Vegetative Compatibility and Pathogenicity of *Verticillium dahliae* from Spearmint and Peppermint

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ABSTRACT

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The vegetative compatibility of 128 isolates of *Verticillium dahliae* from spearmint and peppermint in the western and midwestern United States was determined. *Nit* mutants were used to assign isolates to vegetative compatibility groups (VCGs). All isolates were assigned to VCG 2B except for one assigned to VCG 2A and two assigned to VCG 4A. VCG 2 isolates were found in all commercial mint growing regions, while the two VCG 4A isolates originated from southern Idaho. Pathogenicity assays on mint were performed using isolates from mint and other hosts, and pathogenicity assays were conducted on the potato cultivar Russet Norkotah using mint and potato isolates. Isolates originating from mint were significantly more aggressive on mint than were other host isolates, indicating that mint isolates were host-adapted. VCG 4A isolates from mint and potatoes were significantly more aggressive on potato than VCG 4B potato isolates and VCG 2B mint isolates. We speculate that the low VCG diversity of mint isolates may be due to the introduction of a single aggressive strain into Washington State mint fields via infected rhizomes.

Additional keywords: *Mentha*, pathotypes, *Verticillium* wilt

Verticillium wilt, caused by the vascular wilt pathogen *Verticillium dahliae* Kleb., is an economically important disease of peppermint (*Mentha × piperita* L.) and Scotch spearmint (*M. × gracilis* Sole), two of the three main mint species produced commercially for essential oils in western and midwestern states. The third mint species, Native spearmint (*M. spicata* L.), can be infected by *V. dahliae* but is less susceptible to wilt damage than the other two mint species (4). During the late 1940s, the majority of mint production shifted from midwestern to western states due to *Verticillium* wilt (23,27). *V. dahliae* reduces oil production by limiting plant growth or killing plants. *Verticillium* wilt is managed by planting disease-free mint rhizomes in noninfested soil and rotating with nonsusceptible hosts (16,25). In Washington and Idaho, mint is occasionally grown in soil previously planted with susceptible crops such as potato. Knowledge of host-specificity of strains of *V. dahliae* would assist in making disease management decisions.

V. dahliae isolates are thought to have a wide host range and lack host specificity. However, pathotypes have been reported

for potato (22,29,36) and cotton (34), and physiological races have been shown for tomato (1). Nelson (27) considered the microsclerotial form of *Verticillium* from peppermint to be host-specific and reclassified *V. dahliae* (18) as *V. albo-atrum* var. *menthae*. Subsequent work demonstrated that host specificity of *V. dahliae* isolates was not as specific as Nelson (27) had reported (14,15,17,19).

Vegetative compatibility is a useful marker for determining the genetic structure of some fungal populations (24) and, in fungi, is controlled by multiple genetic loci. Hyphae of strains that have identical alleles present at all compatibility loci can anastomose to form a viable heterokaryon. Isolates that share compatibility loci and can anastomose with one another belong to a subpopulation designated as a vegetative compatibility group and are genetically isolated from other VCGs (24,30).

Four or more VCGs (21,35) have been detected among *V. dahliae* isolates from various hosts (3,8,11,13,21,22,26,29,30,32,37,38). Several studies have correlated VCGs with pathogenicity on different hosts (11,22,36). For example, Joaquim and Rowe (22) separated VCG 4 isolates into two subgroups, VCG 4A and VCG 4B. The subgroups were classified as pathotypes and were separated based on pathogenicity experiments on potatoes and the positive reactions with different tester strains. When inoculated to potatoes, VCG 4A isolates were more aggressive than VCG 4B isolates.

