Distribution of *Verticillium* in Stems of Resistant and Susceptible Species of Mint

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ABSTRACT

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Two methods were used to ascertain whether the ascent or proliferation of *Verticillium dahliae* is retarded in the stems of the resistant *Mentha crispa* compared to that in the susceptible *Mentha piperita*. In the greenhouse, young cuttings of both mint species were planted in soil artificially infested with *V. dahliae*. Surface-sterilized nodes of these plants were plated out on agar weekly and the presence of *V. dahliae* was ascertained for each node. In the field, rooted cuttings were planted into heavily infested soil; once a week, the stem of each sampled plant was cut into segments and the segments were individually fragmented in a small amount of distilled water. The resulting suspension was plated out and the relative amounts of propagules of *V. dahliae* were ascertained. We detected no difference in the ascent of *V. dahliae* in stems of either mint species. However, two-thirds of the infected plants of *M. piperita* yielded from 100 to 15,000 propagules per millimeter of stem length. Most plants of *M. crispa* yielded fewer than 10 with a maximum of 57 propagules per millimeter of stem. Evidently, *M. crispa* suppresses the proliferation of *V. dahliae*, but not the ascent of the fungus, within the stems.

Additional key words: host resistance, proliferation, vascular wilt.

Peppermint, *Mentha piperita* L., is susceptible to the soilborne fungus, *Verticillium dahliae* Kleb. However, plants of *M. crispa* L. (a mint species not used for mint oil) remains healthy-looking and vigorous in the midst of dying plants of *M. piperita*.

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Lacy and Horner (8) sought, but did not find evidence for resistance to *V. dahliae* either in the rhizosphere or at the root surfaces of *M. crispa*, but they reported that fragmented roots of *M. crispa* contained fewer propagules than those of *M. piperita*.

The study reported here had two main purposes: to ascertain whether *V. dahliae* ascends stems of *M. crispa* as readily as it does those of *M. piperita*; and to ascertain whether *V. dahliae* proliferates to the same extent within stems of *M. crispa* and *M. piperita*.

MATERIALS AND METHODS

Qualitative assays of roots and nodes. Coarse sand was placed about 2 cm deep in 0.7-L (1.5–pint), wide-mouthed glass jars. Next, a uniform, screened soil was added to a level 7.6 cm below the tops of the jars. Inoculum (130 mg) consisting primarily of microsclerotia from peppermint stems was then spread over the soil surface. *Mentha piperita* L. and *M. crispa* L. stem cuttings that were 4 wk old and had roots at least 7.6 cm long were then planted and soil was added to fill the remaining space in the jars. Each jar received a total of 625 gm (dry wt) of soil. The plant root extremities were placed into contact with the inoculum layer in each jar, and the roots subsequently grew down through the inoculum layer. The jars were placed into soil temperature tanks held at 24 C in a greenhouse. Water was added periodically to the jars in amounts calculated to approach, but not to exceed, the amount necessary to reach field capacity.

At weekly intervals, three plants of each species and all the soil were carefully removed from the jars and placed into a dishpan of water to loosen the soil. The remainder of the soil was removed from the roots with a gentle spray. After the leaves were removed with a sterile razor blade, the stems and roots were immersed in 10% Clorox (0.525% sodium hypochlorite) for 1 min. Root and stem pieces were cut on a disinfected surface with a sterile razor blade. Four sections of tissue were removed from each node and were plated in a petri dish containing quarter-strength potato-dextrose agar (ie, 50 gm of potatoes and 5 gm of technical-grade glucose per liter) plus 0.5 ml of streptomycin-ethanol mixture (12). The roots were put, without cutting, into a sterile petri dish. Cooled (but still liquid) water agar containing 0.5 ml of the ethanol-streptomycin mixture was added to each dish and allowed to solidify. The cultures were incubated at room temperature (about 20–30 C) for about 2 wk then were examined for colonies of *Verticillium*.

