Plant Pathology (2004) **53**, 669–676 Doi: 10.1111/j.1365-3059.2004.01070.x

Verticillium longisporum and *Fusarium solani*: two new species in the complex of internal discoloration of horseradish roots

M. Babadoost[a]*†, W. Chen[b], A. D. Bratsch[c] and C. E. Eastman[d]

[a]*Department of Crop Sciences, University of Illinois, Urbana, IL 61801;* [b]*USDA-ARS, Department of Plant Pathology, Washington State University, Pullman, WA 99664;* [c]*Department of Horticulture, Virginia Polytechnic Institute, Blacksburg, VA 24061; and* [d]*Illinois Natural History Survey, Champaign, IL 61820, USA*

This study was conducted to determine the causal agent(s) of internal discoloration of horseradish roots. In 1999, 133 roots from 31 fields, and in 2000, 108 roots from nine fields, were assayed to determine the incidence and severity of internal discoloration of horseradish roots as well as the pathogen(s) associated with discoloured tissue. *Verticillium dahliae*, *Verticillium longisporum* and *Fusarium solani* were isolated from 14, 16 and 23% of roots in 1999, and from 24, 20 and 19% of roots in 2000, respectively. *Verticillium longisporum* on horseradish was identified for the first time. Pathogenicity tests of isolated microorganisms were conducted on horseradish in the glasshouse. In one experiment on the susceptible cultivar 1573, roots (sets) were inoculated by dipping the sets in a suspension of either *V. dahliae* microsclerotia, *V. longisporum* microsclerotia, or *F. solani* conidia and then grown in a soil mix over 5 months. Plants inoculated with any of the three species developed root discoloration similar to that observed in commercial fields. Internal root discoloration symptoms developed over a period of 5 months. For all three pathogens, severity of root discoloration was significantly higher after 5 months compared with 2 months from inoculation. In another experiment on cultivar 1590, tissue culture-generated seedlings and sets were planted in an infested soil mix with *V. dahliae* or *V. longisporum* and grown in the glasshouse. Plants developed root discoloration, as observed in the field. The pathogens were reisolated from inoculated plants in both experiments. No pathogen was isolated from the control plants in the experiments. The results of this study suggest that internal discoloration of horseradish roots is a disease complex caused by at least three fungal species.

Keywords: *Armoracia rusticana*, soilborne disease, vascular discoloration, verticillium wilt

Introduction

Horseradish (*Armoracia rusticana*) is grown for its white, fleshy and pungent roots, and is used as a condiment (Kadow & Anderson, 1940; Courter & Rhodes, 1969). Approximately 50% of the total commercial horseradish produced in the USA (12 million kg on 1500 ha) is grown in the Mississippi River Valley, near East St Louis, Illinois (Rhodes, 1977; Babadoost *et al*., 2001). Two other major horseradish-production areas are Eau Claire, Wisconsin and Tule Lake, California.

Internal discoloration of roots (Fig. 1) has been a serious problem for horseradish production in Illinois as well as other areas (Potschke, 1923; Stark, 1961; Gerber *et al*., 1983; Eastburn & Chang, 1994; Babadoost & Islam, 2002). In Illinois horseradish roots (sets) are planted in April or May, harvested in October or later, and stored at 0–4°C until they are processed or planted. Internal discoloration of roots becomes more evident in August and September in the field (M.B., unpublished data). Internal discoloration of the root begins with dark brown to black discoloration of the vascular system and gradually spreads to inward (core) and outward (cortex) areas in the root (Mueller *et al*., 1982; Gerber *et al*., 1983; Percich & Johnson, 1990; Eastburn & Chang, 1994; Babadoost *et al*., 2001). Internally discoloured horseradish roots are not acceptable for processing, and are discarded. Internal root discoloration is usually followed by root rot (Mueller *et al*., 1982; Gerber *et al*., 1983; Percich & Johnson, 1990).

Verticillium dahliae has been reported as the primary causal agent of internal discoloration of horseradish roots

*To whom correspondence should be addressed.