Although a few mint isolates have been included in previous vegetative compatibility studies (20,32), VCG diversity in *V. dahliae* populations infecting mint has not been studied. Additionally, the level of host adaptation in *V. dahliae* populations associated with mint is not clear. Knowledge of VCG diversity and host adaptation would be useful in developing wilt-resistant mint species, designing crop rotations for mint, and determining inoculum sources for *V. dahliae* in mint fields. Mint is rotated with potato in Washington State, and a better understanding of host adaptation and aggressiveness of *V. dahliae* isolates from both hosts would help devise better disease management strategies. The objectives of this study were to: (i) determine VCG diversity of *V. dahliae* isolates collected from naturally infected spearmint and peppermint plants grown throughout the western and the midwestern United States; and (ii) evaluate pathogenicity and aggressiveness of *V. dahliae* isolates from mint and other hosts on mint and potato. A preliminary report of this investigation has been published (12).

MATERIALS AND METHODS

Isolates of *V. dahliae*. *V. dahliae* isolates from mint and other host plants were obtained from plants collected in the field and from collaborators. Mint plants showing *Verticillium* wilt symptoms were collected from randomly selected commercial mint fields in Washington, Idaho, and Oregon between June and September 1996. One diseased stem per plant was surface-sterilized with a 10% commercial bleach solution for 2 min, rinsed with sterile water, and crushed inside a polyethylene plastic bag using a vise. Two milliliters of sterile distilled water was added to the bag, and 0.25 to 0.50 ml of sap solution was spread onto NPX medium (6) and incubated at room temperature (21 to 24°C) for 10 to 15 days. One isolate per stem with up to three isolates per field were randomly chosen from NPX plates to be used in vegetative compatibility analyses. Monoclonal isolates were stored on sterilized filter paper and kept at 5°C.

Vegetative compatibility. Nitrate nonutilizing mutants (*nit*) were generated using a modified version of Puhalla's technique (31). *V. dahliae* isolates were incubated for 1 to 2 weeks at room temperature (21 to 24°C) on cornmeal agar (Difco Laboratories, Detroit, MI) amended with dextrose

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(2 g/liter) and potassium chlorate (25 to 40 g/liter). Conidia from chlorate-resistant sectors were streaked onto Minimal Medium (31) with nitrate as the sole source of nitrogen. *Nit* mutants were identified by their thin, expansive, appressed growth. One *nit* mutant was obtained from each wild-type isolate. Monoconidial cultures of each mutant were stored as described above. *Nit* mutants were separated into two phenotypic classes, *nit1* and *NitM* (9), based on their growth on Basal Media (21) amended with one of four different nitrogen sources: sodium nitrate, hypoxanthine, ammonium-tartrate, or uric acid.

Each *nit* mutant of *V. dahliae* was paired with a known *nit1* and *NitM* VCG testers designated as OARDC tester strains obtained from R. C. Rowe (Ohio State University, Wooster). The tester strains used for each VCG were the following: VCG 1, V-44; VCG 2A, PH; VCG 2B, 115; VCG 3, PCW; VCG 4A, BB; VCG 4B, S39. Mutants were paired with each tester at least twice. Additionally, eight *nit* mutants were tested for complementation across a polycarbonate membrane to ensure that the positive reaction resulted from heterokaryon formation and not cross-feeding. In these tests, each mutant was paired with each tester, which was separated by a 47-mm-diameter polycarbonate membrane with a pore size of 0.2 μ m (Poretics Corp., Livermore, CA).

Pathogenicity assays. Isolates from mint and other hosts were tested for patho-

genicity and aggressiveness on mint and potato in four greenhouse experiments. The first three experiments were conducted on two mint species, Black Mitcham peppermint and Scotch spearmint, and were designated as experiments 1 to 3. The fourth experiment was conducted on the potato cultivar Russet Norkotah and was designated as experiment 4. Each experiment was conducted twice using the same isolates and hosts. Isolates from mint and other hosts were used for the three mint experiments, and isolates from mint and potato were used for the experiment on potato. Experiment 1 included 18 mint isolates and five isolates from other hosts and was replicated four times (Table 1). In experiment 1, a greater number of mint isolates were used to evaluate the range of variation among isolates from mint. Experiments 2 and 3 included four mint isolates and four isolates from other hosts (Tables 2 and 3), and experiment 4 included four mint isolates and four potato isolates (Table 4). Experiments 2 and 3 were replicated eight times, and experiment 4 was replicated 10 times. Additional replicates were added to experiments 2 to 4 to increase statistical power. Three isolates in experiment 1 were not used when the experiment was repeated due to the lack of availability of inoculum but were used in experiment 2. Isolates for each experiment were chosen by species origin and geographic location. Treatments were arranged in the greenhouse in a randomized com-

plete block to reduce variability among the experimental units within a block.

