Host Range of *Verticillium dahliae* from Horseradish and Pathogenicity of Strains

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ABSTRACT

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The pathogenicity of *Verticillium dahliae* isolated from horseradish (*Armoracia rusticana*) was evaluated on nine herbaceous plant species. A range of disease reactions, from highly susceptible to highly resistant, was observed following root-dip inoculation, and foliar symptoms developed on China aster, eggplant, horseradish, potato, and sunflower. No external symptoms were seen on brussels sprouts, but root and/or stem tissues had slight to moderate vascular discoloration. Pepper, tomato, and watermelon were asymptomatic, but the pathogen was recovered from roots and/or basal stem areas from each of them. Eleven strains of *V. dahliae* isolated from a wide range of hosts were also evaluated for their ability to cause symptoms on a susceptible cultivar of horseradish. On the basis of root and foliar symptoms, the strains ranged from highly virulent to nearly avirulent. The time from inoculation to symptom appearance ranged from 35 to 110 days, depending on the strain of *V. dahliae*. The shortest incubation (35–40 days) was observed for the horseradish strain.

Verticillium wilt of horseradish (*Armoracia rusticana* P. Gaertn., B. Mey., & Scherb.), caused by *Verticillium dahliae* Kleb, is a serious problem for commercial growers in Illinois and Wisconsin (6,18,21). The disease is characterized by stunted plants, temporary wilting of leaves, a one-sided pattern of foliar chlorosis and necrosis that often occurs around veins, black streaking of leaf petioles, and root discoloration (17,18). Because the deterioration and rotting of roots are often associated with this disease (6,18), the yield losses attributable to Verticillium wilt have been substantial.

The economic importance of this disease to horseradish growers and the lack of highly resistant or tolerant cultivars (18) prompted our investigation to develop effective control measures. Crop rotation to decrease initial inoculum of *V. dahlia* in infested fields has been included as a part of disease management strategies (5). Most horseradish growers in Illinois follow at least short-term crop rotation. However, some growers have experienced heavy losses when potato was grown prior to replanting horseradish (D. M. Eastburn, *unpublished*). Appropriate crop rotation sequences can be determined by studying host range and survival of *V. dahliae* in soil (4,10,11).

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Although *V. dahliae* infects a wide range of herbaceous and woody plants (7,20), host specificity of this pathogen has been recognized on brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.) (12,13) and peppermint (*Mentha piperita* L.) (9,19). Ashworth (2) described a continuous variation in virulence among strains of *V. dahliae* on cotton (*Gossypium hirsutum* L.). However, pathotypes of *V. dahliae* have been reported on cotton (23), and physiologic races of the pathogen have been differentiated on tomato (*Lycopersicon esculentum* Mill.) using the *Ve* gene (1).

The objectives of this investigation were 1) to assess the susceptibility of herbaceous plant species known to be hosts of *V. dahliae* (7) to a strain of the pathogen from horseradish and 2) to test the virulence of strains from various hosts on horseradish.

MATERIALS AND METHODS

Fungal strains and preparation of inoculum. The 11 strains of *V. dahliae* used in this study were isolated from both herbaceous and woody hosts from diverse geographic locations (Table 1). All cultures were grown on potato-dextrose agar (PDA) at 20 C prior to inoculation. All strains produced microsclerotia except for the strain isolated from black gum (*Nyssa sylvatica* Marsh.). Inoculum was prepared by blending a 4-wk-old PDA culture with 200 ml of distilled water in a Waring blender at high speed for 2 min. The resulting suspension was passed through a nested series of 250-, 180-, and 38-μm sieves. The materials on the 180- and 38-μm sieves were collected and rinsed in running tap water. The collected microsclerotia were resuspended in distilled water and adjusted to a concentration of approximately 400 microsclerotia per milliliter. Inoculum of the black gum strain was prepared by filtering a conidial suspension through a double layer of cheesecloth. The resulting suspension was adjusted to a concentration of approximately 10^6 conidia per milliliter of water as described by Schnathorst and Mathre (23). A hemacytometer was used to estimate inoculum density by counting the number of microsclerotia or conidia. The final concentration was determined by dilution plate counts on ethanol medium (3). The inoculum of horseradish strain was a mixture of equal proportions (v/v) of two isolates.