†E-mail: babadoos@uiuc.edu

Accepted 28 May 2004

(Percich & Johnson, 1990; Eastburn & Chang, 1994; Atibalentja & Eastburn, 1998). *Fusarium*, *Pseudomonas* and *Erwinia* species have also been found associated with discoloured roots in Illinois and Wisconsin (Percich & Johnson, 1990; Eastburn & Chang, 1994). Percich & Johnson (1990) reported discoloration and rot of horseradish roots as a complex disease incited by *Fusarium roseum* '*acuminatum*', *Verticillium dahliae* and *Pseudomonas fluorescens*.

During 1999–2002 *Verticillium* and *Fusarium* species were frequently isolated from internally discoloured horseradish roots grown in commercial fields in Illinois. *Verticillium longisporum* comb. nov. (Karapapa *et al*., 1997), reported on cruciferous plants from several crucifer-growing areas of the world, was identified for the first time on discoloured horseradish roots. The present study was conducted to determine the association of *Verticillium* and *Fusarium* species with horseradish roots showing internal discoloration, and to determine their pathogenicity.

Materials and methods

Field survey

Field surveys were conducted in 1999 and 2000 to determine the incidence and severity of internal discoloration of horseradish roots in commercial fields in Illinois. In 1999, root samples of nine different horseradish cultivars, grown in 31 commercial fields in the Mississippi River Valley near East St Louis (Madison and St Clair Counties) and Decatur (Macon County), Illinois, were collected at harvest during autumn (Table 1). The roots were selected at random. Collected roots were stored at 4°C (up to 4 weeks) until they were assayed for symptoms and tested for the presence of fungi and bacteria. Three to six roots (each root representing one plant) from each field were evaluated for incidence and severity of internal discoloration. The roots were washed under tap water, blotted dry, cut into five sections of equal length, and rated for severity of the symptoms on the cross-sections. Severity of discoloration was rated as 0 = no discoloration; 1 = 1–10; 2 = 11–25; 3 = 26–50; and 4 = 51–100% discoloration of vascular tissue, core and cortex separately in the cross-section. The root sections were then used for pathogen isolation.

In September 2000, nine horseradish fields in the East St Louis growing area were surveyed. In each field, 10 sampling sites were randomly selected by walking across the longest diagonal of the field. At each sampling site, five randomly selected plants were dug up and the roots were collected and examined for the incidence of discoloration of vascular tissue, core and cortex in the cross-section. A total of 108 roots were used for pathogen isolation.

Pathogen isolation

Roots collected during the surveys were used to isolate associated pathogens. Root sections (5 cm long) were cut and peeled. The sections were surface-sterilized by soaking in 5·25% NaOCl (full strength commercial bleach) solution for 1 min, followed by soaking in 95% ethanol for 2 min. Each surface-sterilized section was then rinsed three times in sterile distilled water (SDW). The section was blotted with sterilized blotter paper and cut into *c*. 3–5 mm pieces. The pieces were placed on acidified potato dextrose agar with lactic acid (PDA-LA), water agar (WA), Nash medium (NM; Nelson *et al*., 1983) and nutrient agar (NA) in Petri plates, and incubated at 22°C without illumination. The plates were examined weekly for 4 weeks. Emerging fungal and bacterial colonies were transferred onto PDA-LA and NA, respectively.

Identification of isolates

Fungal and bacterial colonies isolated from horseradish roots were purified by single-spore and single-cell culturing, respectively, and identified. *Fusarium* isolates grown on PDA were identified according to Nelson *et al*. (1983). Colony growth and production of microconidia, macroconidia and chlamydospores on PDA were evaluated. Morphology of conidia was determined, and length and

Table 1 Incidence of internal discoloration of horseradish roots collected at harvest from commercial fields in Illinois in 1999

Cultivar	Number fields surveyed	Number roots assayed	Percentage of roots in each symptom severity class[a]				
			0	1–10	11–25	26–50	51–100
1573	13	58	62	15	7	9	7
1590	6	30	80	17	0	0	3
1038	4	13	92	8	0	0	0
1722	2	13	23	46	8	8	15
647	2	7	57	43	0	0	0
1635	1	3	67	0	0	0	33
1069	1	3	67	33	0	0	0
1005	1	3	100	0	0	0	0
Poag	1	3	100	0	0	0	0
Total	31	133	67	19	4	4	6

[a]Class of symptom severity is based on percentage of area discoloured in the cross-section of roots, e.g. roots in class 1–10 would contain 1–10% discoloured area in the cross-section.

width of 20 microconidia, 20 macroconidia and 20 chlamydospores, produced on conventional PDA, were measured.

