency of isolation from asymptomatic roots was similar to that from roots with symptoms. The overall frequency of isolation for *F. solani* was never greater than 21% for any symptom category. Therefore, there is no strong evidence that *Fusarium* spp. play a major role in disease development. *V. dahliae* was consistently isolated from symptomatic roots and was isolated only once from an asymptomatic root, which was from a field that had a very high incidence of root discoloration.

F. solani and *Trichoderma* spp. were commonly recovered from roots grown in steam-sterilized potting mixes in the greenhouse, and *F. solani* was consistently associated with a brown discoloration of vascular elements near the cut ends of these set root segments. The rapid growth of these fungi on isolation media made it difficult to reisolate the slower growing *V. dahliae* and was probably the primary reason for the lower than expected frequency of recovery from inoculated roots grown in the greenhouse. More recent attempts to isolate *V. dahliae* from discolored sections of petioles taken from inoculated plants, and the use of ethanol medium (1), have resulted in a substantial reduction in contamination by *F. solani* and *Trichoderma* spp. and an increase in the percent recovery of *V. dahliae* (R. J. Chang and D. M. Eastburn, *unpublished*).

As with previous studies (8,9), the artificial inoculation of horseradish roots with *V. dahliae* resulted in typical symptoms of vascular discoloration, but the peg or rot symptoms that are also observed in the field did not develop. Therefore, other organisms may be responsible for these phases of the disease. The frequent isolation of *V. dahliae* from tissues with peg and rot symptoms suggests that infection by *V. dahliae* may have preceded the development of these additional symptoms. Further research on the causes of these distinct symptomologies is warranted.

It was common to isolate bacteria, including *P. fluorescens,* from both clean and symptomatic root tissues. Dual inoculations of these bacteria with *V. dahliae* need to be conducted to determine the role of these bacteria in the development of symptoms. However, a culture identified as *E. carotovora* isolated from a horseradish root was able to cause a soft rot of both horseradish roots and potato tubers, which supports the hypothesis that bacteria could be involved as secondary pathogens.

Foliar symptoms have been used as an initial indicator of infection by *V. dahliae* (8), but we found the development of foliar symptoms unreliable. When characteristic foliar symptoms were observed, vascular root discoloration was almost always present, but in several instances root discoloration was evident on plants that had no apparent foliar symptoms, and *V. dahliae* was isolated from these roots. The absence of foliar symptoms, therefore, does not necessarily indicate the absence of root discoloration caused by *V. dahliae.*

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