Occurrence of *Fusarium commune* and *F. oxysporum* in Horseradish Roots

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Abstract

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This study was conducted to investigate the etiology of internal discoloration of horseradish roots. Several species of *Fusarium* and *Verticillium* were isolated from internally discolored horseradish roots collected from commercial fields in Illinois and research plots in Wisconsin during 2008 and 2009. Eleven isolates of *Fusarium*, identified as *Fusarium oxysporum* based on morphological features, were characterized by DNA sequencing of the nuclear translation elongation factor 1 α (EF-1 α) and mitochondrial small-subunit ribosomal DNA (mtSSU rDNA). Maximum parsimony analyses of DNA sequences from these two regions and the combined data set revealed that six isolates were clearly separated into a common clade that contained *F. commune*, with the remaining five isolates being grouped into a common clade with *F.*

oxysporum. Based on the DNA sequence data, we considered the six isolates grouped into a common clade with *F. commune* to be *F. commune*. Pathogenicity tests of *F. commune* and *F. oxysporum* were conducted on two horseradish cultivars, '1573' and 'Big Top Western', in a greenhouse. Root segments were inoculated by dipping them in a conidial suspension and then growing them in pots in a greenhouse for 4 months. For plants inoculated with *F. commune*, internal root discoloration and root rot developed 1 month after inoculation and almost all roots of the plant were completely rotten 4 months after inoculation. Inoculation of the plants with *F. oxysporum* resulted in only internal root discoloration but not root rot symptoms. This is the first report of *F. commune* causing internal discoloration and root rot of horseradish.

Horseradish (*Armoracia rusticana* Gaertn., Mey. & Scherb.), a member of the Brassicaceae family, is grown for its white, fleshy, and pungent roots, which are used as a condiment (9,17,27). Approximately 50% of the total horseradish in the United States is grown in the Mississippi River Valley, near East St. Louis, IL (5,26). Two other major horseradish production areas in the United States are Eau Claire, WI and Tule Lake, CA (4,5).

Internal discoloration of horseradish roots occurs throughout the world and is the main limiting factor in the production of horseradish (4,10,13,24). In the past 30 years, growers in North America have experienced significant reductions in the marketable yield of horseradish, resulting from internal discoloration and rotting of roots (4,10). In Illinois, yield losses up to 100% in commercial fields, caused by internal discoloration and rotting of roots, have occurred (4).

Internal discoloration of horseradish root was first reported in Germany in the 1890s (24). Mueller et al. (21) reported *Verticillium dahliae* as the primary causal agent of internal discoloration of horseradish roots. In 1986, B. J. Jacobson (*unpublished data*) isolated *Fusarium oxysporum* from internally discolored horseradish roots in Illinois. Percich and Johnson (23) reported discoloration and rot of horseradish roots as a complex disease in Wisconsin, caused by *F. roseum* 'Acuminatum', *V. dahliae*, and *Pseudomonas fluorescens*. Eastburn and Chang (10) reported *V. dahliae* as the primary causal agent of root discoloration in Illinois. Babadoost et al. (4) concluded that internal root discoloration of horseradish is a disease complex, caused by at least three fungi: *V. dahliae*, *V. longisporum*, and *F. solani*.

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 (10,13,21). Internally discolored horseradish roots are useless for industrial purposes such as preparing horseradish sauce (4).

Gerber et al. (13), Mueller et al. (21), and Percich and Johnson (23) reported that internal root discoloration in horseradish is usu-

ally followed by root rot. However, according to Percich and Johnson (23), infection by *V. dahliae* alone did not result in root rotting, and a combination of *V. dahliae* with *F. roseum* 'Acuminatum' caused root rot. Also, Atibalentja and Eastburn (2,3) and Eastburn and Chang (10) did not report any root rot caused by *V. dahliae*. Furthermore, Babadoost et al. (4) did not observe any root rot caused by *V. dahliae*, *V. longisporum*, or *F. solani*. Although root rot is a major problem of horseradish in Illinois (5) and other horseradish-growing areas in North America (M. Babadoost, *unpublished data*), its causal agent is not yet determined. It was suspected that root rot of horseradish is caused by a *Fusarium* sp. other than *F. solani*.