Host range. Nine species of plants reported to be hosts of *V. dahliae* (7) were used: brussels sprouts, China aster (*Callistephus chinensis* (L.) Nees), eggplant (*Solanum melongena* L.), horseradish, pepper (*Capsicum annuum* L.), potato (*S. tuberosum* L.), sunflower (*Helianthus annuus* L.), tomato, and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). Seeds of test plants were sown at depths of 1.5–2.0 cm in a sterilized mixture of equal portions (v/v) of soil, sand, and vermiculite in cells of Styroform trays. Segments of horseradish set root (about 2 cm in diameter and 10 cm long) were dipped in 70% ethanol for 15 sec, soaked in 0.5% sodium hypochlorite for 5 min, and rinsed in tap water. Horseradish root segments and potato seed pieces were planted at a depth of 2 cm in the planting mixture described above in 35 × 50 × 9.9 cm flats. Trays and flats were kept in a greenhouse, with a daytime temperature of 25 C, for 5–6 wk after planting. Seedlings were then gently removed from trays and flats, and the roots were washed to remove as much of the soil mixture as possible. In the first trial, the seedlings were inoculated by dipping the roots in the inoculum suspension described previously for 15 sec. Because of low dis-

ease incidence, the root-dipping period was extended to 5 min in the second trial. Control seedlings were dipped in sterile water. After root dipping, seedlings were replanted in the sterilized soil mixture as described above in 15-cm clay pots. The seedlings were placed in the greenhouse and arranged in a completely randomized design with five replicates. Each experimental unit consisted of one inoculated and one noninoculated seedling of the same cultivar. Seedlings were watered daily to maintain adequate soil moisture levels. Every 4 wk, 20 g of a slow-release fertilizer 13-13-13 (Osmocote) was applied to each pot.

The severity of foliar and vascular symptoms of Verticillium wilt were rated 3 mo after inoculation using a modified disease assessment key (12), where 0 = no external or internal symptoms, 1 = vascular discoloration but no external symptoms, 2 = slight leaf chlorosis and/or necrosis involving one lower leaf, 3 = two to fewer than one-half of the leaves showing advanced chlorosis and/or necrosis with some defoliation, 4 = one-half to three-quarters of the leaves showing chlorosis and/or necrosis and/or wilt with advanced defoliation, and 5 = more than three-quarters of the leaves showing symptoms or lethal reaction.

V. dahliae was reisolated from each of the inoculated plants by cutting a 2-cm section of symptomatic or asymptomatic tissue from roots and basal stem areas. The sections were soaked in 0.5% sodium hypochlorite for 90 sec and rinsed in sterile distilled water. Each section was then cut into eight pieces, four of which were aseptically transferred to PDA medium amended with streptomycin (100 mg/L) and the remaining four to an ethanol medium (3). Plates were incubated at 20 C, and fungal colonies were identified after 10–14 days. The experiment was done twice. Data from the two trials were not combined.

Virulence test. The 11 strains of *V. dahliae* listed in Table 1 were tested for their pathogenicity on horseradish. Seedlings of the susceptible horseradish cultivar 647 were grown in the greenhouse and inoculated 4 wk after emergence. Treatments and the sterile water control were arranged in a completely randomized design with four replicates. Each experimental unit consisted of two seedlings inoculated with the same strain. The incubation period was recorded for each seedling as the time from inoculation to the first appearance of foliar symptoms with a disease rating of 2. The severity of foliar symptoms also was rated 90–110 days after inoculation using the disease assessment keys described above.

Symptoms of *V. dahliae* infection on horseradish roots were categorized as "pepper" for roots with black discoloration, "peg" for roots with brown necrotic tissue that extended from the vascular region into the surrounding pith and cortex, and "rot" for roots with internal areas that had begun to decompose (6). The severity of root discoloration/symptoms on cross sections of horseradish roots was rated on a 0–3 scale, where 0 = no internal symptoms, 1 = trace to <10% of the root cross section with either pepper, peg, or rot symptoms; 2 = 10–50% of the root cross section with either pepper, peg, or rot symptoms; and 3 = >50% of the root cross section with either pepper, peg, or rot symptoms. Roots exhibiting more than one symptom were rated in each of the appropriate categories.

