Evaluation of Verticillium Wilt Resistance in *Mentha arvensis* and *M. longifolia* Genotypes

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ABSTRACT

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Verticillium wilt, caused by *Verticillium dahliae*, is a major constraint to mint (*Mentha* spp.) production in the United States, and the use of resistant cultivars is an important component of Verticillium wilt management. Two *Mentha arvensis* and four *M. longifolia* genotypes were evaluated for resistance to Verticillium wilt in the greenhouse using *V. dahliae* isolates obtained from different hosts and belonging to different vegetative compatibility groups. Isolates of *V. dahliae* obtained from peppermint (*M.* × *piperita*) caused significantly higher disease severity, plant mortality, and yield reduction than isolates obtained from other hosts. Disease severity, plant mortality, and pathogen incidence in aboveground stems were higher and yields lower in peppermint, the susceptible standard, compared with the resistant standard, native spearmint (*M. spicata*). Root-dip inoculations of *M. arvensis* and *M. longifolia* with isolates of *V. dahliae* obtained from peppermint produced severe symptoms; however, both species displayed the ability to recover from infection and produce asymptomatic growth from rhizomes. Both *M. arvensis* cultivars exhibited lower mean disease severity ratings following cutback and regrowth and were not significantly different than native spearmint. The restriction of pathogen movement in aboveground tissue and ability to recover from infection may be important components of *V. dahliae* resistance in perennial mint cropping systems.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major disease affecting mint (*Mentha* L.) production in the United States. Symptoms of Verticillium wilt in mint can include anthocyanescence, bronzing or curling of the apical leaves, chlorosis, stunting, wilt, necrosis, and premature senescence (20). Losses occur due to decreased oil production and stand reduction, which can worsen over the lifetime of the perennial crop. Fields of peppermint (*M.* × *piperita* L.) and Scotch spearmint (*M.* × *gracilis* Sole) can be severely affected by Verticillium wilt while another commercially grown species, native spearmint (*M. spicata* L.), is more resistant (5).

The host range of *V. dahliae* includes hundreds of dicotyledonous species in various genera (1,9,40). Despite its broad host range, some isolates of *V. dahliae* exhibit varying degrees of aggressiveness on certain host species (6,8). In addition, isolates can be separated into vegetative compatibility groups (VCGs) based on their ability to undergo hyphal anastomosis and form stable heterokaryons with other isolates (9,23). Most *V. dahliae* isolates collected from mint belong to VCG 2B, are highly aggressive on mint, and can interact synergistically with the root lesion nematode *Pratylenchus penetrans* (14,25), indicating the possible presence of a mint pathotype (12,16,25).

Initial inoculum of *V. dahliae* consists primarily of soilborne microsclerotia, which form in senescing plants and can survive in soils for over 10 years (18,32,40). Microsclerotia germinate in response to plant root exudates (33) and hyphae colonize the root surface and cortex. Verticillium wilt symptoms occur when the pathogen penetrates the stele and invades the xylem, where it is systemically translocated through the host vascular system (15). The pathogen is capable of colonizing the roots of resistant and nonhosts but it appears that the fungus is restricted from extensively colonizing the cortex and is unable to infiltrate the xylem (2,3,15,45). Microsclerotia are produced at host senescence, and colonized plant debris can contribute to future inoculum levels if incorporated into soil. Conidia can also be produced during host senescence but do not survive as long as microsclerotia and are not known to play a significant role in the disease cycle (18,45). Rhizomes used for planting may also be infected by *V. dahliae*, providing a potential source of primary inoculum in fields previously not planted to mint (30).

Verticillium wilt is managed primarily through the use of pathogen-free planting stock, rotations with nonsusceptible monocotyledonous crops, and preplant fumigation (17,34). Environmental and economical concerns may limit the future use of chemical controls. Crop rotation is of only limited benefit, largely due to the ability of *V. dahliae* to colonize and survive on numerous hosts and nonhosts and its long persistence in soil. The development of resistant cultivars offers a promising tool to manage Verticillium wilt in other host systems (11,27,44). Unfortunately, both peppermint and Scotch spearmint are sterile hybrids and conventional breeding is not possible. Mint mutants derived from irradiation treatment produced mixed results (21,24,35,42); however, other characteristics such as plant vigor, oil composition, and yield are of critical importance during cultivar development and may not be retained using nonspecific mutation techniques (31). Recent advances in *Agrobacterium tumefaciens*-mediated transformation of peppermint offers the opportunity to introduce Verticillium wilt resistance genes from other *Mentha* spp. into sterile mint hybrids without altering desirable oil composition or quality characteristics (36,46,50).