F. commune is a recently characterized species closely related to the *F. oxysporum* species complex (28). This fungus has been isolated from soil and plants, including white pine, Douglas fir, carnation, corn, carrot, and barley (28). Recently, this fungus was also reported to cause crown and root rot in tomato (16) and root rot in forest nurseries (18,30). The morphological differences between conidia and conidiophores of *F. commune* and *F. oxysporum* are subtle (18,30) and may not provide reliable characteristics to morphologically distinguish these species from each other. DNA sequences from the translation elongation factor 1 α (EF-1 α) and the mitochondrial small-subunit ribosomal DNA (mtSSU rDNA) regions have been shown to be suitable for distinguishing *F. commune* from *F. oxysporum* (18,30).

During 2008 and 2009, *Fusarium* and *Verticillium* spp. were isolated from almost all horseradish roots collected from Illinois and Wisconsin. In addition to three previously-reported *Fusarium* spp. (*F. oxysporum*, *F. acuminatum*, and *F. solani*) in horseradish roots, *F. commune* has been isolated for the first time in the present study. The purpose of this study was to identify *F. commune* based on molecular characterization from roots of horseradish and to determine the association of *F. commune* and its sister taxon *F. oxysporum* with internal discoloration of horseradish roots.

Materials and Methods

Sampling and fungal isolation. During 2008 and 2009, 306 horseradish roots were collected from 20 commercial fields (10 fields each year) in Illinois at harvest. A minimum of 10 roots and a maximum of 20 roots were collected from each field, which included 72 roots from 'Big Top Western' (BTW) and 234 roots from '1573'. In addition, 120 roots were collected from horseradish

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research plots in Eau Clair, WI in 2008 and 2009 (60 roots each year), which included 40 and 80 roots from BTW and '15K'. BTW, 1573, and 15K are major horseradish cultivars grown in Illinois and Wisconsin. All collected roots had internal discoloration symptoms, with severity of discoloration of 5 to 50% at the cross-sections (2). The roots were either tested immediately for the presence of pathogens or stored at 4°C until they were tested within 2 months from the date of collection.

Fungal isolations were carried out on collected roots using the procedure developed by Babadoost et al. (4). A 5-cm segment was cut from the middle of each collected root. The outer layers (approximately 1 mm) of the segments were peeled to remove fungal and bacterial contaminants on the root surface. The peeled segments were surface sterilized by dipping them in a 6% NaClO (full-strength commercial bleach) solution for 1 min followed by dipping in a 95% ethanol concentration for 3 min, then rinsed in sterile-distilled water (SDW) three times (1 min each). Each surface-sterilized segment was blotted with sterilized blotter paper, and five small segments (5 mm thick) from each root were cut and placed onto potato dextrose agar (PDA; Difco) with lactic acid (PDA-LA) in petri plates. PDA-LA was made by adding 4 ml of 25% lactic acid to PDA after autoclaving and cooling the culture medium to 50°C. The plates were incubated at 22 to 24°C with 12 h of light and 12 h of darkness for 15 days. After 5, 10, and 15 days of incubation, plates were examined and individual hyphal tips of the developing fungal colonies were removed and placed onto PDA in petri plates. The plates were incubated at 22 to 24°C for 8 days. Single-spore cultures were prepared from the isolates and the cultures were saved on PDA slants for further studies.

Using morphological characteristics, isolated fungi were first identified to genus. Isolates identified as *Fusarium* were then further identified to species based on their morphological characteristics (7,14). Eleven isolates identified as *F. oxysporum* were selected for molecular analysis to verify their identification.

DNA extraction and polymerase chain reaction amplification. The isolates were cultured in potato dextrose broth (Difco) in flasks for 7 to 10 days at 25°C on a shaker. Mycelia were collected from the cultures by filtration and transferred to 1.5-ml tubes. Total DNA was extracted from fungal mycelia using the Fast DNA Spin kit (MP Bio), according to the manufacturer's recommended protocol. The mtSSU rDNA and EF-1 α regions were amplified using primers NSM1/NSM2 (CAGCAGTGAGGAATATTGGTCAATG/GCGGATCATCGAATTAAATAACAT) (20) and EF-1/EF-2 (ATGGGTAAGGARGACAAGAC/GGARGTACCAGTRATCATG TT) (22), respectively. A 50- μ l polymerase chain reaction (PCR) mixture was prepared, which contained 1 $\times$ PCR buffer, 1.5 mM MgCl₂, 200 μ M dNTPs, 5 pmol of each primer, 2 units of *Taq* polymerase (Promega Corp.), and 10 ng of DNA extract.