V. dahliae was reisolated from roots, basal stem areas, and/or petioles of each inoculated horseradish plant as described previously. The experiment was repeated once. Data from the two trials were analyzed separately.

Statistical analyses. Because of considerable variation between the two trials, data from each trial were analyzed by ANOVA with cultivar and strain as qualitative independent variables. Waller-Duncan Bayesian least significant difference (BLSD) values with $k = 100$ were used to compare the effects of treatments ($P < 0.05$). F statistics ($P < 0.05$) were used to test the significance of the regression models and independent variables. Correlation coefficients (r) were calculated to determine the correlations between two dependent variables.

RESULTS

Host range. The plant species exhibited a wide range of disease reactions, from highly susceptible to highly resistant (Table 2). Disease incidence, disease severity, and pathogen reisolation were higher in the second trial, and only data from this trial are shown (Tables 2 and 3). China aster, eggplant, horseradish, potato, and sunflower exhibited external symptoms, with a relatively high percentage of inoculated plants developing symptoms typical of Verticillium wilt. Brussels sprouts did not show any external symptoms, but root and/or stem tissues showed slight to moderate vascular discoloration. The other three species (pepper, tomato, and watermelon) showed neither external nor internal symptoms, but the pathogen was reisolated from roots and/or basal stem areas in the proximity of the taproots.

Of the five externally symptomatic hosts, potato was the most susceptible species. Both a susceptible (Norland) and a moderately resistant (Atlantic) potato cultivar (25) were evaluated in this study. Symptoms of yellowing and wilting were first observed 21–28 and 49–56 days after inoculation, respectively, and plants of both cultivars were dead by the end of the trial. Recovery of the pathogen from potato was relatively low because of infection by a *Colletotrichum* sp. that colonized the potato tissues.

Eggplant also was a highly susceptible host, and disease reactions of the three eggplant cultivars were similar. All of the inoculated plants were infected, and pathogen recovery was 100% (Table 2). Symptoms of leaf chlorosis and necrosis were first observed 21–42 days after inoculation. Infected plants usually were stunted, and leaf wilting, curling, and defoliation developed along with vascular discoloration in all three cultivars.

Sunflower and China aster plants developed moderate symptoms of leaf chlorosis and necrosis 28–48 and 56–63 days after inoculation, respectively. Growth of the infected plants was not noticeably affected when compared with the noninoculated plants, and none of the inoculated plants died.

Virulence test. The 11 strains of *V. dahliae* differed significantly in disease ratings on foliage and roots (Table 3). Foliar symptoms ranged from 0.5 to 4.0 on the 0–5 scale, and root symptoms ranged from 0.5 to 2.3 on the 0–3 scale. The incidence of infected horseradish plants with external symptoms ranged from 25 to 100%, and recovery of the pathogen ranged from 0 to 100%. On the basis of disease ratings of foliar and root symptoms, the horseradish strain was the most virulent. The strain from black gum, which did not produce microsclerotia in culture, was the least virulent on horseradish. None of horseradish plants were killed as a result of infection. Some control plants exhibited black flecks in the roots, but foliar symptoms were never observed on these plants (Table 3). Length of incubation period ranged from 35 to 110 days depending on the strain of the pathogen, but the

Table 1. Strains of *Verticillium dahliae* used to assess virulence on horseradish