Quantitative assays of stem segments. *Mint plants and field plots.* Forty-eight rooted cuttings of *M. piperita* and 48 rooted cuttings of *M. crispa* were planted in two field plots infested with *V. dahliae* pathogenic to peppermint. Each plot received 24 plants of each species. The rows were 76 cm apart and the cuttings were 61 cm from each other in the rows. The species were alternated so that adjacent plants were different species.

Sampling. A sample consisted of five pairs of plants, each pair consisting of one member of each mint species. Three of the pairs came from one plot, while two of the pairs came from the other plot. The plants of *M. piperita* were selected with a table of random numbers and the *M. crispa* member of each pair had grown adjacent to the plant of *M. piperita* selected for analysis.

Harvesting and sterilizing. On the day of analysis, the disease symptoms of each plant to be analyzed and its height (measured from the soil surface) were recorded. Each plant was dug up, excess soil was knocked from the roots, and it was put into an individual flask of tap water.

The stems were cut off below soil level and were washed with fresh tap water. Leaves and branches were cut off close to the stem, the stems were surface-sterilized for 1 min in 10% Clorox (0.525% sodium hypochlorite), and then were rinsed twice in sterile, distilled water. With sterile scalpels, each stem was cut into segments—10 mm long when the stems were short; 20 mm for the 36th and 43rd days' sampling; and four segments, one-third, one-third, one-sixth, and one-sixth of the total stem height for the 50th and 57th days'

TABLE 1. Recovery of *Verticillium dahliae* from roots and stems of *Mentha piperita* (susceptible) and *M. crispa* (resistant) growing in infested soil in a greenhouse

Weeks after planting in infested soil	Occurrence of *V. dahliae* in roots and stem sections of mint plants[a]			
	Mentha piperita		*Mentha crispa*	
	Root	Nodes[b]	Root	Nodes[b]
1	−	− − − −	−	− − − −
	−	− o − −	−	− − − −
	−	− − − o −	−	− − − −
2	−	− − − − − −	−	− − − − −
	−	− − − − − − −	+	− − − − −
	+	− − − − −	−	− − − − −
3	+	− − + − − − −	+	− − − − − − −
	+	− − − − − − −	+	− − − + + +
	+	+ + + + + + +	+	− − − − − − −
4	+	− − − − − − − −	+	+ − + − − − −
	+	+ + + + + + + +	+	− − − − − + + −
	+	+ + + + + + + +	+	− − + − + + + −
5	+	+ + + + + + + +	+	− − − − − − − − −
	+	+ + + + + + + + + + −	+	+ + + + o + + + +
	+	+ + + + + + + + + − −	+	− − − + + + + −
6	+	+ − + + + + + + + + − −	+	− + + + + + + − − −
	+	+ + + + + + + + + + + + +	+	− − − − − − − − −
	+	+ + + + + + + + + − − −	+	+ + + + + + − − − −
7	+	− − − − − − − − − − − −	+	+ + + + + + − + + + + −
	+	+ + + + + + + + + + + +	+	− + + + + + − − − − − −
	+	+ + + + + + + + + + + −	+	− − + + + + + + − − −
8	−	+ + + + + + + + + + + + + + + +	+	+ + − + − + + − − − − −
	+	+ + + + + − − − − − − − −	+	+ + + + + + + + + + +
	+	+ + + + + + + + + + + + + −	+	− − + + + + + + + +

[a] Symbols: − = *Verticillium* not recovered; + = *Verticillium* recovered from the roots or from at least one of the four stem sections taken from the node; o = results obscured or not available.

[b] Each row of symbols represents analyses of one plant. The symbols from left to right represent the results consecutively from the lowest node to the highest node.

samplings. Each segment was cut into ~2-mm pieces on a sterile surface with a sterilized scalpel.

Fragmenting and incorporating into agar medium. All the 2-mm pieces of each segment were added to 10 ml of filter-sterilized, distilled water in the sterilized 50-ml cup of a high-speed blender (Sorvall Omnimixer) and were fragmented at maximum speed for 30 sec. Preliminary work showed that fragmenting with an Omnimixer yielded many more propagules of *V. dahliae* than did grinding the stem segments in a mortar with liquid nitrogen or with distilled water.