The genus *Mentha* includes at least 18 species, 11 hybrids, and numerous varieties (43), all of which are potential sources of Verticillium wilt resistance. *Mentha arvensis* L. is the most widely grown mint in the world, and menthol derived from *M. arvensis* is used in a variety of cosmetics, foods, and tobacco products (10). India is now the leading producer of *M. arvensis* menthol, mostly due to the development and integration of an annual mint cropping system with existing food production systems, an efficient distilling infrastructure, and the development of high-yielding, disease-resistant cultivars (4,28,29,41). A prior study investigated the impacts of powdery mildew (*Erysiphe cichoracearum*), rust (*Puccinia menthae*), and leaf spot (*Alternaria alternata*) on *M. arvensis* cultivars and genotypes (26); however, the potential effects of *V. dahliae* infection of *M. arvensis* are not known.

Another *Mentha* sp., *M. longifolia* (L.) L., is a wild relative of cultivated mint with a wide geographic range and a relatively

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high degree of intraspecific variation (43,49). Verticillium wilt resistance-like sequences, similar to the tomato *Ve* gene effective against *V. dahliae* race 1 (27,39), were previously identified in *M. longifolia* using degenerate polymerase chain reaction primers. Differences in Verticillium wilt response were also demonstrated in various genotypes of *M. longifolia* using a single *V. dahliae* isolate (48,49). The objectives of this greenhouse study were to evaluate responses of *M. arvensis* cvs. Paraguayan and Shivalik and *M. longifolia* accessions CMEN 584 and CMEN 585 (previously described as susceptible and resistant to Verticillium wilt, respectively) using *V. dahliae* isolates from various hosts and VCGs. In addition, a single progeny from a cross of the resistant and susceptible *M. longifolia* accessions (F_1) and a single progeny from an F_1 self-cross (F_2) were evaluated. Plants were inoculated using a root-dip method and Verticillium wilt symptoms, mortality, stem colonization, and yield were measured over successive croppings and compared with susceptible (*M.* × *piperita* 'Black Mitcham') and resistant (*M. spicata*) standards.

MATERIALS AND METHODS

Plant material and *V. dahliae* isolates. In total, five isolates of *V. dahliae* were used in the *M. arvensis* experiments. Isolates 109 and 111 (both VCG 2B) were both obtained from peppermint. Isolate 109 was previously demonstrated to be highly aggressive on *M.* × *piperita* Black Mitcham and *M.* × *gracilis* (12,24). Isolate VSP 695, also VCG 2B, was obtained from spinach seed and kindly provided by L. du Toit (Washington State University NWREC, Mt. Vernon). Isolate 601 (VCG 4A) was obtained from cherry (*Prunus* sp.) and isolate 240 (VCG 4B) was obtained from potato (*Solanum tuberosum* L.). An additional isolate from potato, isolate 653 (VCG 4A), was also used in the *M. longifolia* experiments. All isolates used were obtained from hosts in Washington State. The identities of isolates were confirmed as *V. dahliae* using polymerase chain reaction and species-specific primers based on the β-tubulin 2 gene as described by Atallah et al. (2) Disease assays on *M. arvensis* cultivars were conducted independently of *M. longifolia* assays in separate greenhouse trials and both experiments were repeated. The first experiment consisted of two Mint Industry Research Council lines of *M. arvensis,* Paraguayan and Shivalik, while the second experiment consisted of *M. longifolia* United States Department of Agriculture accessions CMEN 584 (plant introduction [PI] 557769) and CMEN 585 (PI 557767), a single progeny from a CMEN 584 × CMEN 585 cross (designated "F_1"), and a single progeny from an F_1 self-cross (designated "F_2"). *M. arvensis* cultivars were obtained from Summit Laboratories, Inc. (Fort Collins, CO) and *M. longifolia* genotypes were obtained from Kelly Vining (University of New Hampshire, Durham). All experiments consisted of four replicates and included *M.* × *piperita* Black Mitcham and *M. spicata* as susceptible and resistant standards, respectively. Mint plants were vegetatively propagated by treating 7- to 10-cm apical cuttings with Root-Tone (0.20% 1-Naphthaleneacetamide and 4.04% tetramethylthiuram-disulfide; Black Leaf Products, Louisville, KY) and rooted in flats filled with Sunshine LC1 peat-based media (SunGro, Bellevue, WA) for 4 to 6 weeks.