All isolates of *Verticillium* produced microsclerotia. *Verticillium* isolates were identified based on conidial morphology and extracellular polyphenol oxidase (PPO) activity (Hawksworth & Talboys, 1970; Howel, 1970; Karapapa *et al*., 1997). Morphological comparisons among the isolates were made by measuring the length and width of at least 20 conidia from each culture grown on PDA. Polyphenol oxidase activity was tested in a modified medium containing tannic acid (PPO medium) (Karapapa *et al*., 1997). Isolates of *V. dahliae* produce extracellular PPO which oxidizes tannic acid and causes the medium to turn black. Isolates of *V. longisporum*, however, lack the enzyme and cause no change in the colour of the medium (Howel, 1970; Karapapa *et al*., 1997). *Verticillium* isolates were grown on PDA at 24°C under 12 h fluorescent light/12 h darkness for 10 days. Agar disks from the actively growing colonies were transferred onto the PPO medium (1·36 g KH_2PO_4, 1·68 g K_2HPO_4, 5 g peptone, 3 g yeast, 20 g agar L^{-1} distilled water; 1% filter-sterilized tannic acid added after autoclaving). The PPO plates were incubated at 24°C and inspected for colour change at 4-day intervals for up to 16 days. Additional isolates of *V. longisporum* from broccoli and cabbage (Subbarao *et al*., 1995) and one isolate of *V. dahliae* from a maple tree (Chen, 1994) were included in this study for comparison.

Pathogenicity assays

Pathogenicity assays of *V. dahliae*, *V. longisporum* and *F. solani* were carried out on horseradish plants in two different experiments.

Experiment I Root-dip inoculation

Horseradish plants were produced from root segments (*c*. 2 cm diameter by 10 cm long) of cv. 1573 (a cultivar susceptible to internal root discoloration). Root segments were washed in tap water, dipped in 70% ethanol for 25 s, soaked in 0·5% NaOCl for 5 min, and rinsed in tap water. Segments were then planted in 50 × 35 × 10 cm trays containing a pasteurized soil : peat : perlite mix (1 : 1 : 1). The trays were placed in a glasshouse at 20–26°C. After 30 days, when the roots had produced lateral roots and a few leaves, plants were removed from the trays, most of the soil was removed from the main root and lateral root system, and plants were inoculated. Three isolates of each of *V. dahliae*, *V. longisporum* and *F. solani* were used in this experiment. Three isolates of each species separately and combined together (four treatments for each species) were tested on horseradish plants in the glasshouse.

For producing inoculum, PDA cultures of *V. dahliae* and *V. longisporum* were macerated in a small amount of SDW, spread on the surface of PDA in 90 × 15 mm Petri plates, and inoculated at 20°C under 12 h fluorescent light/12 h darkness for 3 weeks. These cultures were then blended with 200 mL distilled water (DW) at high speed for 30 s in a Waring Blender (Dynamics Corporation of America, New Hartford, CT, USA). The resulting suspension was passed through a series of 500, 180 and 38 μm sieves. The material (mostly microsclerotia) on the 38 μm sieve was rinsed with DW, collected from the sieve and resuspended in DW to obtain a final concentration of 500 microsclerotia mL^{-1}. A conidial suspension of *F. solani* was prepared from 7- to 10-day-old culture plates of the fungus grown on PDA at 20°C under 12 h fluorescent light/12 h darkness. Ten mL SDW were added to each plate and the conidia were dislodged using a soft brush. The concentration of conidia (microconidia and macroconidia) was adjusted to 10^5 spores mL^{-1} using a haemocytometer.

To provide a sticky, thick consistency of inoculum suspension, both microslerotial and conidial inoculi were prepared in 0·5% agar solutions (Virgin & Maloit, 1947; Wiles, 1960; Atibalentja & Eastburn, 1998). Roots of plants grown in the glasshouse were dipped in the suspension of microsclerotia of either *V. dahliae* or *V. longisporum* or conidia of *F. solani* for 15 s, then planted in 15 cm plastic pots containing the pasteurized soil : peat : perlite mix (1 : 1 : 1). Roots of control plants were dipped in SDW containing 0·5% agar. Pots were placed in the glasshouse at 20–26°C under a combination of 1000 W high pressure sodium and mercury vapour lamps with a 12 h day^{-1} photoperiod.