The suspension of fragmented stem pieces was next poured into 15 ml of melted, double-strength, ethanol-streptomycin agar (12) held at 44–46 C. The cup was rinsed with 5 ml of filter-sterilized water and the rinse was added to the melted agar medium. Beginning with the analysis on the 36th day, a series of 10-fold dilutions of the suspension were made in sterile, distilled water prior to incorporation into the melted agar. We made no dilutions for plants without symptoms and made dilutions up to 10,000-fold for plants that had severe symptoms.

Between the fragmenting of one segment and the next, the cup and shaft of the Omnimixer were immersed in 70% ethanol and were then rinsed twice with sterile, distilled water. This procedure, plus timely replacement of worn shaft bearings, reduced the carryover of propagules to fewer than one per 10,000 in 23 of 25 tests and to fewer than one per 1,000 in two tests.

Incubating and counting. The petri dishes were incubated in darkness at 25 ± 1 C for 12 days or more. Only colonies that produced microsclerotia were counted.

Repetition. The quantitative assay was repeated in abbreviated form the following year. Essentially the same methods were employed as in the last two samplings of the initial experiment. The rooted cuttings were, however, planted in bottomless wooden boxes set in turf and containing soil infested with *V. dahliae*. Cuttings were planted in pairs of species and were selected for analysis with the aid of a table of random numbers. Five pairs were dug up and were analyzed 43 days after planting, and five more pairs were dug up and analyzed 82 days after planting.

RESULTS

Qualitative assays of roots and nodes. After 3 wk in infested soil in the greenhouse, every plant of *M. piperita* assayed contained *V. dahliae* either in its roots or stems (Table 1).

V. dahliae was recovered from the stems of 16 of the 19 plants of *M. piperita* that were infected. In seven of these 16 plants, the fungus reached the top node; in six others, it reached the second, third, or fourth node from the top.

After 3 wk in infested soil, every *M. crispa* plant also contained *V. dahliae* in roots or stems. *V. dahliae* was recovered from the stems of 14 of the 19 infected plants. In four of these 14 plants, the fungus reached the top node; in six other plants, it reached to the second, third, or fourth node from the top. Discontinuities were apparent in the vertical distribution of *V. dahliae* in the stems of both *M. crispa* and *M. piperita*.

Quantitative assays of stem segments from field-grown mint. *V. dahliae* ascended inside the stems of both resistant and susceptible species of mint. Where the plants averaged 5–100 propagules per millimeter of stem length, *V. dahliae* ascended to the top segment in four of five resistant plants and in three of five susceptible plants (Fig. 1A and B). Where the plants yielded fewer than four propagules per millimeter of stem length, *V. dahliae* appeared to ascend about as far in resistant plants as it did in susceptible plants; the resistant plants had, on the average, about 32% of their top parts free of *V. dahliae*, while the susceptible plants had an average

TABLE 2. Relation of symptoms of Verticillium wilt to numbers of propagules of *Verticillium dahliae* recovered from stems of individual plants of a susceptible species of mint (*Mentha piperita*) planted in infested field plots

Time after planting in infested soil (days)	Symptoms[a]	Average number of propagules recovered (per mm of stem)
22	none	16[b]
36	none	5[b]
50	mild	0
43	mild	602
50	mild	0.5[b]
57	mild	57[b,c]
36	moderate	3,243
50	moderate to severe	4,303
57	severe	3,830
36	severe	4,343
50	severe	9,341
43	severe	12,750
57	severe	14,867

[a] The independent judgements of two observers were the same for each plant listed.
[b] The distribution of propagules appears in Fig. 1A.
[c] The taller of the two plants (Fig. 1A) that averaged 57 propagules per millimeter of stem length.