The PCR conditions for mtSSU were initial denaturing at 94°C for 3 min; 35 cycles of denaturing at 94°C for 35 s, annealing at 52°C for 55 s, and extension at 72°C for 2 min; and final extension at 72°C for 5 min. For EF-1 α , the PCR conditions were initial denaturing at 94°C for 2 min; 30 cycles of DNA denaturing at

92°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min; and final extension was at 72°C for 5 min. All reactions were conducted with an MJ PTC-200 thermal cycler (MJ Research Inc.).

DNA sequencing and phylogenetic analysis. The PCR products were purified using the Wizard SV gel and the PCR Clean-Up system (Promega Corp.), according to the manufacturer's protocol. Purified PCR products were sequenced in an automated sequencer at the Core DNA Sequencing Facility of the University of Illinois at Urbana-Champaign. Sequencing was performed using the same forward and reverse primer sets used for the PCR amplification (32).

Raw sequences from both strands were aligned and edited using BioEdit version 7.0.9 (15). Using BLAST, the edited sequences were compared to the ID region available in the National Center for Biotechnology Information GenBank database (<http://www.ncbi.nlm.nih.gov>). Sequences generated from materials in this study and retrieved from GenBank were aligned using the program CLUSTAL X (31) and the alignment was refined using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit>) (8). Maximum parsimony analysis was estimated using heuristic searches consisting of random addition and tree bisection-reconnection branch swapping. Bootstrap analysis using 1,000 replications was performed to assess the relative stability of the branches. The sequences used in this analysis were deposited in GenBank (Table 1).

Pathogenicity tests. Pathogenicity tests were conducted using a root-dip inoculation method of Babadoost et al. (4) and Eastburn and Chang (10). Two isolates each of *F. commune* and *F. oxysporum* identified by morphological and molecular methods were tested for pathogenicity on 1573 and BTW horseradish.

Asymptomatic horseradish sets of 1573 and BTW were obtained from commercial growers. To eliminate possible set-borne inoculum, the sets were treated at 47°C for 20 min (11) prior to inoculation. The sets were dipped in 70% ethanol for 25 s, soaked in 0.6% NaClO solution for 5 min, and rinsed in tap water. Segments were then planted in 50-by-35-by-10-cm pots containing a pasteurized mix of soil/peat/perlite (1:1:1) (4,10), and were grown in a greenhouse at 20 to 26°C. After 30 days, plants were removed from the pots, soil was gently removed from the roots, and roots were inoculated with *Fusarium* isolates.

F. commune and *F. oxysporum* isolates were grown on PDA in 90-mm petri plates in an incubator at 24°C with 12 h of light and 12 h of darkness. A conidial suspension of each isolate was prepared from 10-day-old cultures by adding 10 ml of SDW to each plate and dislodging the conidia using a soft brush. The concentration of conidia (macroconidia and microconidia) was adjusted to 10⁵ spores/ml using a hemocytometer (4). To prepare the sticky inoculum suspension, *Fusarium* inoculum was prepared in a sterilized 0.5% agar solution (2).

Roots of plants grown in the greenhouse were inoculated by dipping them in the 0.5% agar suspension of conidia for 15 s. Inoculated plants were planted in plastic pots (50 by 35 by 10-cm) containing the pasteurized soil/peat/perlite (1:1:1) mix. Roots of

Table 1. Isolates of *Fusarium commune* and *F. oxysporum* from horseradish roots collected from Illinois and Wisconsin in 2008 and 2009

Identified species	Isolate	Horseradish cultivar	Geographic origin	GenBank accession number ^z	
				NMS	EF-1 α
<i>F. commune</i>	SD4139	1573	Illinois	HM804944	HM804935
<i>F. commune</i>	SC4015	Big Top Western	Illinois	HM804946	HM804937
<i>F. commune</i>	SC4025	Big Top Western	Illinois	HM804947	HM804938
<i>F. commune</i>	WA4023	15K	Wisconsin	HM804948	HM804939
<i>F. commune</i>	WA4069	15K	Wisconsin	HM804949	HM804940
<i>F. commune</i>	WB4031	Big Top Western	Wisconsin	HM804950	HM804941
<i>F. oxysporum</i>	SD3183	1573	Illinois	HQ114271	HQ114266
<i>F. oxysporum</i>	WA3037	15K	Wisconsin	HQ114272	HQ114267
<i>F. oxysporum</i>	WA3065	15K	Wisconsin	HQ114273	HQ114268
<i>F. oxysporum</i>	SD3152	1573	Illinois	HQ114274	HQ114269
<i>F. oxysporum</i>	WA3008	15K	Wisconsin	HQ114275	HQ114270

^z NMS = mitochondrial small-subunit ribosomal DNA and EF-1 α = translation elongation factor 1 α .

control plants were dipped in sterilized 0.5% agar solution. Pots were placed in the greenhouse at 20 to 26°C under a combination of 1,000-W high-pressure sodium and mercury vapor lamps with a photoperiod of 12 h per day. Each inoculation treatment included six replications, with four plants in each replication. The experiment was performed twice.