Conidial suspensions of each *V. dahliae* isolate were prepared by inoculating 125 ml of Czapeks-Dox broth (MP Biomedicals, Solon, OH) with 1-cm^2 plugs taken from single-spore isolates grown on potato dextrose agar (PDA). Liquid cultures were incubated on a 150-rpm shaker at 22 to 23°C in the dark for 5 to 7 days. Conidia were strained through four layers of cheesecloth to remove mycelia. Conidial concentrations were quantified with a hemacytometer and adjusted to 1×10^6 conidia/ml by adding sterile distilled water (sdH_2O). Rooted cuttings were uplifted and potting media gently rinsed from the roots prior to inoculation. Rooted cuttings were soaked for 5 min in 100 ml of conidial suspension, with control inoculations consisting of a 5-min soak in sdH_2O. Plants were transplanted into 10-cm^2 pots (J. M. McConkey & Co., Inc., Puyallup, WA,) filled with Sunshine LC1 media and arranged in a randomized complete block design. Natural light was supplemented to achieve a photoperiod of at least 15 h when necessary. Temperatures in the greenhouse during the *M. arvensis* experiments ranged diurnally from 8.6 to 25.6°C (trial 1) and 9.8 to 32.8°C (trial 2), while temperatures during the *M. longifolia* experiments ranged from 6.9 to 32.8°C (trial 1) and 9.4 to 32.8°C (trial 2).

Disease severity, stem assays, and yield measurements. Verticillium wilt symptoms were assessed approximately 4 weeks postinoculation (wpi) and weekly thereafter using the following disease severity index (DSI): 0 = no visible symptoms; 1 = mild chlorosis, <10% of plant; 2 = distinct chlorosis, 10 to 20% of plant; 3 = asymmetrical apical growth, chlorosis 20 to 40% of plant, or stunting (<80% height of control plants); 4 = chlorosis on >40% of plant, or severe stunting (<60% height of control plants); 5 = necrosis on >40% of plant; and 6 = dead or nearly dead plant. At 8 wpi, plants were cut at 1 to 2 cm above the soil line and allowed to regrow for an additional 8 weeks, at which point disease ratings were recorded and plants cut back again.

Stem assays were conducted following each 8-week growth period. Two 4-cm basal stem sections were arbitrarily sampled from each plant, surface sterilized in 0.5% NaOCl for 3 min, and plated onto Whatman filter paper moistened with sdH_2O. Plates were incubated for 5 days in the dark and checked for *V. dahliae* conidiophores and microsclerotia formation. Identification was performed by microscopic examination of conidiophores, conidia, and microsclerotia and verified by subplating onto PDA when necessary. Following regrowth ratings and stem assays, the remaining aboveground tissue (stems, leaves, and flowers) was cut to 1 to 2 cm above the soil line and dried for 2 weeks, and the total mass (yield) was recorded. Yields were converted to yield ratios using the formula $\text{yield ratio}_{n(x)} = \text{yield}_{n(x)}/\text{mean yield}_{\text{control}(x)}$, where $n(x)$ = individual yield observation of mint species (x) and control(x) = the mean yield of the noninoculated mint species (x) control treatment. A yield ratio <1 indicates a reduced yield compared with the mean yield of the noninoculated control treatment of the same mint genotype.

Data analysis. Areas under the disease progress curve (AUDPCs) were calculated for ratings taken during the first 8 weeks using the formula $\Sigma_i^{n-1}\,[(Y_i + Y_{i+1})/2](t_{i+1} - t_i)$, where Y_i = cumulative disease severity at the ith observation, t_i = time (days postinoculation) at the ith observation, and n = number of observations. Analysis of variance (ANOVA) was performed using PROC GLM in SAS (version 9.1; SAS Institute, Cary, NC). AUDPC data, regrowth ratings, and yield ratios were analyzed separately, and comparisons were made between *V. dahliae* isolates and *Mentha* spp. using Tukey's honestly significant difference test at $P < 0.05$.

RESULTS

***M. arvensis* trials.** Root-dip inoculations of *M.* × *piperita* Black Mitcham and both *M. arvensis* cultivars with isolates 109 and 111, both obtained from peppermint, resulted in significantly higher AUDPC values than inoculations with other isolates in both trials (Table 1). Inoculations with isolate 240 caused significantly higher AUDPC values than water-inoculated controls for Paraguayan in both trials. Overall, Shivalik and *M. spicata* exhibited higher and lower AUDPC values, respectively, during the first 8-week growth period. *M.* × *piperita* Black Mitcham exhibited the highest DSI following regrowth in both trials (Table 1). Only *M.* × *piperita* Black Mitcham inoculated with isolates 109 and 111 exhibited significantly higher DSI ratings than water-inoculated controls following harvest and regrowth in both trials. There was considerable variation in DSI values among replicates of *M. arvensis* inoculated with isolates 109 and 111, with DSI values ranging between 0 (no symptoms) to 6 (dead plant). Only *V. dahliae* isolates 109 and 111 caused plant senescence and mortality was highest for *M.* × *piperita* Black Mitcham (Table 2).