Host or substrate	Isolate	Origin	Source
Black gum (*Nyssa sylvatica*)	1990-9	Ohio	W. Chen
Cotton (*Gossypium hirsutum*)	SS-4	California	J. E. DeVay
	T-9	California	J. E. DeVay
Horseradish (*Armoracia rusticana*)	HR001, HR015	Illinois	D. M. Eastburn
Japanese maple (*Acer palmatum*)	1990-1	Illinois	W. Chen
Littleleaf linden (*Tilia cordata*)	1972-1	Illinois	W. Chen
Potato (*Solanum tuberosum*)	V-13	Idaho	L. H. Sorensen
Potato field soil	V-9	Idaho	L. H. Sorensen
Sugar maple (*Acer saccharum*)	1990-2	Illinois	W. Chen
Sumac (*Rhus* sp.)	1991-1	Illinois	W. Chen
Viburnum (*Viburnum* sp.)	1991-2	Illinois	W. Chen

interaction between incubation period and strain of the pathogen was not significant. The shortest incubation period of 35–40 days was recorded from horseradish plants inoculated with the horseradish strain.

Incubation period was highly correlated with disease rating of foliar symptoms ($r = -0.87$). There was no significant correlation between incubation period and disease rating of root symptoms ($r = -0.24$) or between ratings of foliar and root symptoms ($r = 0.30$).

DISCUSSION

Severity of symptoms differed on nine plant species inoculated with a strain of *V. dahliae* from horseradish as did virulence of 11 strains of the pathogen from various hosts on horseradish. The results indicate that while the strain of *V. dahliae* from horseradish has a broad host range, it is also more virulent on horseradish than the other strains tested.

China aster, eggplant, horseradish, potato, and sunflower showed external symptoms typical of Verticillium wilt, such as leaf chlorosis and necrosis with vascular discoloration. Pepper, tomato, and watermelon were asymptomatic hosts, since plants were colonized by the pathogen without showing any symptoms. Similar results were obtained by Stark (24), who reported that the pathogen isolated from horseradish was pathogenic to potato but failed to produce symptoms in tomato. Recovery of *V. dahliae* from symptomless plants has been reported (6,16,20,26). These asymptomatic hosts may serve as sources of inoculum to maintain or increase pathogen populations if they are grown in rotation with horseradish. The disease reaction of brussels sprouts was intermediate between the externally symptomatic and asymptomatic hosts. Infected plants of brussels sprouts did not exhibit any external symptoms but did develop discoloration of the vascular tissue.

The 11 strains of *V. dahliae* from herbaceous and woody hosts varied in their ability to induce Verticillium wilt symptoms on horseradish plants. Although horseradish was susceptible to all 11 strains of the pathogen, the horseradish strain proved to be the most virulent. Mueller et al (18) inoculated horseradish with strains of *V. dahliae* from potato and velvetleaf (*Abutilon theophrasti* Medik.) and found only 10% of the horseradish plants were infected by the potato strain, whereas the velvetleaf strain was not pathogenic on horseradish. In our experiments, 50% of the horseradish plants were systemically infected by a potato strain (V-13), and the pathogen was recovered from 75% of the inoculated plants.

The origin of the horseradish strain of *V. dahliae* is not known. The pathogen either could have been introduced on infected planting materials or was indigenous to soil in the area (18,20). Following its introduction, intensive cultivation of horseradish may have selected strains that were more virulent to horseradish. Determining the vegetative compatibility relationships (14,15) between the horseradish strains and other strains of *V. dahliae* may support the hypothesis that these strains are genetically distinct (22).

Throughout the experiment the pathogen was reisolated from inoculated plants with some difficulty. Predictions of infection based on symptom appearance did not always correlate well with pathogen recovery. The problem with recovery of the pathogen was complicated by infection of the horseradish roots by *Fusarium* spp. Although symptoms of *Fusarium* spp. infection in horseradish usually can be distinguished as scattered brown spots in the root and brownish discoloration in the crown (18), colonization by *Fusarium* spp. did occasionally produce black flecks in the root that were similar to those caused by *V. dahliae*. Thus, disease ratings of root symptoms did not correlate well with disease ratings of foliar symptoms. Other factors associated with isolation difficulties could be attributed to the degree to which the pathogen had systemically spread through the host or to the uneven distribution of the pathogen within the host tissues (26). In our reisolation study, recovery of the pathogen varied not only between two different media but also on the same medium. Sometimes only one of eight infected pieces yielded the pathogen on either one

Table 2. Susceptibility of nine plant species to a *Verticillium dahliae* strain from horseradish