Peppermint and Scotch spearmint plants were clonally propagated in flats by cutting the top 7.5 to 10 cm of stems from Verticillium-free repository mint stock, dipping cut stems into Root tone (NAA, Madison, GA), and planting the stems in sterilized potting soil. The cuttings were grown in the greenhouse until the plants were approximately 3 to 5 cm tall and had well-developed root systems. Potato plants were prepared by planting a 3 $\times$ 1.5 cm bud piece from generation-one certified seed tubers into sterilized potting soil and grown in the greenhouse until the plants were 8 to 10 cm. Seventy-five percent of the potatoes from the lot used in this experiment were tested and found to be free of *V. dahliae*. The tubers were tested by placing a small sliver of the stem end onto NPX media and examining the plates for *V. dahliae* at approximately 10 to 14 days.

Conidial inoculum of *V. dahliae* isolates were prepared by placing colonized agar plugs from wild-type isolates into 125 ml of Czapeks-Dox broth (Difco) or potato dextrose broth (Difco) and incubating at room temperature on a shaker for 7 days. Conidia were filtered through cheesecloth, quantified with a hemacytometer, and adjusted with sterile water to a concentration of 5 $\times$ 10⁵ conidia per ml for experiments 2 and 3 and 1 $\times$ 10⁶ conidia per ml for experiments 1 and 4. Preliminary data (not

Table 1. Mean disease severity values when spearmint and peppermint were inoculated with *Verticillium dahliae* isolates VCG 2B and VCG 4A from mint and other hosts in experiment 1

Isolate no.	VCG	Host origin	Geographic origin	Mint species		Mean
				Black Mitcham peppermint	Scotch spearmint	
123	2B	Spearmint	WA	5.4 ^{a,b}	5.0	5.2 ^c
29	2B	Peppermint	MT	5.8	4.5	5.1
46	2B	Spearmint	WA	5.2	5.0	5.1
104	2B	Spearmint	WA	6.0	4.5	5.1
47	2B	Spearmint	WA	5.1	4.8	5.0
21	2B	Spearmint	WA	4.9	4.9	4.9
141	2B	Peppermint	WA	5.3	4.4	4.8
84	2B	Peppermint	WA	5.5	4.0	4.8
36	2B	Peppermint	WA	4.9	4.6	4.7
151	2B	Peppermint	OR	5.3	4.1	4.7
57	2B	Spearmint	WA	5.1	4.2	4.6
75	2B	Spearmint	WA	4.7	4.5	4.6
127	2B	Spearmint	WA	4.7	4.6	4.6
136	2B	Peppermint	MT	4.6	4.3	4.4
130	2B	Spearmint	WA	3.2	4.6	3.9
49	2B	Peppermint	WA	4.0	4.1	3.9
98	2B	Spearmint	WA	2.4	3.9	3.2
868 ^d	2A	Okra	AK	2.8	3.0	2.7
155	4A	Peppermint	ID	2.7	3.0	3.2
601 ^e	4A	Cherry	WA	3.0	3.7	3.1
629 ^e	4A	Potato	BC	2.4	3.4	2.9
653 ^e	4A	Potato	ID	2.5	3.3	2.8
97-147-2	4A	Watermelon	OR	1.8	2.8	2.3
LSD (<i>P</i> = 0.05)						0.9

^a All values were rounded to the nearest one-tenth.

^b Symptoms recorded as 1 = no visible symptoms to 6 = dead or nearly dead.