Yield ratios for *M.* × *piperita* Black Mitcham inoculated with isolate 111 were reduced significantly in both trials (Table 3). Isolate 109 caused a significant reduction in mean yield ratio in *M.* × *piperita* Black Mitcham during the first trial but mean yield reduction was not significant in the second trial. Although isolates obtained from other hosts sometimes resulted in higher or lower mean yield ratios than controls, these differences were not significant in either trial. Stem assays resulted in the recovery of *V. dahliae* predominantly from plants inoculated with isolates obtained from mint, and assays of *M.* × *piperita* Black Mitcham plants inoculated with isolates 109 and 111 resulted in the recovery of the pathogen from 100% of stems (Table 2).

***M. longifolia* trials.** Consistent with both *M. arvensis* trials, isolates 109 and 111, both obtained from peppermint, caused significantly higher AUDPC values than all other isolates for all six mint lines tested in both *M. longifolia* trials (Table 4). The resistant standard *M. spicata* exhibited significantly lower overall AUDPC values than all other mint lines following inoculations with isolates 109 and 111 in both trials. Isolate 111, obtained from peppermint, caused significantly higher DSI values than water-inoculated controls following regrowth for all mint lines except for *M. spicata* in both trials (Table 4). Isolate 109 caused significant DSI values following regrowth in *M.* × *piperita* Black Mitcham, CMEN 584, and F_1 in both trials. As with *M. arvensis*, DSI values ranging between 0 and 6 were observed following regrowth of *M. longifolia* replicates inoculated with peppermint isolates 109 and 111. Senescence (a disease rating of 6) only occurred in plants inoculated with these isolates obtained from peppermint; however, differences in plant mortality were not significant (Table 2).

Yield ratios were significantly reduced for *M.* × *piperita* Black Mitcham, CMEN 584, and F_1 by isolates 109 and 111 in both trials (Table 3). Isolate 111 also significantly reduced mean yield ratios for F_2 in both trials. Other significant reductions in mean yield ratio were observed but were not consistent among trials. Higher yield ratios were observed in CMEN 585 inoculated with isolates from other hosts in both trials. Stem assays recovered the pathogen primarily in plants inoculated with isolates 109 and 111 (Table 2). Pathogen recovery was relatively low for CMEN 585 and *M. spicata* in both trials.

DISCUSSION

Approximately 3 to 4 weeks following root-dip inoculation, observable differences in resistance were evident among mint cultivars as well as aggressiveness among the *V. dahliae* isolates. Inoculations of mint lines with both *V. dahliae* isolates obtained from peppermint often resulted in complete wilt, necrosis, and senescence of the inoculated stem by the conclusion of the first 8-week growth period. The exception was *M. spicata*, which exhibited only mild chlorosis and crescent leaf symptoms and only on rare occasions. Isolates obtained from hosts other than peppermint (VSP 695, 240, 601, and 653) were able to cause mild symptoms in all mint lines, including slight to moderate chlorosis and mild stunting or asymmetric apical growth. However, symptoms caused by isolates obtained from non-mint hosts were not as severe as those caused by isolates obtained from peppermint, and necrosis or plant mortality due to Verticillium wilt was not observed. Overall, isolates 109 and 111, both obtained from peppermint, produced

Table 2. Incidences of plant mortality and *Verticillium dahliae* recovery from aboveground stems of mint plants inoculated with isolates from peppermint[z]

	Incidence (%)					
	Isolate 109			Isolate 111		
Mint	Mortality	Trial 1	Trial 2	Mortality	Trial 1	Trial 2
Experiment 1						
Black Mitcham	30	100 a	100 a	50	100 a	100 a
Shivalik	0	19 b	13 b	10	6 b	29 b
Paraguayan	0	6 b	13 b	10	19 b	7 b
Mentha spicata	0	0 b	0 b	0	0 b	13 b
Experiment 2						
Black Mitcham	30	100 a	94 a	30	100 a	94 a
M. longifolia 584	30	58 ab	69 ab	30	67 ab	86 ab
M. longifolia 585	40	17 bc	14 c	50	13 c	6 d
584 × 585 (F_1)	30	58 ab	79 a	30	64 ab	50 bc
$F_1 \times F_1$ (F_2)	30	21 bc	79 a	50	25 bc	60 ab
M. spicata	0	0 c	31 bc	0	13 c	13 cd

[z] Mortality values indicate the total percentage of plants with a disease rating of 6 (dead or nearly dead plant) by the end of both trials. Values within a column followed by the same letter are not significantly different within trials using Tukey's test at $P = 0.05$.