Plants were removed at monthly intervals for 5 months. Roots were washed, cut at three sites (2·5 cm from each end and in the middle), and evaluated for severity of internal discoloration using the 0–4 scale described previously. Root sections were then surface sterilized by soaking in 5·25% NaOCl solution for 1 min, soaking in 95% ethanol for 2 min and rinsing in SDW for three times. Root sections were tested for presence of the pathogens by placing sections on PDA-LA and incubating the plates at 22°C without illumination. Plates were examined weekly for 4 weeks. Emerging fungal colonies were transferred onto PDA-LA and identified.

The experiment was performed in a split-plot design with five randomized complete blocks, with fungal species as main plots, isolates as subplots, and time as a repeated measure. Each replication time included one pot with a single plant. The experiment was repeated once. Data were analysed using the ANOVA procedures of SAS (SAS Institute, Cary, NC, USA).

Experiment II Infested soil inoculation

Two isolates of *V. dahliae* (one from horseradish and one from maple) and four isolates of *V. longisporum* (two from horseradish, one from broccoli and one from cabbage) were used in this experiment. Soil inoculum was prepared by adding 100 g diced potato cubes to 500 mL soil : sand mix (1 : 1) in a flask, inoculating the mixture with the fungal isolate, incubating it under 12 h fluorescent light/12 h darkness at 22°C for 4 weeks, and shaking periodically. This inoculum was then mixed with a potting soil (soil : peat : perlite; 1 : 1 : 1) at the rate of 100 mL inoculum with 900 mL potting soil.

In this experiment, horseradish cv. 1590 (a cultivar susceptible to internal root discoloration) was used. Plants inoculated were either tissue culture-generated seedlings

or grown from root segments produced in a commercial field. In one study, 8-week-old tissue culture-generated seedlings (Norton *et al*., 2002) were transplanted into 15 cm plastic pots containing infested soil mix. Control seedlings were transplanted into pots containing noninfested soil mix. In another study, segments of roots (2 cm diameter, 10 cm long), grown in a field with no history of root discoloration and with no symptoms of discoloration, were used. The segments (sets) were surface disinfected as previously described and planted in 15 cm pots containing infested soil mix. Control sets were planted in pots containing noninfested soil mix. Pots from both studies were placed in the glasshouse at 20–26°C. After 4 months plants were removed from pots and roots were washed with tap water. Roots were then cut in the middle, and the severity of discoloration in cross section was assessed using the 0–4 scale described previously. Roots were tested for presence of the pathogens.

The tests were performed on a split-plot design with five randomized complete blocks. Pathogen was considered the main plot and isolates as subplots. Each replication was one pot with a single plant. Data were analysed using the ANOVA procedures of SAS.

Results

Field survey

There was no significant difference in incidence or severity of symptoms of discoloration on cross-sections of different parts of each root; thus only the results of incidence and severity of disease on the cross-sections at the middle of roots are presented. In 1999 internal discoloration was observed in roots collected from 17 of 31 fields. Overall, 33% of 133 roots examined were discoloured (Table 1). In 2000 root discoloration was observed in all nine fields surveyed (Table 2). The percentage of roots with a discoloured core (central), vascular (middle), and cortex (outer) regions ranged from 0–14% (mean 4·5%), 0–52% (mean 24·0%), and 2–28% (mean 11·6%), respectively. Core discoloration (75%) and cortex discoloration (91%) were associated with vascular discoloration. Attempts to isolate *Verticillium* or *Fusarium* species from roots with discoloured core or cortex, but without vascular discoloration, were unsuccessful.

Table 2 Incidence of internal discoloration of horseradish roots in commercial fields in Illinois in 2000[a]

	Roots with symptoms (%)		
Cultivar	Core discoloration	Vascular discoloration	Cortex discoloration
1069[b]	14	8	8
1069	10	52	28
1573	6	12	14
1573	0	24	2
1573	2	8	14
Jersey	0	28	14
1590	4	34	8
Eastern	4	0	2
647	0	50	6
Average	4·5	24·0	11·6

[a]Results of a survey conducted in September 2000.
[b]Each row represents one commercial field. In each field 50 roots (each representing one plant) were assessed for internal discoloration.