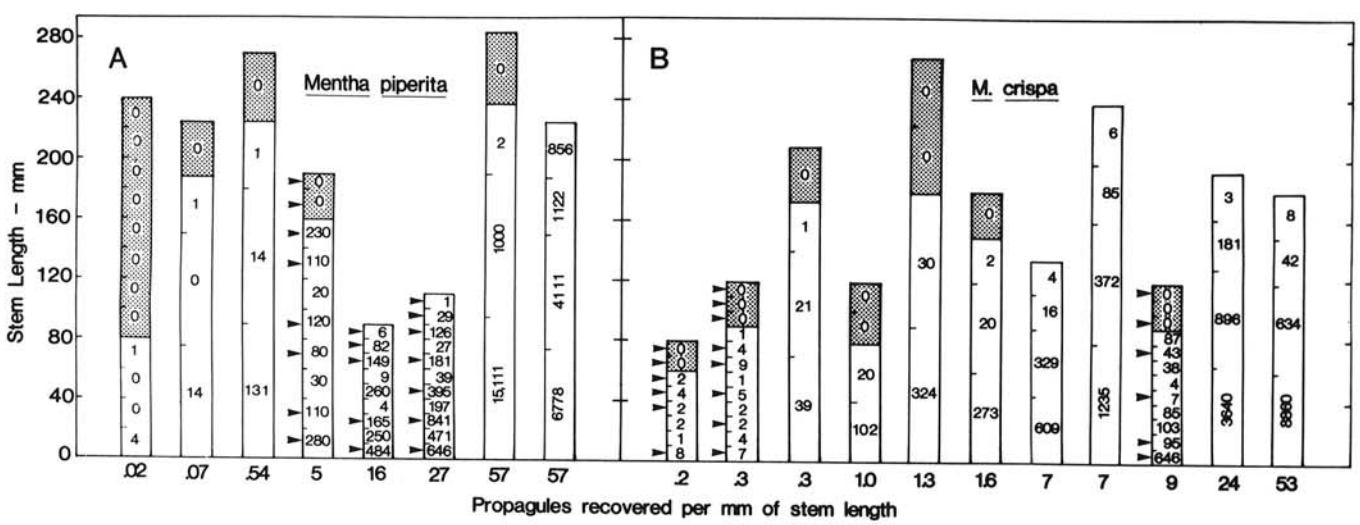

Fig. 1. Distribution of propagules of *Verticillium* recovered from serial segments of stems of mint planted in infested field plots. Each bar represents the stem of one plant. The numbers within the bars specify the number of propagules recovered from each segment of the stems. Horizontal lines delimit the segments. Shading indicates the topmost segments that failed to yield propagules. When collected and assayed, these plants had either mild symptoms of Verticillium wilt or no symptoms at all; only plants that averaged fewer than 100 propagules per millimeter of stem are represented. The arrows next to some bars indicate segments that had one or two nodes. **A,** Propagules from plants of the susceptible species, *M. piperita*. **B,** Propagules from plants of the resistant species, *M. crispa*.

of about 34% of their top parts free of *V. dahliae* (Fig. 1A and B). *V. dahliae* was recovered from the topmost segment of all plants of *M. piperita* that averaged more than 100 propagules per millimeter of stem. No plant of *M. crispa* yielded more than 100 propagules per millimeter.

Apparent discontinuities in the vertical distribution of *V. dahliae* in stems occurred in two plants of *M. piperita* (Fig. 1A). In other plants, the number of propagules varied in successive 1-cm or 2-cm segments of both *M. piperita* and *M. crispa* (Fig. 1A and B). In some plants, nodes occurred in the segments from which the higher number of propagules were recovered; in other plants, relatively high numbers of propagules came from stem segments that lacked nodes (Fig. 1A and B).

The symptoms of Verticillium wilt in *M. piperita* included (more or less in order of occurrence): lateral curving of the younger leaves, chlorosis of the leaves, shortened internodes, bronze color of the leaves, epinasty, and (finally) necrosis. Wilting did not appear until the plants were nearly dead. Except for occasional curving of the younger leaves, symptoms of Verticillium wilt did not appear in *M. crispa*.