Tested plant	External disease incidence (%)	Internal disease incidence (%)		Foliar rating[x]	Recovery (%)
		Stem	Root		
Brussels sprouts cv. Valiant Hybrid	0	80	100	1.0 d[y]	100
China aster cv. Crego Mix	60	100	100	1.8 c	80
Eggplant cv. Burpee Hybrid	100	100	100	3.8 b	100
cv. Japanese Long Purple	100	80	100	3.6 b	100
cv. Millionaire	100	100	100	4.0 b	100
Horseradish cv. 647	100	100	100	3.6 b	100
Pepper cv. California Wonder	0	0	0	0.0 e	40
Potato cv. Atlantic	100	ND[z]	ND	5.0 a	40
cv. Norland	100	ND	ND	5.0 a	40
Sunflower cv. Giant Greystripe	60	80	100	2.0 c	100
Tomato cv. Rutgers	0	0	0	0.0 e	40
Watermelon cv. Charleston Gray	0	0	0	0.0 e	40

[x]Based on a scale of 0–5, where 0 = no symptoms and 5 = more than three-quarters of the leaves show symptoms or lethal reaction.
[y]Values in each column followed by the same letter do not differ significantly according to the Waller-Duncan test ($P < 0.05$).
[z]ND = not determined.

Table 3. Disease development on susceptible horseradish cultivar 647 following inoculation with 11 strains of *Verticillium dahliae*

Strain origin	External disease incidence (%)	Disease rating		Incubation period (days)	Recovery (%)
		Foliar[v]	Root[w]		
Black gum	25	1.3 bc[x]	0.8 de	70	0
Cotton (isolate T-9)	50	2.3 abc	2.0 ab	49	100
Cotton (isolate SS-4)	50	1.8 bc	1.8 abc	90	100
Horseradish	100	4.0 a	2.3 a	35	100
Japanese maple	50	1.8 bc	1.3 cd	70	75
Littleleaf linden	50	2.0 bc	1.8 abc	63	100
Potato (isolate V-13)	50	1.5 bc	0.8 de	77	75
Potato field soil	25	1.3 bc	1.8 abc	110	50
Sugar maple	75	2.5 ab	2.0 ab	69	100
Sumac	75	2.5 ab	2.0 ab	72	100
Viburnum	50	2.3 abc	1.5 bc	53	75
Control[y]	0	0.5 c	0.5 e	ND[z]	0

[v]Based on a scale of 0–5, where 0 = no symptoms and 5 = more than three-quarters of the leaves show symptoms or lethal reaction.
[w]Based on a scale of 0–3, where 0 = no internal symptoms and 3 = more than 50% of the root cross section shows symptoms.
[x]Values in each column followed by the same letter do not differ significantly according to the Waller-Duncan test ($P < 0.05$).
[y]Control plants were root-dipped in sterile water.
[z]ND = not determined.

of the media.

Effective strategies for controlling Verticillium wilt of horseradish should include attempts to avoid spreading the pathogen to noncontaminated fields (18,20). Planting noninfected set roots in noninfested soil provides a practical approach for control (17,18). However, because plant certification programs and soil detection methods for the pathogen are not available at the present time for horseradish growers in Illinois, the disease is causing significant problems in this region. Therefore, any measures that can effectively reduce populations of the pathogen would be desirable. So far, seed treatment with fungicides has been ineffective, the use of soil fumigants has been impractical, and cultivars with high levels of resistance have not been identified (18). Crop rotation has been recommended as part of an integrated pest management program for controlling Verticillium wilt (5), but its effects in reducing inoculum levels of the pathogen are inconsistent and depend on the amount of time between the presence of highly susceptible hosts and the choice of crops used in the rotation sequence (4,8,10,16). The results from our study indicate that crop rotation may have been an unrealistic control practice, because the pathogen exhibits a wide host range and because horseradish is susceptible to various strains of *V. dahliae* that survive well in soil (11,13). In addition, asymptomatic weed hosts and infected volunteer horseradish plants may serve as reservoirs of inoculum that may negate the benefits of crop rotation in horseradish fields.

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