Six plants were removed at monthly intervals until 4 months after inoculation. Roots were washed, cut, and assessed for severity of internal discoloration at the cross-section at the middle of the root, as described by Babadoost et al. (4). Severity of discoloration was rated using a scale of 0 to 4, where 0 = no discoloration and 1 = 1 to 10 (5.0), 2 = 11 to 25 (17.5), 3 = 26 to 50 (37.5), and 4 = 51 to 100% (75%) discoloration of root in the cross-section. The root sections were then used for pathogen isolation as previously described. Species of the isolated fungi from greenhouse-grown roots were identified using morphological characteristics and molecular methods reported by Skovgaard et al. (28), as previously described. Each pathogen was reisolated from its respective inoculated plant in both pathogenicity assays (*data not shown*).

Statistical methods. Statistical analyses were performed using SAS 9.3 (SAS Institute). Incidence and severity of the internal discoloration of the roots and incidence of root rot were analyzed using analysis of variance procedures. To calculate and compare severity of the internal root discoloration, the average of each severity index was considered, as follows: 0 = 0.0, 1 = 5.0, 2 = 17.5, 3 = 37.5, and 4 = 75.0%.

Results

Morphological identification of fungi. Fungi were isolated from 357 of 426 roots, which included 250 of 306 and 107 of 120

roots collected from Illinois and Wisconsin, respectively. In total, 444 fungal isolates were collected, which comprised 328 and 116 isolates from Illinois and Wisconsin, respectively. Two and occasionally three different fungal species were isolated from some of the roots. Based on morphological characteristics, isolated fungi belong to seven genera, including *Alternaria*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Pyrenochaeta*, *Rhizopus*, and *Verticillium* (1,6). The most commonly isolated genera were *Verticillium* and *Fusarium*. *Verticillium* spp. were isolated from 45 and 22% of roots collected from Illinois and Wisconsin, respectively. In contrast, *Fusarium* spp. were isolated from 31 and 64% of roots collected from Illinois and Wisconsin, respectively. In total, 175 isolates were identified as *Fusarium*. Isolated *Fusarium* spp. included *F. acuminatum*, *F. equiseti*, *F. oxysporum* complex, *F. proliferatum*, and *F. solani* (19,28), with 72, 12, 32, 3, and 59 of isolates, respectively (*data not shown*). *F. solani* was the most frequently isolated species (43%) from samples collected from Illinois. In contrast, *F. acuminatum* was the most frequently isolated species (57%) from roots collected from Wisconsin (*data not shown*). Eleven isolates (five from Illinois and six from Wisconsin) identified as *F. oxysporum* based on morphological characteristics were selected for molecular species identification.

Phylogenetic analysis. The PCR amplification of the mtSSU rDNA region for all 11 isolates generated 687- to 715-bp fragments, and alignment of the sequences resulted in a dataset of 643 characters. Based on 38 parsimony-informative characters, three most-parsimonious trees were located with tree length of 76 steps (consistency index [CI] = 0.8947, retention index [RI] = 0.9590; Fig. 1). All 11 isolates of *Fusarium* used in this study, morphologically placed in the *F. oxysporum* species complex, were

Fig. 1. One of three parsimonious trees inferred from sequence of mitochondrial small-subunit ribosomal DNA (NMS) gene of *Fusarium* spp. Numbers next to branches represent bootstrap values of 1,000 replicates calculated with maximum parsimony. Only bootstrap values higher than 50% are shown.

divided into two clades. The first clade included six isolates along with reference strains of *F. commune* (NRR28387, Q2, and Q29), with strong bootstrap support (100%). The second clade contained five isolates and reference strains of the *F. oxysporum* species complex (0349, 0350, 9411C, 9418C, and NRRL26409), with strong bootstrap value (98%) (Fig. 2). The mtSSU rDNA sequences of the six isolates were deposited in the GenBank under the accession numbers HM804944 to HM804950 for *F. commune* and HQ114271 to HQ114275 for *F. oxysporum* (Table 1).

