Development of Wilt in Mint in Response to Infection by Two Pathotypes of *Verticillium dahliae* and Co-infection by *Pratylenchus penetrans*

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ABSTRACT

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Two isolates of *Verticillium dahliae* in vegetative compatibility group (VCG) 2B and one isolate in VCG 4A were tested for aggressiveness on peppermint, Scotch spearmint, and native spearmint alone and in combination with the lesion nematode *Pratylenchus penetrans*. The VCG 2B isolates were more aggressive than the VCG 4A isolate on all three mint species. Severity of wilt was greatest on peppermint and Scotch spearmint with the VCG 2B isolates, and CFU of *V. dahliae* recovered from infected stems were greater in Scotch spearmint when inoculated with *V. dahliae* and *P. penetrans* than when inoculated with VCG 2B alone. Wilt severities and CFU in peppermint and Scotch spearmint were not increased when inoculated with VCG 4A and *P. penetrans* compared with VCG 4A alone. These data indicate that *V. dahliae* isolates from VCG 2B but not VCG 4A interact synergistically with *P. penetrans* on *Verticillium* isolate-susceptible mints. The resistance to *Verticillium* isolates in native spearmint was not affected by the presence of lesion nematodes.

Verticillium wilt, caused by *Verticillium dahliae*, is a limiting disease of cultivated peppermint, *Mentha × piperita*, and Scotch spearmint, *M. × gracilis* (*M. cardiaca*), in North America. A third cultivated mint, native spearmint, *M. spicata*, is more resistant to wilt and is not as severely damaged by the disease as the other two commercial species. Symptoms of the disease are characterized by chlorosis, stunting, asymmetric development of apical leaves, shortening of internodes, and plant mortality (10). Co-infection by the lesion nematode, *Pratylenchus penetrans*, and *V. dahliae* in peppermint and Scotch spearmint can enhance disease severity (1,6,20). *P. minyus* (syn. *P. neglectus*) was reported to increase the incidence and severity of *Verticillium* wilt on peppermint (6–8); however, field observations and greenhouse studies show that mint is not a suitable host for *P. neglectus* (G. S. Santo, unpublished data). Co-infection of potato by *V. dahliae* and *P. penetrans* enhances onset and severity of wilt symptoms (2,3,12,18,19). Although

the mechanism of this interaction is not fully understood, it appears the nematode may alter or delay host response to infection and colonization by *V. dahliae*, rather than just causing root wounds that facilitate entrance of the fungus into the vascular system (3,7).

Green (9) demonstrated that *V. dahliae* from peppermint was not host specific to the genus *Mentha*, as once thought (16), and presented evidence for the existence of strains of *V. dahliae* that varied in host specificity and aggressiveness (9). Although *V. dahliae* has a wide host range, isolates differ in aggressiveness on various host species (5,9,13–15,17). Vegetative compatibility analysis and pathogenicity tests of *V. dahliae* isolates from peppermint, spearmint, and other hosts have shown that isolates belonging to vegetative compatibility group (VCG) 2B are more prevalent in infected mint plants in commercial fields and more aggressive in mint than isolates belonging to VCG 4A and VCG 4B (5). Moreover, VCG 4A of *V. dahliae* appears to be a host-adapted pathotype of potato in North America, in that VCG 4A was isolated more frequently from potato stems and tubers, and was more aggressive on potato than VCG 4B (17). VCG 4A isolates and *P. penetrans* interact synergistically, resulting in severe disease and reduced tuber yields on potato; VCG 4B isolates and *P. penetrans* did not result in a synergistic infection (2). In a recent survey of *V. dahliae* isolates from potato in Israel, VCG 4B and VCG 2A were recovered, whereas VCG 4A was not (14).

This study investigated the interaction of isolates of *V. dahliae* from VCG 2B and VCG 4A with *P. penetrans* on the severity of *Verticillium* wilt of peppermint, Scotch spearmint, and native spearmint.