Isolation of fungi and bacteria

All the fungal colonies that emerged in the horseradish culture plates were identified. The range of fungal colonies recovered on PDA-LA cultures encompassed those isolated on WA, NM and NA media. Thus only the results of PDA-LA cultures are presented. Fungi included *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Trichoderma*, *Verticillium*, and bacteria of *Erwinia* and *Pseudomonas* species emerged from cultured root tissues. The frequency of isolation of *Alternaria*, *Aspergillus*, *Penicillium*, *Rhizoctonia*, *Trichoderma*, *Erwinia* and *Pseudomonas* was <10%, and they emerged from the tissues that were colonized by *Fusarium* and *Verticillium* species.

Almost all the *Fusarium* isolates were identified as *F. solani* based on the morphological characteristics described by Nelson *et al*. (1983). On PDA colony growth was rapid, with abundant aerial mycelium, white to pale buff, reaching to 5–7·5 cm diameter in 6 days at 24°C. The surface of the colony was covered with confluent sporodochia that gave the appearance of pionnotes with a cream to yellow colour. Microconidia developed in culture, varying from sparse to abundant, single-celled, oval to kidney-shaped, 4·2–12·6 × 2·5–5·0 μm, formed on elongated branched or unbranched conidiophores. Macroconidia developed abundantly from monophialides of conidiophores, hyaline, cylindrical, slightly curved, with a beak apical cell and foot-shaped basal cell, mostly 3–7 septate, 38–72 × 3–5 μm. Chlamydospores formed in boths hyphae and macroconidia, terminal or intercalary, thick-walled, solitary, in pairs or in chains, 5–12 μm diameter.

Based on conidial length, *Verticillium* isolates were divided into two groups. One group with conidia of length >7 μm fitted the description of *V. longisporum* (Karapapa *et al*., 1997); the other group with conidia of length <7 μm fitted the description of *V. dahliae* (Hawksworth & Talboys, 1970) (Table 3). However, PPO activities were minimal for the *Verticillium* isolates from horseradish, regardless of conidial length.

In 1999 *V. dahliae*, *V. longisporum* and *F. solani* were isolated from 14, 16 and 23% of roots assayed, respectively (Table 4). In 2000 *V. dahliae*, *V. longisporum* and *F. solani* emerged from 24, 20 and 19% of roots cultured, respectively (Table 5). These fungi were isolated from 26, 61, 82 and 28% of roots with no obvious symptoms, and with discoloured core, vascular, and cortex region, respectively (Table 5). More than one species of *Verticillium* and/or *Fusarium* emerged from 29, 27, 11 and 37% of roots with no symptoms and discoloured core, vascular, and cortex region, respectively.

Table 3 Conidial length and standard deviation ($N = 20$) of *Verticillium* isolates from horseradish and their polyphenol oxidase (PPO) activity

Strain	Spore length: Mean length (μm)	Spore length: Standard deviation	PPO[a] activity (medium colour)	Species
HR-1	8·77	1·19	Unchanged	*V. longisporum*
HR-2	4·66	1·23	Unchanged	*V. dahliae*
HR-3	5·67	1·13	Unchanged	*V. dahliae*
HR-4	8·47	1·21	Unchanged	*V. longisporum*
HR-5-2	8·18	1·83	Unchanged	*V. longisporum*
HR-5-3	7·33	1·28	Unchanged	*V. longisporum*
HR-6	4·99	0·92	Unchanged	*V. dahliae*
HR-7	5·07	1·27	Unchanged	*V. dahliae*
HR-8	4·99	1·41	Unchanged	*V. dahliae*
HR-9	8·25	2·18	Unchanged	*V. longisporum*
HR-11	5·38	2·11	Unchanged	*V. dahliae*
HR-14	4·33	0·91	Unchanged	*V. dahliae*
HR-15	8·32	1·44	Unchanged	*V. longisporum*
HR-16	5·35	0·76	Unchanged	*V. dahliae*
HR-17	8·70	1·60	Brown	*V. longisporum*
HR-18	8·75	1·81	Brown	*V. longisporum*
VdBOB-70[b]	9·25	1·76	Brown	*V. longisporum*
VdBOC-74[bc]	9·32	1·91	Unchanged	*V. longisporum*
90-1[bc]	4·9	0·97	Black	*V. dahliae*

[a]Unchanged = no visible PPO activity; brown = intermediate PPO activity; black = high level of PPO activity.
[b]Strains VdBOB-70, VdBOC-74, and 90-1 were from broccoli, cabbage and maple, respectively.
[c]Type control isolates of *V. longisporum* and *V. dahliae*.