Table 1. Mean area under disease progress curve (AUDPC) and disease severity index (DSI) ratings of 'Black Mitcham' peppermint (susceptible), *Mentha spicata* (resistant), and two *Mentha arvensis* cultivars (Shivalik and Paraguayan) following root-dip inoculation with *Verticillium dahliae* isolates from different hosts and vegetative compatibility groups (VCGs)[z]

	V. dahliae isolates									
	VCG 2B						VCG 4A		VCG 4B	
	Peppermint				Spinach		Cherry		Potato	
	109		111		695		601		240	
Rating, mint	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
AUDPC										
Black Mitcham	85 a*	80 ab*	62 b*	77 b*	19 a	17 a	22 ab	9 a	16 a	9 a
Shivalik	100 a*	95 a*	95 a*	104 a*	15 a	13 a	11 b	8 a	15 a	23 a*
Paraguayan	57 b*	68 b*	68 ab*	84 ab*	17 a	23 a*	32 a*	15 a	25 a*	24 a*
M. spicata	51 b*	21 c*	43 b*	23 c*	17 a	24 a*	9 b	7 a	17 a	8 a
DSI										
Black Mitcham	1.7 a*	1.8 a*	2.0 a*	1.8 a*	0.8 a*	0.0 b	0.9 a*	0.0 a	1.0 a*	0.0 a
Shivalik	1.0 b*	0.8 b	0.8 b*	0.5 ab	0.5 a	0.0 b	0.7 a	0.0 a	0.5 ab	0.0 a
Paraguayan	0.6 b	0.6 b	1.0 b*	0.9 ab	0.8 a	0.5 a	0.4 a	0.4 a	0.2 b	0.5 a
M. spicata	0.7 b*	0.2 b	0.8 b*	0.2 b	0.5 a	0.2 ab	0.4 a	0.0 a	0.5 ab	0.2 a

[z] Disease severity index (DSI): 0 = no visible symptoms; 1 = mild chlorosis, <10% of plant; 2 = distinct chlorosis, 10 to 20% of plant; 3 = asymmetrical apical growth, chlorosis on 20 to 40% of plant, or stunting (<80% height of control plants); 4 = chlorosis on >40% of plant or severe stunting (<60% height of control plants); 5 = necrosis on >40% of plant; and 6 = dead or nearly dead plant. Values within a column followed by the same letter are not significantly different within trials using Tukey's test at $P = 0.05$; * indicates a significant difference from water-inoculated controls within mints using Tukey's test at $P = 0.05$. Plants were harvested at 8 weeks postinoculation and allowed to grow for an additional 8 weeks prior to disease severity index ratings. DSI ratings were $\log_e$-transformed for analysis.

significantly higher AUDPC ($P < 0.0001$) and DSI ratings following regrowth ($P < 0.0001$) than isolates from other hosts in all four trials (Tables 1 and 4). Water-inoculated control plants did not exhibit any Verticillium wilt symptoms. Some variation in AUDPC values was observed between *M. arvensis* trials, particularly in *M. spicata* plants inoculated with isolates obtained from peppermint. The exact cause of the trial variability is not known but the timing of experiments may have played a role. AUDPC values for the first *M. arvensis* trial were recorded during July and August, whereas AUDPC readings for the second *M. arvensis* trial were recorded between March and May. Although the greenhouse environment is regulated, differences in diurnal temperature fluctuations or the amount and intensity of solar irradiation may have influenced the interaction between these isolates and *M. spicata*, impacting disease development.