MATERIALS AND METHODS

Pathogenicity and aggressiveness of three isolates of *V. dahliae* was evaluated on three mint species by inoculating each with specific fungal isolates alone or in combination with *P. penetrans*. *V. dahliae* isolates 50 and 109 (both VCG 2B) were obtained from peppermint collected in 1996 in Benton and Grant Counties, WA, respectively. Isolate 653 (VCG 4A) was obtained from potato cv. Russet Burbank collected in 1995 in southern Idaho. Isolates were stored in soil at 4°C. Conidia and microsclerotia from pure cultures of each isolate were increased in Czapek-dox broth in shake culture at 21 to 23°C for 9 days. The suspension was filtered through four layers of cheesecloth and then mixed with sterile sand. This mixture was then spread about 1 cm deep in galvanized flats and dried in a greenhouse under natural light at 18 to 26°C for 5 weeks to allow death of conidia. The sand-microsclerotial mixture was then incorporated into a standard potting medium (3:1:1, vol/vol/vol, peat moss:pumice:sand) at 100, 200, and 300 microsclerotia/cm³ soil in one experiment; at 30 and 60 microsclerotia/cm³ in a second experiment; and at 30 microsclerotia/cm³ in a third experiment. These inoculum concentrations reflect the range of *V. dahliae* microsclerotia detected in central Washington soils (D. A. Johnson, unpublished data). A relatively high inoculum level was selected in the first experiment to increase the chance for infection of native spearmint and infectivity by the VCG 4A isolate. Concentration of microsclerotia was determined by plating a series of dilutions from a known volume of the sand-microsclerotial mixture on polygalacturonic acid medium (NPX) (4), incubating in darkness for 2 weeks at 21 to 23°C, then counting the number of *V. dahliae* colonies that formed. Appropriate volumes of the microsclerotial mixture were mixed with the potting mix just before transplanting test plants.

Six-week-old cuttings, with well-developed root systems, of peppermint cv. Black Mitcham, Scotch spearmint, and native spearmint, were transplanted into infested soil treatments (height, width, and depth of

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pots were 10 by 10 by 7 cm, respectively). *P. penetrans* was increased and maintained on excised corn roots in tissue culture. The nematode inocula were collected from 2- to 3-month-old cultures by dissecting the roots in distilled water and incubating at 21°C overnight. A 5.0-ml suspension of 5,000 *P. penetrans* vermiforms per pot was pipetted around the root system of each plant during transplanting. Treatments were arranged on greenhouse benches as a randomized complete block with nine replicates (each pot was an experimental unit). Black Mitcham peppermint and native spearmint were used in the first experiment and Black Mitcham peppermint and Scotch spearmint were used in the second and third experiments. Plants grown in pots without *V. dahliae* and *P. penetrans* served as nontreated controls. The three mint species also were inoculated with *P. penetrans* alone. Temperatures were 19 to 26°C during the day and 16 to 19°C at night. Natural light supplemented with sodium lamps (approximately 210 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic active radiation) provided a photoperiod of 14 to 15 h.

Verticillium wilt symptoms were assessed 9 weeks after inoculation when plants had begun to flower. After disease ratings were taken, stems were cut near the soil line and plants were allowed to regrow. Wilt severity was assessed again 6

weeks later on the regrowth. The following scale was used for wilt assessments: 0 = no visible symptoms; 1 = mild chlorosis of upper one-tenth of the plant; 2 = distinct chlorosis in upper one-tenth of the plant; 3 = asymmetric growth in leaves or stems, stunting (80% height of nonaffected stems), and chlorosis of upper one-fifth of plant; 4 = severe stunting (60% height of nonaffected stems) and two-fifths or more of the tissue chlorotic; and 5 = severe stunting and 40% or more necrotic tissue.

Quantity of *V. dahliae* in mint stems was determined after the second wilt assessment (11). A stem section, 3 cm in length, was cut from each plant beginning 1 cm above the soil line, then washed, disinfested in 0.5% NaOCl for 3 min, placed in a polyethylene bag, and crushed in a vise. Distilled water (1 ml) was added to the plant sap, after which 0.5 ml of the resultant suspension was spread evenly on NPX agar medium (4) and incubated in darkness at 21 to 23°C for 2 weeks. Colonies of *V. dahliae* were then counted.

Nematodes were extracted by washing roots free of soil and incubating them for 1 week in plastic bags with enough water to cover the roots. Nematodes were collected by vigorously shaking each bag, then passing the suspension through 35- and 500-mesh sieves. The nematodes collected on the 500-mesh sieve were counted, and the

number of *P. penetrans* per gram of dry root weight was determined. Roots were dried in an oven at 55°C for 48 h.

Data for wilt severity and numbers of CFU of *V. dahliae* were analyzed as a randomized complete block design using SAS general linear models procedure (version 6.12; SAS Institute, Cary, NC). Data for CFU were log transformed to satisfy normality and variance assumptions, but all data are reported as nontransformed values. Mean separations were done using Fisher's protected least significant difference and single degree of freedom contrasts.