Table 4 Frequency of isolation of *Verticillium dahliae*, *V. longisporum* and *Fusarium solani* from roots of horseradish cultivars grown in Illinois in 1999

Cultivar	Number roots assayed	Isolation frequency (%)[a]: *Verticillium dahliae*	*Verticillium longisporum*	*Fusarium solani*
1573	58	10	9	16
1590	30	3	7	7
1038	13	15	0	15
1722	13	31	54	31
647	7	29	0	57
1635	3	0	100	100
1069	3	33	33	67
1005	3	67	33	67
Poag	3	33	67	67
Total	133	14	16	23

[a]Two or three species were isolated from some roots.

Pathogenicity assays

Vascular discoloration was consistently observed in inoculated plants in the glasshouse (Tables 6 and 7), which also showed core and cortex discoloration in some inoculated roots. Discoloration was also observed in the core region of 10% of noninoculated plants 5 months after inoculation.

Experiment I Root-dip inoculation

There was no significant difference in the incidence or severity of root discoloration among three isolates of each pathogen used separately or combined together to inoculate plants. Also, there was no significant difference in disease incidence or severity of root discoloration between two experiments. Therefore only the results from plants inoculated with combined isolates from two experiments

Table 5 Frequency of isolation of *Verticillium dahliae*, *V. longisporum* and *Fusarium solani* from horseradish roots with different symptoms in commercial fields in Illinois in 2000

Root symptom	Number roots tested	Number roots with fungal colony	Isolation frequency[a]: *Verticillium dahliae*	*Verticillium longisporum*	*Fusarium solani*
No symptoms	27	26	5	11	7
Core discoloration	18	61	33	28	22
Vascular discoloration	34	82	41	32	18
Cortex discoloration	29	28	7	10	28
Total	108	50	24	20	19

[a]Two or three species were isolated from 29, 27, 11 and 37% of roots without symptoms and discoloured core, vascular and cortex, respectively.

Table 6 Frequency of root discoloration (n = 10) and reisolation of the pathogens from horseradish roots artificially inoculated with *Verticillium dahliae*, *V. longisporum* and *Fusarium solani*

Pathogen[b]	Incidence/severity/ pathogen reisolation	Time after inoculation (month)[a] 1	2	3	4	5
Verticillium dahliae	Incidence (%)[c]	30 a[d]	60 ab	70 ab	90 b	80 b
	Severity[c]	0·2 a	0·8 ab	1·8 bc	2·7 c	2·8 c
	Pathogen reisolation (%)[e]	67 a	50 a	86 a	67 a	86 a
Verticillium longisporum	Incidence (%)	30 a	70 b	70 b	80 b	90 b
	Severity	0·3 a	1·1 ab	1·6 bc	2·6 cd	3·1 d
	Pathogen reisolation (%)	67 a	71 a	86 a	75 a	67 a
Fusarium solani	Incidence (%)	50 a	70 a	80 a	70 a	90 a
	Severity	0·9 a	1·1 a	1·5 a	1·3 a	3·1 b
	Pathogen reisolation (%)	100 a	86 a	88 a	86 a	78 a
Control	Incidence (%)	0 a	0 a	0 a	10 a	10 a
	Severity	0 a	0 a	0 a	0·4 b	0·2 ab
	Pathogen reisolation (%)	0	0	0	0	0

[a]Plants were assessed for incidence and severity of disease every month until 5 months after inoculation.
[b]Three isolates of each pathogen were mixed and used in inoculation.
[c]Plant infection was assessed in the cross-section of the middle of growing root. Incidence = percentage roots discoloured; severity = percentage area of roots discoloured (on a scale of 0–4).
[d]Combined results of two experiments. Values in each row with a letter in common are not significantly different from each other according to Fisher's protected LSD (P = 0·01).
[e]Frequency of reisolation of the pathogen from roots with symptoms.