The PCR amplification of EF-1 α for all 11 isolates generated 623- to 646-bp fragments, and alignment resulted in a 624-character dataset. Based on 85 parsimony-informative characters, 12 most-parsimonious trees were generated with tree length of 201 steps (CI = 0.8905, RI = 0.9275). The EF-1 α trees had topology similar to that revealed in analysis of the mtSSU sequences, except with greater genetic diversity among isolates of the two clades. Six isolates fell into four distinct groups, including reference strains of *F. commune* (NRR28387, Q2, and Q29), and formed a monophyletic clade with strong bootstrap support (100%). The second clade included five isolates and reference strains of *F. oxysporum* species complex (0348, 0349, 0350, 9411C, 9418C, and NRRL26409), which were supported with strong bootstrap value (100%) (Fig. 3). The full lengths of EF-1 α sequences of the isolates were deposited in GenBank under accession numbers HM804935 to HM804941 for *F. commune* and HQ114266 to HQ114270 for *F. oxysporum* (Table 1).

Phylogenetic analysis based on combined sequences of mtSSU rDNA and EF-1 α yielded four most-parsimonious trees (steps =

278, CI = 0.8885, RI = 0.9369). Parsimony analysis of the combined dataset revealed a tree with topology similar to that revealed in independent analyses of mtSSU rDNA and EF-1 α (Fig. 3). All isolates were divided into two distinct clades: one contained six isolates (*F. commune*) and the other five isolates (*F. oxysporum*). Sequences of *F. commune* strains (NRR28387, Q2, and Q29) obtained from GenBank clustered together with six isolates of *Fusarium* from horseradish roots with strong bootstrap support (100%), whereas sequences of *F. oxysporum* species complex strains (NRRL26409, 0348, 0349, 0350, 9411C, and 9418C) obtained from GenBank clustered together with five separate isolates of *Fusarium* from horseradish with strong bootstrap value (100%). The phylogenetic tree revealed complete separation between isolates of *F. commune* and the *F. oxysporum* species complex, and indicated that six of the isolates of *Fusarium* (SC4015, SC4025, SD4139, WA4023, WA4069, and WB4031) from horseradish roots were *F. commune*, distinguished from its sister taxon *F. oxysporum* (28).

Pathogenicity. There was no significant difference in the incidence or severity of root discoloration and rot among two isolates of each pathogen used separately or combined together to inoculate plants.

All of the roots inoculated with *F. commune* were diseased 4 months after inoculation (Table 2; Fig. 4). Incidence of internal discoloration and root rot increased significantly by time over the period of 4 months. Internal discoloration was observed 1 month after inoculation of roots (Fig. 4C), and root rot was observed 2 months after inoculation (Fig. 4D). Three months after inoculation,

Fig. 2. One of 12 parsimonious trees inferred from sequence of translation elongation factor 1 α (EF-1 α) gene of *Fusarium* spp. Numbers next to branches represent bootstrap values of 1,000 replicates calculated with maximum parsimony. Only bootstrap values higher than 50% are shown.

roots of 100 and 83.3% of plants of 1573 and BTW, respectively, were either internally discolored or rotten. Four months after inoculation, roots of 100% of plants of 1573 were rotten and roots of 16.7 and 83.3% of plants of BTW were internally discolored and rotten, respectively. Severity of internal discoloration in inoculated

plants increased significantly over the 3-month period. *F. commune* was isolated from all inoculated symptomatic roots of both cultivars. Isolation of the pathogen from most of the completely rotten main roots was not possible. However, the fungus was isolated from symptomatic lateral roots of the rotten main roots.

Fig. 3. One of four parsimonious trees based on the combined analysis of mitochondrial small-subunit ribosomal DNA (NMS) and translation elongation factor 1α (EF-1α) gene sequences from *Fusarium* spp. Numbers next to branches represent bootstrap values of 1,000 replicates calculated with maximum parsimony. Only bootstrap values higher than 50% are shown.