RESULTS

Severity of Verticillium wilt and CFU of *V. dahliae* did not differ significantly ($P > 0.05$) among inoculum levels within the first and second experiments; thus, data for inoculum level within an experiment were combined and only one inoculum level was used for the third experiment (Table 1). Mean values for wilt severity among mint species and treatments increased between the first and second growth periods. Treatment effects were the same for both disease assessments within an experiment; therefore, data from the second assessment are presented for the three experiments (Table 1).

Wilt symptoms were greater ($P < 0.05$) in Black Mitcham peppermint and native

Table 1. Severity of wilt symptoms and CFU of *Verticillium dahliae* from stems of Black Mitcham peppermint, native spearmint, and Scotch spearmint inoculated with three isolates in two different vegetative compatibility groups (VCGs) alone and in combination with *Pratylenchus penetrans*^a

Mint species/isolate ^c	VCG	Wilt severity index ^b		CFU/cm of stem	
		<i>V. dahliae</i>	<i>V. dahliae</i> + <i>P. penetrans</i>	<i>V. dahliae</i>	<i>V. dahliae</i> + <i>P. penetrans</i>
First experiment					
Black Mitcham					
Isolate 50	2B	4.1	4.2	369	498
Isolate 109	2B	3.9	4.0	131	198
Isolate 653	4A	2.3*	2.7*	0*	73*
Native spearmint					
Isolate 50	2B	3.6	3.5	0	0
Isolate 109	2B	3.0	3.2	22	3.6
Isolate 653	4A	2.5*	2.7*	0	0
Second experiment					
Black Mitcham					
Isolate 50	2B	4.0	4.0	39	20
Isolate 109	2B	4.1	4.5**	117	267
Isolate 653	4A	1.9*	2.1*	0.04*	0.04*
Scotch spearmint					
Isolate 50	2B	2.8	3.2**	0.6	16**
Isolate 109	2B	2.6	3.3**	0.4	13**
Isolate 653	4A	2.3*	2.1*	2.0	0.04*
Third experiment					
Black Mitcham					
Isolate 50	2B	2.8	3.2**	238	154
Isolate 109	2B	3.4	3.8**	282	248
Isolate 653	4A	1.3*	1.6*	0*	0*
Scotch spearmint					
Isolate 50	2B	2.1	3.3**	0	7**
Isolate 109	2B	2.2	3.4**	0	42**
Isolate 653	4A	1.2*	1.3*	0	0*

^a Values are means of nine replicates; * indicates value for isolate 653 (VCG 4A) is significantly less ($P < 0.05$) than values for VCG 2B isolates; ** indicates significantly greater ($P < 0.05$) when co-inoculated than when only inoculated with *V. dahliae*.

^b Wilt severity index: 0 = healthy; 5 >40% necrotic tissue.

^c Inoculum concentration levels: first experiment, 100, 200, and 300 microsclerotia/cm³ of soil; second experiment, 30 and 60 microsclerotia/cm³ of soil; third experiment, 30 microsclerotia/cm³ of soil. Wilt severity and CFU did not differ significantly among inoculum levels within each experiment; thus, data for inoculum levels within an experiment were combined.

spearmint in the first experiment and in Black Mitcham peppermint and Scotch spearmint in the second and third experiments when inoculated with the two VCG 2B isolates compared with the VCG 4A isolate (Table 1). Numbers of CFU of *V. dahliae* in stems were greater ($P < 0.05$) in peppermint in the three experiments when inoculated with the VCG 2B isolates than with the VCG 4A isolate (Table 1). Numbers of CFU obtained from native spearmint were low, and no difference ($P > 0.05$) between VCG 2B and VCG 4A isolates was observed. Numbers of CFU in Scotch spearmint were higher for the VCG 2B isolates than the VCG 4A isolate when in the presence of *P. penetrans*, but were similar when plants were inoculated only with *V. dahliae* (Table 1).

Wilt severity and number of CFU from plant stems were greater ($P < 0.05$) for Black Mitcham peppermint than for native spearmint in the first experiment and greater for Black Mitcham peppermint than for Scotch spearmint in the second and third experiments with the VCG 2B isolates, but not with the VCG 4A isolate (Table 1).