Although *V. dahliae* generally exhibits a wide host range, some studies demonstrated variation in pathogen–host interactions among *V. dahliae* isolates and the presence of host-adapted populations capable of causing more severe symptoms on certain hosts (6,38). Correlations between aggressiveness and VCG have been found to exist in some cases (37) and most *V. dahliae* isolates collected from peppermint belong to VCG 2B and exhibit increased aggressiveness on peppermint (12). In this study, root-dip inoculations of *M.* × *piperita* Black Mitcham and all genotypes of *M. arvensis* and *M. longifolia* with *V. dahliae* isolates from peppermint resulted in significantly higher disease severity than inoculations with isolates obtained from other hosts, including a VCG 2B isolate collected from spinach seed. These results support previous studies suggesting that *V. dahliae* isolates collected from mint may be a host-adapted group within VCG 2B (6,12,16) and suggest that knowledge of both host origin and VCG are important in

Table 3. Mean yield ratios of 'Black Mitcham' peppermint (susceptible), *Mentha spicata* (resistant), two *Mentha arvensis* cultivars, and four *Mentha longifolia* genotypes following inoculation with *Verticillium dahliae* isolates from various hosts and vegetative compatibility groups (VCGs), harvest and regrowth[z]

	***V. dahliae* isolates**											
	VCG 2B						**VCG 4A**				**VCG 4B**	
	Peppermint				**Spinach**		**Cherry**		**Potato**		**Potato**	
	109		**111**		**695**		**601**		**653**		**240**	
Mint	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
Experiment 1												
Black Mitcham	0.4 a	0.3 b*	0.0 c	0.4 b*	1.1 a*	1.2 a	1.6 a*	0.9 a	Nt	Nt	1.4 a*	0.9 a
Shivalik	0.9 a	0.5 b	1.1 a	0.4 b	1.4 a	1.2 a	0.9 a	1.1 a	Nt	Nt	1.3 a	0.9 a
Paraguayan	0.8 a	1.0 a	0.6 b	0.5 ab	0.8 a	0.9 a	0.8 a	1.1 a	Nt	Nt	0.8 a	1.0 a
M. spicata	1.0 a	1.1 a	0.9 ab	1.1 a	0.9 a	1.1 a	1.0 a	1.0 a	Nt	Nt	1.0 a	0.9 a
Experiment 2												
Black Mitcham	0.2 a*	0.3 b*	0.1 b*	0.3 b*	0.7 a	1.1 a	1.1 ab	1.0 b	0.8 a	1.2 a	1.0 ab	1.2 a
M. longifolia 584	0.1 a*	0.2 b*	0.1 b*	0.3 b*	0.7 a	1.2 a	0.8 b	1.0 b	0.7 a	1.2 a	0.8 b	1.0 a
M. longifolia 585	0.5 a	0.9 ab	0.0 b*	1.2 a	1.2 a	1.1 a	1.4 a*	1.1 ab	1.4 a*	1.3 a	1.5 a*	1.1 a
584 × 585 (F_1)	0.3 a*	0.2 b*	0.3 ab*	0.4 b*	0.8 a	0.9 a	0.9 b	1.0 b	1.1 a	1.0 a	0.8 b	1.0 a
$F_1 \times F_1$ (F_2)	0.5 a	0.5 ab	0.2 b*	0.4 b*	0.9 a	1.0 a	0.8 b	1.1 ab	1.1 a	1.1 a	0.9 b	1.1 a
M. spicata	0.7 a*	1.1 a	0.7 a	1.2 a	0.7 a	1.1 a	1.0 b	1.2 a*	0.7 a	1.2 a	0.8 b	1.2 a

[z] Values within a column followed by the same letter are not significantly different within trials using Tukey's test at $P = 0.05$; * indicates a significant difference from water-inoculated controls within mints using Tukey's test at $P = 0.05$; Nt = not tested. Plants were cut back at 8 weeks postinoculation and allowed to grow for an additional 8 weeks prior to yield ratio measurements.

Table 4. Mean area under disease progress curve (AUDPC) and disease severity index (DSI) ratings of 'Black Mitcham' peppermint (susceptible), *Mentha spicata* (resistant),and four *Mentha longifolia* genotypes following root-dip inoculation with *Verticillium dahliae* isolates from different hosts and vegetative compatibility groups (VCGs)[z]