Table 2. Occurrence of internal root discoloration and root rot caused by artificial inoculation of *Fusarium commune* and *F. oxysporum* on horseradish ‘1573’ and ‘Big Top Western’

Treatment ^x	Disease ^y	Occurrence ^z	Disease development in months ^w									
			1573					Big Top Western				
			1	2	3	4	LSD	1	2	3	4	LSD
<i>F. commune</i>	IRD	Incidence	33.3	58.3	50.0	0.0	NS	33.3	25.0	33.3	16.7	NS
		Severity	1.7 ab	8.9 bc	11.7 c	0.0 a	7.7	1.7	3.0	4.0	14.5	NS
	RR	Incidence	0.0 a	16.7 a	50.0 b	100 c	26.8	0.0 a	33.3 ab	50.0 ab	83.3 c	33.6
		Severity	0.0 a	16.7 a	50.0 b	100 c	26.8	0.0 a	33.3 ab	50.0 ab	83.3 c	33.6
<i>F. oxysporum</i>	IRD+RR	Incidence	33.3 a	75.0 b	100 b	100 b	27.5	33.3 a	58.3 ab	83.3 bc	100c	33.4
		Severity	16.7 a	58.3 b	83.3 bc	91.7 bc	33.2	16.7 a	41.7 a	83.3 b	83.3 b	34.9
	RR	Incidence	0.8 a	7.42 ab	15.2 bc	25.3 c	8.6	0.8 a	6.4 b	12.3 b	33.7 c	14.6
		Severity	0.0	0.0	0.0	0.0	NS	0.0	0.0	0.0	0.0	NS
Control	IRD	Incidence	0	0	8.3	16.7	NS	0	0	0	8.3	NS
		Severity	0	0	0.4	0.8	NS	0	0	0	1.5	NS
	RR	Incidence	0	0	0	0	NS	0	0	0	0	NS
		Severity	0	0	0	0	NS	0	0	0	0	NS

^w Values within each row and for each cultivar with a letter in common are not significantly different from each other according to Fisher’s protected least significant difference (LSD; $P = 0.05$). NS = not significant.

^x *F. commune* strain SC4015 and *F. oxysporum* strain SD3183.

^y IRD = internal root discoloration, RR = root rot, and IRD+RR = percentage of plants with both internal root discoloration and root rot.

^z Disease occurrence. Incidence = percentage of plants with discolored root or with rotten root. Data represent average of eight treatments (isolates), each with 48 plants in two experiments. Severity = percent root area at the cross-section discolored. Severity of discoloration was based on 0-to-4 scales, where 0 = 0 (0), 1 = 1 to 10 (5), 2 = 11 to 25 (17.5), 3 = 26 to 50 (37.5), and 4 = 51 to 100% (75%).

Internal discoloration developed in the roots inoculated with *F. oxysporum* isolates (Fig. 2B). The symptoms were observed 1 month after inoculation of roots and incidence of root discoloration was 83.3% in both cultivars by the end of 4 months from inoculation (Table 2). Severity of the discoloration increased over time but averages of the discoloration severity in treatments were less than those of *F. commune* and did not exceed 3% in the cross-sections of the roots. No root rot was observed in the roots inoculated with *F. oxysporum* isolates.

At 4 months after inoculation, roots of more than 83.3% of uninoculated plants were asymptomatic (Fig. 4A). However, 16.7 and 8.3% of roots of control plants of 1573 and BTW, respectively, showed minor internal discoloration in core areas of roots. Neither *F. commune* nor any other pathogens were isolated from these roots or any of the asymptomatic roots. Similar discoloration has been observed in some commercial fields with no detection of pathogens in the roots.

Discussion

We isolated six species of *Fusarium* (*F. acuminatum*, *F. commune*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, and *F. solani*) from internally discolored horseradish roots. We also found that *F. commune* caused not only internal discoloration but also rot of horseradish roots. Previous investigators have reported *V. dahliae*

(10,21,23), *V. longisporum* (4), *F. acuminatum* (*F. roseum* 'Acuminatum') (23), and *F. solani* (4) as the causal agents of the internal discoloration of horseradish roots. Also, B. J. Jacobson (*unpublished data*) isolated *F. oxysporum* from internally discolored roots in Illinois. However, he did not report its pathogenicity in horseradish. In this study, we identified *F. commune* in horseradish roots as a sister taxon of *F. oxysporum* based on sequence analyses of EF-1 α and mtSSU rDNA regions.