together are presented (Table 6). Discoloration symptoms of roots developed over a period of 5 months (Table 6). In treatments with *V. dahliae* and *V. longisporum* inoculation, a significantly higher percentage of plants had discoloured roots 4 months after inoculation than 1 month after inoculation. Severity of root discoloration was significantly higher 5 months after inoculation compared with 2 and 3 months for *V. dahliae* and *V. longisporum*, respectively. For plants inoculated with *F. solani*, the percentage of plants with root discoloration increased from 50% (1 month postinoculation) to 90% (5 months postinoculation). Severity of root discoloration was significantly higher 5 months after inoculation compared with 4 months or less. Incidence and severity of root discoloration in inoculated plants were significantly higher than those of control plants. *Verticillium dahliae*, *V. longisporum* and *F. solani* were reisolated from 75, 74 and 87% of plants inoculated with these fungi, respectively. The results were consistent across runs of the experiments.

Experiment II Infested soil inoculation

There was no significant difference in incidence and severity of root discoloration between tissue culture-generated plants and plants generated from horseradish roots grown in the commercial field. Therefore the results of these tests are combined and presented together (Table 7). Incidence and severity of root discoloration were significantly higher in inoculated plants compared with control plants. Severity of discoloration of roots of plants inoculated with *Verticillium* isolates from horseradish was significantly higher than that of plants inoculated with *Verticillium* isolates from maple, broccoli or cabbage (Table 7).

Table 7 Incidence and severity of discoloration of horseradish roots (n = 10) following inoculation with *Verticillium dahliae* and *V. longisporum* from various host plants

Strain	Species	Original host	Horseradish root discoloration Incidence (%)[a]	Severity[b]
HR-14-S5	*V. dahliae*	Horseradish	90 b[c]	2·0 c
90-1	*V. dahliae*	Maple	90 b	1·2 b
HR-5-1-S4	*V. longisporum*	Horseradish	100 b	2·6 c
HR-15-S4	*V. longisporum*	Horseradish	100 b	2·7 c
VdBOB-70	*V. longisporum*	Broccoli	90 b	1·2 b
VdBOC-74	*V. longisporum*	Cabbage	100 b	1·2 b
Control	–	–	30 a	0·3 a

[a]Percentage of plants with discoloration symptoms. Plants were assessed for incidence and severity of disease 4 months after inoculation. Combined results of two experiments.
[b]Discoloured area at cross-section, rated using a scale of 0–4: 1 = 1–10; 2 = 11–25; 3 = 26–50; and 4 = 51–100% discoloration of root in the cross-section.
[c]Values in each column with a letter in common are not significantly different from each other according to Fischer's protected LSD (P = 0·05).

Discussion

The incidence and severity of internal discoloration of roots in commercial fields indicate that this disease is a serious threat to horseradish production in Illinois, as previously reported by Rhodes (1977); Gerber *et al*. (1983); Eastburn & Chang (1994). Also, internal discoloration of roots has been a major problem in other horseradish-growing

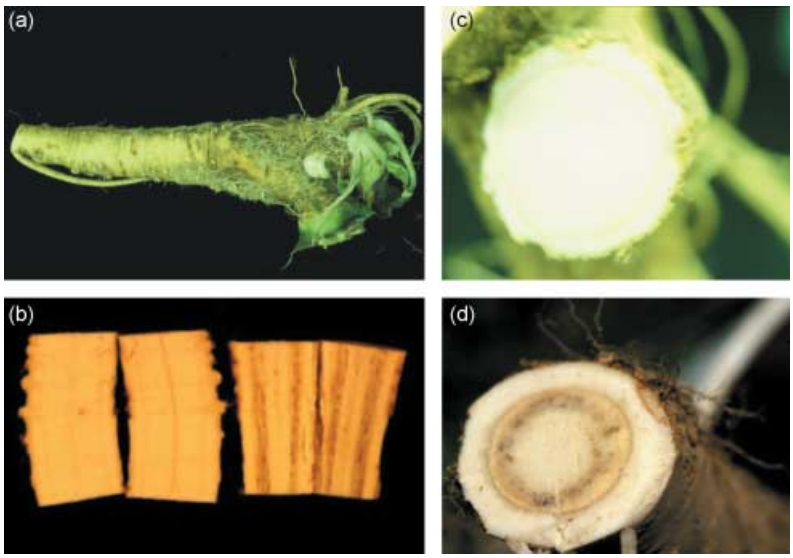

Figure 1 (a) Horseradish root; (b) longitudinal sections of symptomless (left) and discoloured (right) roots; (c) cross-section of a symptomless root; (d) cross-section of a discoloured root.