	***V. dahliae* isolates**											
	VCG 2B						**VCG 4A**				**VCG 4B**	
	Peppermint				**Spinach**		**Cherry**		**Potato**		**Potato**	
	109		**111**		**695**		**601**		**653**		**240**	
Mint	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
AUDPC												
Black Mitcham	70 b*	100 a*	70 bc*	98 a*	3 a	16 a*	2 a	10 a	16 ab	18 a*	10 a	28 a*
M. longifolia 584	113 a*	110 a*	102 a*	109 a*	16 a	11 a	21 a	14 a	18 a	11 a	10 a	14 a
M. longifolia 585	83 ab*	64 b*	84 ab*	68 b*	12 a	15 a	11 a	17 a	1 b	25 a	5 a	17 a
584 × 585 (F_1)	111 a*	107 a*	104 a*	96 a*	7 a	12 a	8 a	16 a	6 ab	12 a	7 a	28 a*
$F_1 \times F_1$ (F_2)	84 ab*	97 a*	92 ab*	109 a*	9 a	14 a	9 a	14 a	3 ab	14 a	7 a	32 a*
M. spicata	36 c*	37 b*	46 c*	34 c*	10 a	12 a	2 a	13 a	8 ab	19 a*	2 a	19 a*
DSI												
Black Mitcham	1.8 a*	1.8 a*	1.9 a*	1.8 a*	0.0 a	0.0 a	0.2 a	0.0 b	0.2 a	0.0 a	0.0 a	0.0 a
M. longifolia 584	1.7 a*	1.6 a*	1.8 a*	1.6 ab*	0.0 a	0.2 a	0.0 a	0.0 b	0.2 a	0.0 a	0.2 a	0.2 a
M. longifolia 585	1.0 a	1.1 ab*	2.0 a*	1.1 b*	0.2 a	0.4 a	0.4 a	0.0 b	0.2 a	0.2 a	0.0 a	0.4 a
584 × 585 (F_1)	1.2 a*	1.6 a*	1.7 a*	1.4 ab*	0.0 a	0.2 a	0.0 a	0.5 a	0.2 a	0.4 a	0.5 a	0.7 a*
$F_1 \times F_1$ (F_2)	1.0 a	1.6 a*	1.3 a*	1.6 ab*	0.0 a	0.4 a	0.2 a	0.2 ab	0.2 a	0.4 a	0.2 a	0.5 a
M. spicata	0.4 a	0.5 b	0.4 b	0.2 c	0.4 a	0.0 a	0.4 a	0.0 b	0.2 a	0.2 a	0.6 a	0.4 a

[z] Disease severity index (DSI): 0 = no visible symptoms; 1 = mild chlorosis, <10% of plant; 2 = distinct chlorosis, 10 to 20% of plant; 3 = asymmetrical apical growth, chlorosis on 20 to 40% of plant, or stunting (<80% height of control plants); 4 = chlorosis on >40% of plant or severe stunting (<60% height of control plants); 5 = necrosis on >40% of plant; and 6 = dead or nearly dead plant. Values within a column followed by the same letter are not significantly different within trials using Tukey's test at $P = 0.05$; * indicates a significant difference from water-inoculated controls within mints using Tukey's test at $P = 0.05$. Plants were harvested at 8 weeks postinoculation and allowed to grow for an additional 8 weeks prior to disease severity index ratings. DSI ratings were $\log_e$-transformed for analysis.

predicting the aggressiveness of a certain isolate on a given host species, and mint in particular. The presence of *V. dahliae* populations with different VCGs, each potentially containing subpopulations with varying degrees of aggressiveness, may complicate efforts to manage and quantify the pathogen in field soils. In addition, results from this study demonstrate that isolates collected from *M.* × *piperita* are capable of causing disease symptoms in two other mint species: *M. arvensis*, the most widely cultivated *Mentha* sp. in the world, and *M. longifolia*, a wild mint species with a global distribution.

In addition to differences in isolate aggressiveness, variation in disease resistance among *Mentha* spp. was evident. Disease severity, plant mortality, and pathogen isolation frequencies were higher and yield ratio lower in the susceptible standard *M.* × *piperita* Black Mitcham compared with the resistant standard *M. spicata*. Although root-dip inoculations of *M. arvensis* and *M. longifolia* genotypes with peppermint isolates of *V. dahliae* resulted in AUDPC values similar to or higher than *M.* × *piperita* Black Mitcham at 8 wpi, *M. arvensis* and *M. longifolia* genotypes exhibited a range of symptoms following cutback and regrowth, ranging from 0 (no symptoms) to 6 (dead plant), whereas 96% of *M.* × *piperita* Black Mitcham plants exhibited moderate to severe symptoms (DSI ≥ 4) at the completion of all trials. The reasons for the observed variation in disease severity and plant mortality among replications of *M. arvensis* and *M. longifolia* are not known; however, the inherent rhizomatous and stoloniferous growth of *Mentha* spp. may allow plants to recover from initial infection, particularly if the rate of pathogen colonization is restricted or host growth is vigorous. Incorporating inoculum in the soil may help reduce this variation by constantly challenging the plant with the pathogen as new rhizomes and stolons grow and root through the soil.