^c Treatment means were compared according to Fischer's protected LSD (*P* < 0.05).

^d VCG designation of isolate 868 was determined by R. S. Potti and G. J. Weidemann, University of Arkansas, Fayetteville.

^e Isolates were not included in the repeated experiment.

published) showed no statistical difference between the two inoculum amounts; therefore, the amount of conidia used for experiments 2 to 4 was determined based on availability.

Plants were inoculated by uprooting them, gently shaking off soil, and soaking roots in 500 ml of prepared conidial suspension for approximately 5 min. Control plants were soaked in distilled water. All plants were planted in sterilized soil and placed in the greenhouse. Day–night temperatures for all experiments varied from 13 to 30°C. Natural light was supplemented with sodium lamps to achieve a photoperiod of at least 15 h. Light intensities ranged from 94 to 132 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR with sodium lights. After transplanting, mint plants were cut back to 1 to 2 cm above the soil line to promote new shoot growth.

Verticillium wilt symptoms were assessed in mint just before flowering at approximately 6 to 8 weeks after inoculation. After disease evaluations were made, stems were cut near the soil line and plants were allowed to regrow. Wilt severity was assessed again just before flowering at approximately 14 to 16 weeks after inoculation. The cutting and regrowth simulated

seasonal harvest of perennial mint plants in the field and allowed time for continued wilt development. The rating scale used for Verticillium wilt of mint was as follows: 1 = no visible symptoms; 2 = slight chlorosis of upper leaves; 3 = 1 to 30% of foliage with slight chlorosis of upper leaves and mild stunting; 4 = 31 to 60% of foliage with extensive chlorosis of upper leaves, moderate stunting, and few to many crescent leaves; 5 = >60% of foliage with severe chlorosis of upper leaves, severe stunting, and many crescent leaves; 6 = plants dead or nearly dead. Potato plants were evaluated for wilt symptoms four times at 7-day intervals to determine disease progression. The initial rating was taken at disease onset at 30 and 16 days after planting for the first and second repetition, respectively. The rating scale used for Verticillium wilt of potato was: 1 = no visible symptoms; 2 = slight chlorosis of upper leaves and slight stunting; 3 = slight chlorosis of upper leaves and mild stunting; 4 = extensive chlorosis of upper leaves and moderate stunting; 5 = chlorosis of upper and lower leaves; 6 = plants dead or nearly dead. Areas under the senescence progress curves (AUSPC)

were calculated using wilt severity values (7,22) using the following formula:

$$\sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

where Y_i = the cumulative disease severity at the i th observation, t_i = time (days postinoculation) at the i th observation, and n = the total number of observations.

All mint stems after the first rating and potato stems after the final rating were assayed for the presence of *V. dahliae* by the same method used initially to obtain *V. dahliae* isolates. If plants were dead, stems were surfaced-sterilized with 10% commercial bleach solution, rinsed in sterile water, and placed on moist filter paper in a glass petri dish. After 5 to 7 days, dead stems were examined for the presence of verticillate conidiophores or microsclerotia.

All experiments were analyzed independently using SAS, v 6.12 (SAS Institute, Cary, NC). The first and the repeat of each experiment were not combined due to statistical interaction between experiments. Mint data were analyzed as a split-plot in time using PROC GLM. Main plots were the mint species and isolates, and subplots

Table 2. Mean disease severity values when spearmint and peppermint were inoculated with *Verticillium dahliae* isolates VCG 2B and VCG 4A from mint and other hosts in experiment 2

Isolate no.	VCG	Host origin	Geographic origin	Mint species		Mean
				Black Mitcham peppermint	Scotch spearmint	
102	2B	Peppermint	WA	4.5 ^{a,b}	3.8	4.2 ^c
83	2B	Peppermint	WA	4.7	3.9	4.3
82	2B	Peppermint	WA	4.9	4.3	4.5
113	2B	Peppermint	WA	4.9	3.8	4.4
601	4A	Cherry	WA	1.6	2.3	2.0
608	4A	Potato	WA	1.6	2.3	2.0
629 ^d	4A	Potato	BC	2.2	1.9	2.0
653	4A	Potato	ID	2.0	1.8	1.9
LSD ($P = 0.05$)						0.7

^a All values were rounded to the nearest one-tenth.