In the first experiment, wilt severity and numbers of CFU in Black Mitcham peppermint and native spearmint were not increased ($P > 0.05$) when inoculated with *V. dahliae* isolates and *P. penetrans* compared with *V. dahliae* alone (Table 1). In the second and third experiments, wilt severity was more severe ($P < 0.05$) in Scotch spearmint when inoculated with the VCG 2B isolates of *V. dahliae* and *P. penetrans* than when inoculated with VCG 2B alone. Wilt severity was significantly greater for Black Mitcham peppermint when inoculated with one of two VCG 2B isolates and *P. penetrans* than with VCG 2B alone in the second experiment, and with both VCG 2B isolates and *P. penetrans* than with VCG 2B isolates alone in the third experiment. Numbers of *V. dahliae* CFU in stems were significantly higher ($P < 0.05$) in Scotch spearmint when inoculated with the VCG 2B isolates and *P. penetrans* than when inoculated with VCG 2B alone. Numbers of CFU did not differ significantly ($P > 0.05$) in Black Mitcham peppermint when inoculated with the VCG 2B isolates and *P. penetrans* compared with VCG 2B alone (Table 1). Wilt severity and CFU in Black Mitcham peppermint and Scotch spearmint were not increased ($P > 0.05$) when inoculated with VCG 4A and *P. penetrans* compared with VCG 4A alone (Table 1).

In all experiments, disease symptoms did not develop on control plants that were not inoculated with either pathogen or those inoculated only with *P. penetrans*. Mean level of *P. penetrans* per gram of dry root weight of plants inoculated with both pathogens was 1,085 for Black Mitcham peppermint and 451 for native spearmint in the first experiment, 1,411 for Black Mitcham peppermint and 1,230 for Scotch

spearmint in the second experiment, and 837 for Black Mitcham peppermint and 1,834 for Scotch spearmint in the third experiment. *P. penetrans* was not recovered from plants inoculated only with *V. dahliae* or from plants not inoculated with either pathogen.

DISCUSSION

A principle finding in this study was that the VCG 2B isolates were capable of interacting with *P. penetrans* on Black Mitcham peppermint and Scotch spearmint, causing increased disease severity, whereas the VCG 4A isolate did not. A similar but opposite finding was reported on potato, where isolates from VCG 4A interacted with *P. penetrans* to increase wilt severity, whereas isolates from VCG 4B did not interact with *P. penetrans* or did so only weakly (2). On potato, the interaction of *V. dahliae* and lesion nematodes also varies with the species of *Pratylenchus* involved (18). A possible explanation for these findings is that *P. penetrans* affects the host defense mechanisms, allowing the most aggressive strains of *V. dahliae* to colonize and reproduce (3,7).

The two isolates of *V. dahliae* from VCG 2B were more aggressive to mint than an isolate from VCG 4A. Wilt severities and numbers of CFU were higher in plants inoculated with VCG 2B isolates. The three mint species, with Black Mitcham peppermint being the most susceptible and native spearmint the most resistant (13), were each more severely affected by the VCG 2B isolates. The relative levels of resistance between native and Scotch spearmint cannot be compared in this study because inoculum levels differed in the two experiments; however, native spearmint has been demonstrated to be less susceptible than Scotch spearmint (13).

The interaction of a specific VCG of *V. dahliae* with *P. penetrans* on either mint or potato supplies additional evidence that VCG 4A is a host-adapted pathotype on potato in North America (17) and VCG 2B is a pathotype on mint (5). We consider a pathotype to be a dominant (frequency of isolation) and highly aggressive strain of a pathogen on a particular host. Other strains of the same pathogenic species may also infect the same host. Supportive evidence for a pathotype concept was originally derived when both VCG 2B and VCG 4A were found to be more prevalent and aggressive on their respective hosts (5,17,21,22). Only a few isolates of *V. dahliae* were used in this study, but this demonstration of increased aggressiveness on mint by isolates from VCG 2B is consistent with recent studies (5,13). This and previous work (2) demonstrated that the association of *P. penetrans* with *V. dahliae* is related to host-adapted strains of *V. dahliae*. In mint and potato, the association is specific to *V. dahliae* strain, *P. penetrans*, and the host.

The resistance in native spearmint in this study was effective against the co-inoculation of both *V. dahliae* and *P. penetrans* and no synergistic effect due to the presence of both pathogens was observed. This agreed with earlier studies (G. S. Santo, unpublished data). Numbers of CFU were low from stems of inoculated native spearmint because of effective host resistance (11,13). In the first experiment, a significant increase in disease severity was not observed on Black Mitcham peppermint co-inoculated with the VCG 2B isolates and *P. penetrans* compared with *V. dahliae* alone, possibly due to the higher range of *V. dahliae* inoculum used. The high numbers of microsclerotia used to infest soils in the test may have overwhelmed potential effects due to the nematode (G. S. Santo, unpublished data). The intent of the first experiment was to provide sufficient inoculum of *V. dahliae* so that plant escapes of native spearmint would not be a factor. This was accomplished. Inoculum levels used in these studies produced severe disease symptoms and were adequate for separating effects of the two pathotypes and levels of host resistance.