F. commune was described as a distinct species in 2003 (28). Morphological characteristics of *F. commune* are very similar to *F. oxysporum* and isolates of this fungus had been identified as *F. oxysporum* until 2003. Both species produce conidia on short monophialides in false heads on the aerial mycelium and chlamydospores singly or in pairs (28). The only distinct morphological characteristics to separate *F. commune* from *F. oxysporum* are the presence of long, slender monophialides in addition to the occasional production of polyphialides (28). However these morphological differences are subtle and can be variable (18,30). Thus, morphological characteristics may not be adequate to distinguish *F. commune* from *F. oxysporum* and DNA sequencing of EF-1 α and mtSSU rDNA for identification of these two species is essential, as previously concluded by other investigators (12,18,28,30). In this study, we also used DNA sequences of the two regions (EF-1 α and mtSSU) and a combined data set to identify *F. commune* and *F.*

Fig. 4. A, Cross-section of an asymptomatic horseradish root; B, cross-section of a horseradish root with internal discoloration caused by *Fusarium oxysporum*; C, cross-section of a horseradish root with internal discoloration caused by *F. commune*; D, rot of main root caused by *F. commune*.

oxysporum. *F. commune* has been reported as an agent of root rot in white pine, Douglas fir, carnation, corn, carrot, and barley (28); root rot in forest nurseries (18,29); and crown and root rot in tomato (16). This is the first report of the occurrence of *F. commune* in horseradish as a root pathogen.

Isolates of *F. commune* caused both internal root discoloration and severe root rot in horseradish. In contrast, *F. oxysporum* caused only internal root discoloration. The isolate, morphologically identified as *F. equiseti*, caused mild internal discoloration but *F. proliferatum* did not cause visible symptoms in horseradish roots in greenhouse studies (*data not presented*). Further studies are needed to fully address the importance of *F. equiseti* and *F. proliferatum* in horseradish roots.

Internal root discoloration is the most destructive disease of horseradish in North America (2–4,10,11,21,23). This problem is now known as the “internal discoloration complex” of horseradish roots. Despite numerous attempts to determine specific symptoms of the internal discoloration complex of horseradish roots caused by each of the above-mentioned pathogenic *Verticillium* and *Fusarium* spp., we have not yet been able to establish species-specific symptom characteristics. Our studies show that internal discoloration of horseradish root begins with dark brown discoloration of vascular system and spreads to inward (core) and outward (cortex) areas. Further studies might result in identification of pathogen-specific symptoms of the internal discoloration of horseradish roots.

Previous researchers reported that foliar symptoms may be used as an initial indicator of internal discoloration of horseradish root (2,10). The major foliage symptom described for this purpose is yellow spots on leaves that start near the leaf margins and progress inward along the veins, resulting in characteristic V-shaped lesions. However, Eastburn and Chang (10) reported that foliar symptoms do not develop in all plants with internally discolored roots. We have rarely observed yellowing foliage symptoms in horseradish plants with discolored roots. Although foliage yellowing may develop in plants with discolored roots in certain cultivars, foliage symptoms are not reliable indications of root discoloration in horseradish. Major symptoms that we have observed in foliage of plants with internally discolored roots in research plots and commercial fields are wilting followed by the death of foliage. These symptoms are caused both by brittle root (*Spiroplasma citri*) (5,25) and root rot (5).

A greater than 20% incidence of root rot has been observed in some commercial horseradish fields in Illinois (5). Although various fungi and bacteria have been isolated from the rotten horseradish roots, the causal agent of the root rot had never been confirmed until now. It is apparent that *F. commune* is a causal agent of root rot of horseradish. In our studies, none of the *Verticillium* spp. or other *Fusarium* spp. isolated from horseradish roots caused root rot. However, some roots infected by *Verticillium* and *Fusarium* spp. may be colonized by opportunistic fungi or bacteria and result in root rot.

In our study, *F. commune* was isolated from 17 of 426 (4%) of internally discolored horseradish roots (*data not shown*). Low incidence of *F. commune* in horseradish roots may indicate less importance of this pathogen in horseradish. As the results of our greenhouse experiments showed, roots of more than 83% of plants inoculated with *F. commune* rotted 4 months after inoculation. Because we collected horseradish root samples at harvest (approximately 5 months after planting propagative roots), it is highly likely that a majority of infected roots with *F. commune* had been rotten before harvest, as we commonly observed in our research plots as well as in commercial fields (5; M. Babadoost, unpublished data). To determine the accurate incidence of *F. commune* in commercial horseradish fields, multi-year studies of monthly sampling of horseradish roots in the fields beginning about 1 month after planting propagative roots is needed.

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