^b Symptoms recorded as 1 = no visible symptoms to 6 = dead or nearly dead.

^c Treatment means were compared according to Fisher's protected LSD ($P < 0.05$).

^d Isolates were not included in the repeated experiment.

Table 3. Mean disease severity values when spearmint and peppermint were inoculated with *Verticillium dahliae* isolates VCG 2B, 2X, 2, and VCG 4A from mint and other hosts in experiment 3

Isolate no.	VCG	Host origin	Geographic origin	Mint species		Mean
				Black Mitcham peppermint	Scotch spearmint	
50	2B	Peppermint	WA	4.9 ^{a,b}	4.3	4.6 ^c
79	2B	Peppermint	WA	5.1	3.3	4.2
109	2B	Peppermint	WA	5.3	3.1	4.2
155	4A	Peppermint	ID	3.9	2.2	3.1
St81	2X ^d	Potato	ID	1.9	1.8	1.9
Le78	2X ^d	Tomato	CA	1.3	1.3	1.3
Fca29	2X ^d	Strawberry	CA	1.4	1.6	1.5
Ca35	2 ^d	Bell pepper	CA	1.8	2.0	1.9
LSD ($P = 0.05$)						0.9

^a All values were rounded to the nearest one-tenth.

^b Symptoms recorded as 1 = no visible symptoms to 6 = dead or nearly dead.

^c Treatment means were compared according to Fisher's protected LSD ($P < 0.05$).

^d VCG designation of VCG 2X and 2 were determined by K. Subbarao, University of CA, Davis, CA, Agricultural Research Station, Salinas, CA.

were disease ratings from the first and second growth. Split-plot analysis was used to obtain information on the differences between the disease ratings of the two growing periods. AUSPC data from the potato experiment were analyzed as a one-way analysis of variance (ANOVA). Single degree of freedom contrasts were used to test differences among treatment means. Fisher's protected LSD at $P = 0.05$ was used to compare treatment means.

RESULTS

Vegetative compatibility. All 128 mint isolates tested were assigned to two VCGs; 125 to VCG 2B, one to VCG 2A, and two to VCG 4A. Among the two phenotypic classes of *nit* mutants recognized, 70% exhibited wild-type growth on all nitrogen sources except nitrate and thus were considered *nit1*. The remaining 30% exhibited wild-type growth on all nitrogen sources except hypoxanthine and nitrate and thus were considered NitM. No prototrophic growth occurred between the testers and eight randomly selected mutants when separated by a membrane, demonstrating that the positive reactions were the result of heterokaryon formation and not cross-feeding.

Pathogenicity assays. All mint pathogenicity experiments showed that when inoculated to mint, isolates from mint were collectively more aggressive ($P < 0.05$) than those isolates from other hosts (Tables

1 to 3). For experiment 1, there were significant ($P < 0.05$) differences among the mint isolates and among the isolates from other hosts (Table 1). The results were the same when the experiment was repeated. For experiment 2, there were no significant ($P > 0.05$) differences among the four mint isolates or among the isolates from potato and cherry (Table 2). The results were the same when the experiment was repeated. In experiment 3, there were no statistical ($P > 0.05$) differences among the VCG 2B mint isolates in the first part, but in the repeat there were significant ($P < 0.05$) differences among the VCG 2B mint isolates. However, VCG 2B consistently produced more disease than isolate 155 from mint belonging to VCG 4A of the first (Table 3) and the repeat of experiment 3.