The quantitative assays allowed comparison of the severity of symptoms of Verticillium wilt and numbers of fungus propagules in the stems of the susceptible species of mint (Table 2). Plants with similar symptoms yielded different numbers of propagules in several instances. Plants without symptoms occasionally yielded propagules from their stems, and plants with symptoms occasionally yielded few or no propagules from their stems.

In all, 100 plants (50 of each species) were assayed. Of these, 30 plants of *M. piperita* and 18 plants of *M. crispa* yielded *V. dahliae*. The roots of *M. crispa* branched and extended much less than did the roots of *M. piperita*. No *V. dahliae* was recovered until 22 days after planting. Of the 10 plants collected and analyzed at that time, only one, a specimen of *M. piperita*, yielded propagules of *V. dahliae*. Even though that plant displayed no symptoms, it averaged 16 propagules per millimeter of stem length and yielded the pathogen from every segment including the topmost (Fig. 1A, the shortest plant).

The number of propagules of *V. dahliae* recovered from the susceptible species, *M. piperita*, generally exceeded the number recovered from the resistant species, *M. crispa* (Table 3). Generally fewer than 10 propagules per millimeter of stem length were recovered from stems of *M. crispa* and in no instance were more than 100 recovered. Contrarily, the number of propagules recovered from stems of *M. piperita* exceeded 100 in 21 of the 30 plants infected. Five plants of *M. piperita* averaged 100–1,000 propagules, 13 plants averaged 1,000–10,000 propagules, and three plants averaged 10,000–15,000 propagules per millimeter of stem length.

TABLE 3. Differences in quantities of propagules of *Verticillium dahliae* recovered from *Mentha piperita* (susceptible) and *M. crispa* (resistant) planted in infested field plots

Season	Time after planting in infested soil (days)	Plants infected[a] (no.)		Propagules recovered (Avg. no. per mm of stem)	
		M. piperita	*M. crispa*	*M. piperita*	*M. crispa*
I	9	0	0	0	0
	15	0	0	0	0
	22	1	0	16	0
	29	3	3	112	4
	36	4	3	2,234[b]	1
	43	5	1	3,092	0.01
	50	4	3	3,650	1
	57	5	4	6,346	21
II	43	3	0	135	0
	82	5	4	2,853	5

[a] Five pairs, each consisting of one of each species, were selected with a table of random numbers and were quantitatively assayed.

[b] Calculated from three of the four infected plants. The count in the omitted one appeared to be not accurate enough.

DISCUSSION

Validity of the quantitative assay. Pegg's (13) work raised the possibility of skewed estimates of populations resulting from effects of host tissue in assays of the kind that we report here. Our assay differed from Pegg's in several respects: For example, the *V. dahliae* we estimated came from natural infections that occurred in the field, whereas Pegg introduced spores and mycelium from laboratory cultures; the hosts in our experiments were species of mint, whereas Pegg used tomato and chrysanthemum; and where Pegg apparently counted any fungal colonies that appeared after 3–5 days, we used longer incubations (12 days) and counted only colonies that formed microsclerotia.

If stem tissue enhanced survival or germination of the propagules in our experiments, one would expect the number of colonies of *V. dahliae* to decrease with increased dilution—we observed no such decrease. Neither did we observe any apparent enhancement or inhibition of colony growth by fragments of mint stems. Therefore, we feel that the procedures reported here yielded reasonably accurate estimates of the quantities of propagules of *V. dahliae* in the mint stems we assayed.

Symptoms and amount of pathogen. Talboys (16) postulated that ". . . the severity of the disease depends on . . . the quantity of the fungus present within the vessels." Our results partly confirmed and partly contradicted this hypothesis. Susceptible plants with symptoms ranging from moderate to severe generally contained 1,000 or more propagules per linear millimeter of stem; plants with no symptoms or with mild symptoms contained fewer than 1,000 propagules per linear millimeter of stem (Table 2). However, the quantity of propagules in stems could not be predicted when the symptoms ranged from absent to mild (Table 2). The mild symptoms of a plant found to contain no propagules in its stem may have resulted from *V. dahliae* having entered the root but not the stem (Table 1).