Yields were consistently reduced only for *M.* × *piperita* Black Mitcham plants inoculated with *V. dahliae* isolates obtained from peppermint, most likely due to its high susceptibility and rate of mortality compared with the resistant standard *M. spicata*. Both *M. arvensis* cultivars exhibited relatively high yield ratios and low mortality rates compared with the standards in response to inoculation with *V. dahliae* isolates 109 and 111. Yield ratios for Shivalik inoculated with isolates 109 and 111 were considerably higher during the first trial compared with the second trial (Table 3). These differences may be related to small differences in initial plant size or variation in greenhouse conditions over the 24-week study. Among *M. longifolia* genotypes, *M. longifolia* CMEN 584 exhibited the lowest yield ratios and *M. longifolia* CMEN 585 the highest; however, CMEN 585 also exhibited the highest mortality rate, especially during the first trial.

Differences in frequencies of pathogen isolations from stems were also evident among cultivars (Table 3). The pathogen was isolated from nearly 100% of stems collected from the susceptible standard *M.* × *piperita* Black Mitcham and less than 10% of stems collected from the resistant standard *M. spicata*. Pathogen reisolation from aboveground stems was relatively low (<15%) in both *M. arvensis* cultivars compared with the other mint lines tested. Among *M. longifolia* genotypes, the pathogen was isolated in highest numbers from *M. longifolia* CMEN 584 and in lowest numbers from *M. longifolia* CMEN 585. As with symptoms and yield ratio, differences in environmental conditions or initial plant size may have contributed to interactions among some cultivars and isolates (Table 2), allowing some plants to recover and grow asymptomatically after initial infection.

Results from this study are consistent with a previous assessment by Vining et al. (49), in which 14 *M. longifolia* accessions were inoculated with a *V. dahliae* isolate from mint using a similar root-dip procedure. They identified CMEN 584 and CMEN 585 as susceptible and resistant *M. longifolia* genotypes, respectively. Vining et al. (47,48) sequenced *mVe*1, a putative homolog of the dominant Verticillium wilt resistance (*Ve*) gene which confers *V. dahliae* race 1 resistance in tomato, in *M. longifolia* CMEN 585. Verticillium wilt resistance screenings of several F_1 and F_2 *M. longifolia* populations suggested that genes other than *mVe*1 are involved in Verticillium wilt-resistant *M. longifolia* genotypes (47). Results from experiments using peppermint plants transformed with *Ve*-like sequences cloned from *M. longifolia* CMEN 585 also suggest that other genes may be involved (13). In this study, the genotype tested from the F_1 generation of a CMEN 584 × CMEN 585 cross exhibited a lower mortality rate than CMEN 585; however, it exhibited reduced yield ratios, higher disease severity, and a higher incidence of pathogen reisolation from aboveground stems compared with CMEN 585. Interestingly, although CMEN 585 consistently exhibited lower DSI ratings and pathogen isolation rates and higher yield ratios than *M. longifolia* CMEN 584, it also exhibited one of the highest mortality rates among all mint lines evaluated. Reduced symptoms, restricted pathogen colonization, sustained yield, and low mortality would all be desirable traits in a Verticillium wilt-resistant mint cultivar.

The identification of resistant phenotypes is a necessary first step in the process of resistant cultivar development. Previous studies on Verticillium wilt resistance in lettuce, potato, and other crops have suggested that host suppression of cortex colonization, stele penetration, and xylem invasion are important components of resistant phenotypes (15,19,22,45). Data from this study are consistent with prior studies demonstrating differences in pathogen incidence within stems of resistant and susceptible mint species (7,20), suggesting that resistance in mint may involve the prevention of vascular or aboveground colonization by the pathogen. Results from this study demonstrate that *M. arvensis* and *M. longifolia* possess the ability to recover from severe *V. dahliae* infection and produce asymptomatic shoots from belowground rhizomes. This may be especially important in mint, considering the rhizomatous and stoloniferous nature of the crop, which may allow the plant to escape from pathogen foci in soil and establish new plants in noninfested areas. The ability to restrict aboveground stem colonization may not only reduce symptoms and yield loss during the current growing season but also could provide a long-term impact by reducing the accumulation of *V. dahliae*-infested debris in the soil. Mint is grown as a perennial in the United States, and the restriction of *V. dahliae* colonization in aboveground stems observed in the *Mentha* spp. tested may be especially significant because repeated harvests present multiple opportunities for infested debris to become incorporated into field soils.

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