Disease symptoms were usually more severe on Black Mitcham peppermint than on Scotch spearmint. No statistical ($P > 0.05$) differences were found between the two mint species in the first part of experiment 1, but in the repeat, Scotch spearmint had significantly ($P < 0.05$) more disease than Black Mitcham peppermint. In experiments 2 and 3, Black Mitcham peppermint had significantly ($P < 0.05$) more disease than Scotch spearmint for the first part of the experiment and the repeat (Table 5). For all the mint experiments, wilt severity was significantly ($P < 0.05$) more severe when mint plants were allowed to regrow after being cut to simu-

late seasonal harvests, reflecting the increased wilt often seen in the field with successive cuttings.

When inoculated to potato, potato and mint VCG 4A isolates were significantly ($P < 0.05$) more aggressive than mint VCG 2B or potato VCG 4B, as indicated by the AUSPC values. Using single degree of freedom contrasts, the VCG 4A isolates were significantly ($P < 0.05$) more aggressive on potato than VCG 2A and VCG 4B isolates. When the experiment was repeated, all three VCGs differed significantly ($P < 0.05$) from one another; however, VCG 4A again was significantly ($P < 0.05$) more aggressive than the other VCGs (Table 4).

DISCUSSION

VCG diversity among 128 *V. dahliae* isolates from naturally infected spearmint and peppermint was low, with 98% of *V. dahliae* isolates belonging to a single VCG. The two isolates assigned to VCG 4A were collected in southern Idaho, whereas the VCG 2B and VCG 2A isolates were collected throughout the Pacific Northwest and northern Midwest, thus demonstrating the widespread distribution of VCG 2B on mint. These results agree with previous studies that have demonstrated low overall VCG diversity within *V. dahliae* within a particular host (29,33).

The predominance and widespread distribution of the VCG 2B mint strain may be attributed to asexual reproduction of *V. dahliae*, vegetative propagation of mint, and the relatively rapid shift of mint production from the U.S. Midwest to the Pacific Northwest. Since sexual reproduction is unknown in *V. dahliae*, it is unlikely that vegetative compatibility genes are recombining. Thus, VCG 2B strains may belong to a subpopulation genetically isolated from other VCGs (24), and the mint isolates may represent a clonal lineage. We speculate that a single strain of *V. dahliae* may have become disseminated in mint rhizomes from the Midwest during the late 1920s to the 1940s when the price of mint oil greatly increased and mint production expanded to the Pacific Northwest (23). Omer et al. (29) found similar results with *V. dahliae* from certified seed potatoes. Their study showed that VCG 4 was the only VCG recovered from the 162 isolates examined with more than half of the western and two-thirds of the eastern isolates belonging to VCG 4A.

V. dahliae is considered to have a wide host range; however, there are documented examples suggesting host-adapted isolates (1,10,22,29,34,36). Isolates within *V. dahliae* are considered to be host-adapted rather than host-specific since the isolates can potentially infect a wide range of hosts but often seem to be adapted as an aggressive pathogen only on certain hosts. In contrast, host-specific isolates by definition are able to infect only certain hosts. This

Table 4. Mean area under the senescence progress curve (AUSPC) values when potato cultivar Russet Norkotah was inoculated with *Verticillium dahliae* isolates from mint and potato belonging to VCG 2, 4A, and 4B in experiment 4

Isolate no.	VCG	Geographic origin	Host origin	Experiment 4 AUSPC ^a	
				4A	4B
145	2B	WA	Peppermint	29.1 ^{b,c}	NT ^d
83	2B	WA	Peppermint	30.8	21.4
82	2B	WA	Peppermint	37.8	21.0
653	4A	ID	Potato	88.9	112.4
155	4A	ID	Peppermint	102.9	109.8
233	4A	OH	Potato	66.2	104.1
235	4B	OH	Potato	NT	77.9
239	4B	OH	Potato	32.2	52.5
LSD ($P = 0.05$)				17.1	15.2

^a AUSPC = $\sum_{i=1}^{n-1} [(Y_i + Y_{i+1})/2](t_{i+1} - t_i)$ where Y_i = the cumulative disease severity at the i th observation, t_i = time (days postinoculation) at the i th observation, and n = the total number of observations.