Wilting not a symptom. Some investigators have listed wilt as ". . . the most conspicuous symptom . . . " of the vascular wilt diseases. In our experiments, however, severely diseased, but apparently turgid plants became necrotic. We did not observe wilting until the plants were virtually dead. Thus, in our opinion, lack of water is not necessarily the primary cause of death in Verticillium wilt of mint. In this conclusion, we concur with Talboys (17).

Escape by *M. crispa*. The reduced frequency of infection of the resistant species, *M. crispa*, in the field (Table 3), might have resulted from resistance in the rhizosphere, resistance at the root surface, or from escape. Previous studies by Lacy and Horner (8) indicated a lack of resistance in the rhizosphere and at the root surface of *M. crispa*. Care to ensure contact of roots with inoculum resulted in equal frequencies of infection in resistant and susceptible species of mint in the greenhouse (Table 1). These data and the less extensive root systems of *M. crispa* plants indicate that the reduced frequency of infection in the field may have resulted from escape.

Lack of resistance to ascent of the pathogen. Explanations of resistance to wilt diseases have invoked physical or chemical barriers that limit the ascent of the pathogen in stems (eg, 1,7,9,10,17,18). Where such a mechanism functioned, one might expect qualitative and quantitative studies to demonstrate a failure of the pathogen to ascend a resistant species in the case of a soilborne disease such as Verticillium wilt. However, Presley et al (14) and Beckman et al (1) found that spores of *Verticillium* moved up stems of resistant cultivars of cotton as readily as they moved up stems of susceptible cultivars. Similarly, our qualitative and quantitative studies showed that *V. dahliae* moved up the stems of the resistant mint species about as readily as it moved up the stems of the susceptible peppermint (Table 1; Fig. 1A and B).

We conclude, therefore, that physical or chemical barriers to ascent do not account for resistance to Verticillium wilt by *M. crispa*. This conclusion, at least in part, is in accord with Dimond's (3) view that gels and tyloses are less likely to cause resistance in

stems and branches of dicotyledonous plants than in banana. The discontinuities and nonuniformity in vertical distribution of *V. dahliae* together with the apparently rapid ascent may have resulted from an upward transport of propagules such as spores in the transpiration stream as Dimond (3) and others (eg, 5,17) have reported.

Lacy and Horner (8) recovered ". . . significantly larger numbers . . ." of propagules of *V. dahliae* from roots of *M. piperita* than from roots of *M. crispa*. In the work reported here, we recovered more propagules of *V. dahliae* from stems of the susceptible species, *M. piperita*, than from stems of the resistant species, *M. crispa* (Table 3). Two other reports have included findings of more propagules of *Verticillium* in susceptible mints than in resistant ones (6,11). Thus, *M. crispa* may resist Verticillium wilt by suppressing proliferation of the pathogen.

Zambino and Anderson (19) found a similar situation in potato; they recovered more colonies of *Verticillium* from susceptible cultivars than from resistant cultivars. Elgersma (4) recovered more propagules from susceptible tomatoes than from resistant ones. Garber and Houston (5) found more conidia in vessels of a susceptible cultivar of cotton than in vessels of a resistant cultivar. Schnathorst et al (15) reported that tracheal fluid from susceptible cultivars of cotton contained many-fold more conidia of *Verticillium* than did tracheal fluid from tolerant cultivars.

We suggest, therefore, that plants other than mint may resist Verticillium wilt by limiting proliferation of the fungal pathogen as apparently happens in mint. Perhaps mint and other plants do so by means of secondary metabolites as Bell and Stipanovic (2) suggest, or perhaps by restricting entry of the pathogen to vessels in the lower part of the host plants or perhaps by both.

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