Management of Verticillium wilt of mint depends on managing the VCG 2B pathotype of *V. dahliae* and the root-lesion nematode, *P. penetrans*. The most aggressive isolates of *V. dahliae* should be used when screening material for resistance (13), and rhizomes used from planting material should be free of VCG 2B. Differentiating among pathotypes of *V. dahliae* in soil, as well as levels of *P. penetrans*, may be beneficial in planning management strategies before planting.

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LITERATURE CITED

1. Bergeson, G. B. 1963. Influence of *Pratylenchus penetrans* alone and in combination with *Verticillium albo-atrum* on growth of peppermint. *Phytopathology* 53:1164-1166.
2. Botseas, D. D., and Rowe, R. C. 1994. Development of potato early dying in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans*. *Phytopathology* 84:275-282.
3. Bowers, J. H., Nameth, S. T., Riedel, R. M., and Rowe, R. C. 1996. Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. *Phytopathology* 86:614-621.
4. Butterfield, E. J., and De Vay, J. 1977. Reassessment of soil assay for *Verticillium dahliae*. *Phytopathology* 67:1073-1078.
5. Douhan, L. I., and Johnson, D. A. 2001. Vegetative compatibility and pathogenicity of *Verticillium dahliae* from spearmint and peppermint. *Plant Dis.* 85:297-302.
6. Faulkner, L. R., and Bolander, W. J. 1969. Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint: Effect of soil temperature. *Phyto-*

- pathology 59: 868-870.
7. Faulkner, L. R., Bolander, W. J., and Skotland, C. B. 1970. Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint: Influence of the nematode as determined by a double root technique. Phytopathology 60:100-103.
 8. Faulkner, L. R., and Skotland, C. B. 1965. Interactions of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint. Phytopathology 55:583-586.
 9. Green, R. J. 1951. Studies on the host range of the *Verticillium* that causes wilt of *Mentha piperita* L. Science 113:207-208.
 10. Horner, C. E. 1954. Pathogenicity of *Verticillium* isolates to peppermint. Phytopathology 44:239-242.
 11. Hoyos, G. P., Zambino, P. J., and Anderson, N. A. 1991. An assay to quantify vascular colonization of potato by *Verticillium dahliae*. Am. Potato J. 68:727-742.
 12. Huan, J., Santo, G. S., and Mojtahedi, H. 1988. Effect of *Pratylenchus penetrans*, *Erwinia carotovora* and *Verticillium dahliae* on potato yield in microplots. J. Nematol. 20:640-641.
 13. Johnson, D. A., and Cummings, T. F. 2000. Evaluation of mint mutants, hybrids and fertile clones for resistance to *Verticillium dahliae*. Plant Dis. 84:235-238.
 14. Korolev, N., Katan, J., and Katan, T. 2000. Vegetative compatibility groups of *Verticillium dahliae* in Israel: Their distribution and association with pathogenicity. Phytopathology 90:529-536.
 15. Lacy, M. L., and Horner, C. E. 1965. Verticillium wilt of mint: Interactions of inoculum density and host resistance. Phytopathology 55:1176-1178.
 16. Nelson, R. 1950. Verticillium wilt of peppermint (*Mentha piperita* L.). Mich. Agric. Exp. Stn. Tech. Bull. 221.
 17. Omer, M. A., Johnson, D. A., and Rowe, R. C. 2000. Recovery of *Verticillium dahliae* from N. American certified seed potatoes and characterization of strains by vegetative compatibility and aggressiveness. Am. J. Potato Res. 77:325-331.
 18. Riedel, R. M., Rowe, R. C., and Martin, M. J. 1985. Differential interactions of *Pratylenchus crenatus*, *P. penetrans*, and *P. scribneri* with *Verticillium dahliae* in potato early dying disease. Phytopathology 75:419-422.
 19. Rowe, R. C., Riedel, R. M., and Martin, M. J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75:412-418.
 20. Santo, G. S., and Skotland, C. B. 1986. Interaction of *Pratylenchus penetrans* and *Verticillium dahliae* on Scotch spearmint in microplots. J. Nematol. 18:631.
 21. Strausbaugh, C. A. 1993. Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. Phytopathology 83:1253-1258.
 22. Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R., and Hancock, J. G. 1992. Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. Phytopathology 82:61-68.