areas (Mueller *et al*., 1982). Control of this disease has been problematic. One reason for lack of effective measures for control of internal discoloration of horseradish roots is insufficient information on the aetiology of this disease.

Eastburn & Chang (1994) cited *V. dahliae* as the primary causal agent of internal discoloration of horseradish roots in Illinois. Percich & Johnson (1990) reported that *F. roseum* '*acuminatum*', *V. dahliae* and *P. fluorescens* incited root discoloration in horseradish. The present study shows that internal discoloration of horseradish roots can be caused by either *V. dahliae*, *V. longisporum* or *F. solani*, which is in agreement with both reports (Percich & Johnson, 1990; Eastburn & Chang, 1994). The results also indicate that internal discoloration of horseradish roots is a disease complex, previously described by Mueller *et al*. (1982) and Percich & Johnson (1990) as root deterioration complex of horseradish.

Verticillium dahliae is clearly a causal agent of internal discoloration of horseradish roots in Illinois (Eastburn & Chang, 1994), while *V. longisporum*, previously reported causing stem discoloration in several cruciferous plants (Subbarao *et al*., 1995; Karapapa *et al*., 1997), also appears to be involved in discoloration of horseradish: *V. longisporum* was described as a distinct species in 1997 (Karapapa *et al*., 1997). It is likely that *V. longisporum* has long been associated with horseradish roots, but misidentified as *V. dahliae*. The latter species and *V. longisporum* isolates from horseradish can be identified easily based on the length of conidia, with *V. dahliae* producing shorter (*c*. 5×3 μm), single-celled, hyaline conidia compared with the longer (*c*. 9×3 μm) conidia of *V. longisporum*. Comparison of extracellular PPO activities, suggested by Karapapa *et al*. (1997) in identification of *V. dahliae* and *V. longisporum* species, was used, but the extracellular activities of the isolates did not correlate with conidial spore length. Appropriate molecular methods may be useful to help distinguish these two species from each other.

Association of *Fusarium* spp. with discoloured roots of horseradish has been reported by other investigators (Percich & Johnson, 1990; Eastburn & Chang, 1994). *Fusarium solani* (Eastburn & Chang, 1994), *Fusarium oxysporum* (B. Jacobson, unpublished data) and *F. roseum* '*acuminatum*' (Percich & Johnson, 1990) have frequently been isolated from discoloured horseradish roots. However, only Percich & Johnson (1990) reported that *F. roseum* '*acuminatum*' caused discoloration of horseradish roots. Almost all the *Fusarium* isolates in the present study were identified as *F. solani*. All three isolates of *F. solani* tested on horseradish roots produced discoloration symptoms similar to those observed in the field.

The results presented here agree with Eastburn & Chang (1994) and Percich & Johnson (1990) that vascular discoloration is the primary symptom of internal discoloration of horseradish roots caused by *Verticillium* and/or *Fusarium* species. Discoloration begins in the vascular system and gradually spreads into the core and cortex regions, with maximum expression of symptoms 4–5 months after inoculation. The frequent isolation of *V. dahliae*, *V. longisporum* and *F. solani* from symptomless roots indicates that such horseradish sets may carry the pathogen(s), resulting in discoloured roots after planting.

Acknowledgements

This research was supported in part by funds from the University of Illinois at Urbana-Champaign and the Horseradish Growers' Association of Illinois. We thank M. A. Norton and R. Skirvin from the Department of Natural Resources and Environmental Sciences of the University of Illinois for supplying tissue culture-generated horseradish plants for inoculation treatments. Also, we are grateful to K. V. Subbarao of the University of

California-Davis for providing *Verticillium* isolates from cruciferous plants for this study.

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