^b Symptoms recorded as 1 = no visible symptoms to 6 = dead or nearly dead.

^c Values with the same letter for one experiment are not significantly different (LSD $P > 0.05$).

^d NT = not tested.

Table 5. Comparisons of mean disease severity values of Black Mitcham peppermint and Scotch spearmint for experiment 1, 2, and 3

Mint species	Experiment 1		Experiment 2		Experiment 3	
	First	Repeat	First	Repeat	First	Repeat
Black Mitcham peppermint	4.20 ^{a,b,c}	3.32	3.30	2.72	3.21	1.99
Scotch spearmint	4.11	3.78	3.01	2.17	2.45	1.67
LSD ($P = 0.05$)	0.25	0.43	0.33	0.36	0.38	0.26

^a All values were rounded to the nearest one-hundredth.

^b Symptoms recorded as 1 = no visible symptoms to 6 = dead or nearly dead.

^c Treatment means were compared according to Fisher's protected LSD ($P < 0.05$).

study demonstrates that mint isolates are not host-specific since mint isolates cause wilt on potatoes (Table 4) and isolates from several hosts can cause slight disease on mint (Tables 1 to 3). However, mint isolates should be considered host-adapted and probably comprise a distinct pathotype since they are collectively more aggressive on mint than isolates from other hosts.

Correlations between pathotype and VCG have been reported for *V. dahliae* (11,22,29,36). Joaquim and Rowe (22) determined that isolates within VCG 4A represented a distinct pathotype since they were collectively more aggressive on potatoes than isolates belonging to either VCG 4B or VCG 2. This study showed similar results. One VCG 4A isolate from mint and two from potato were more aggressive to the potato cultivar Russet Norkotah than VCG 4B isolates from potato and VCG 2 isolates from mint (Table 4). Botseas and Rowe (5) further distinguished VCG 4A isolates from VCG 4B isolates by demonstrating that on potato the root lesion nematode, *Pratylenchus penetrans*, interacted synergistically with VCG 4A *V. dahliae* isolates but either did not interact or did so only slightly with VCG 4B isolates. Schnarhorst and Mathre (34) designated two pathotypes, cotton defoliating and cotton nondefoliating, but Ashworth (2) suggested that there was a continuum of virulence among isolates from cotton rather than two distinct pathotypes. Daayf et al. (11) confirmed the correlation between VCG and the two cotton pathotypes by showing that the defoliating isolates belonged to VCG 1 and the nondefoliating isolates belonged to VCG 2 and VCG 4. Based solely on restriction fragment length polymorphism (RFLP) markers, a distinct host-adapted group from peppermint was recognized (28).

The two mint isolates from this study that belonged to VCG 4A were collected from diseased mint plants in two separate counties in southern Idaho. One of these isolates, 155, was obtained from a first-year mint field that previously had been cropped to potato but never to mint. The planting material originated from stem cuttings produced in sterile soil from *Verticillium*-free mother plants grown in a greenhouse. The stem cuttings were intended for rhizome certification for planting stock and are unlikely to have been the source of the *V. dahliae* isolate. It is more likely that this isolate originated from potato residue in the soil from the previous crop. When inoculated to potato, isolate 155 produced severe symptoms similar to those produced by other VCG 4A isolates from potato.

VCG analysis of *V. dahliae* from mint in this study agrees with previous observations that VCG diversity within *V. dahliae* is limited (3,8,11,13,21,22,26,29,30,32,37,38). The low VCG diversity in mint

isolates may be the result of the introduction and proliferation of a single aggressive strain via infected rhizomes. Pathogenicity experiments suggest that isolates from mint are a distinct pathotype since they are more aggressive on mint than isolates from other hosts. Knowledge of the presence of specific *V. dahliae* strains within mint rhizome planting stock or in the soil may be useful in selecting specific crop rotations involving mint and other crops susceptible to *V. dahliae*.

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