

## Cross Protection in Mints by *Verticillium nigrescens* Against *V. dahliae*

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### ABSTRACT

Peppermint and spearmint were cross protected against disease induced by a virulent isolate of *Verticillium dahliae* when inoculated first with the weak pathogen, *V. nigrescens*. Cross protection effects were observed in peppermint when inoculation with *V. nigrescens* preceded inoculation with *V. dahliae* by 2 days. When inoculations with *V. nigrescens* preceded *V. dahliae* by 7 and 9 days, wilt severity was greatly reduced and many plants were symptomless 4 weeks after inoculation. No cross protection was observed when inoculation with *V. dahliae* preceded inoculation with *V.*

*nigrescens* by 2, 5, 7, and 9 days. In cross-protected peppermint, propagules of *V. dahliae* decreased substantially 22 and 29 days after inoculation. Four weeks after inoculation the number of propagules of *V. dahliae* in stems of cross-protected peppermint were one-fifteenth to one-twentieth of those of nonprotected controls. Numbers of propagules of *V. nigrescens* remained stable, but at a low level, in peppermint plants when *V. dahliae* was the challenger.

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Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major disease of mints in the United States. Commercially grown peppermint (*Mentha piperita* L. 'Mitcham'), and spearmint (*Mentha cardiaca* Baker 'Scotch') are both susceptible to this disease. A chlamydospore-forming isolate of *V. nigrescens* was recovered from peppermint plants showing mild, transient wilt symptoms. This isolate of *V. nigrescens* was weakly pathogenic to spearmint, peppermint, eggplant, tomato, and pepper (3). Skotland (7) reported similar results with another isolate of *V. nigrescens*.

In a preliminary experiment, inoculation of peppermint shoot-tip cuttings with *V. nigrescens* protected them against a virulent isolate of *V. dahliae* (4). Therefore, this study was undertaken to determine the protective effects in mints by inoculation with the mild pathogen *V. nigrescens* on subsequent inoculations with a virulent strain of *V. dahliae*. We studied wilt symptom severity and propagule populations in inoculated plants.

**MATERIALS AND METHODS.**—Cultures of *V. nigrescens* and *V. dahliae* were grown on potato-dextrose agar in the dark for four weeks at 20 C. Conidiospore suspensions were prepared by gently flooding culture plates with distilled sterile water. The suspensions were filtered twice through four layers of cheesecloth to remove larger mycelial fragments and microsclerotia, and the suspension was adjusted to  $5 \times 10^5$  conidiospores/ml.

Mint shoot-tip cuttings, 10-12 cm long, were inoculated by immersing the freshly cut ends into a *V. nigrescens* conidial suspension for 45 minutes under fluorescent lights (6,400 lux) at 20 C. The inoculated shoots were placed in vials containing half-strength Hoagland's solution (2) in a growth chamber at 20 C and a 14-hour per day photoperiod at 6,400 lux. Shoots were then inoculated with *V. dahliae* in the same manner at 0, 2, 5, 7, and 9 days after inoculation with *V. nigrescens*. The opposite inoculation sequence was made in another group of plants in which inoculation with *V. dahliae*

preceded inoculation with *V. nigrescens* by 2, 5, 7, and 9 days. Controls consisted of noninoculated plants and plants inoculated with either *V. nigrescens* or *V. dahliae*. Following the final inoculation, the cuttings were placed in sand for rooting and disease development. The plants were maintained on a greenhouse bench at 21-23 C. Daylight was supplemented by fluorescent lights to provide a 15-hour daylength.

Disease severity, plant height, and quantitative determination of *V. nigrescens* and *V. dahliae* propagules in stems of inoculated plants were the criteria used to assess the extent of protection of mints by *V. nigrescens* against *V. dahliae*.

Verticillium propagule numbers in mint stems were quantitatively determined by fragmentation, dilution, and plating techniques (5). Colonies were counted after 2 weeks of incubation in darkness at 20 C. Each colony of *Verticillium* was assumed to have grown from a single propagule.

**RESULTS AND DISCUSSION.**—Spearmint and peppermint plants inoculated with only *V. nigrescens* were either symptomless or had mild symptoms 4 weeks after inoculation. However, severe wilt symptoms occurred on both mints 4 weeks after inoculations with a virulent isolate of *V. dahliae*. Inoculation of peppermint and spearmint with *V. nigrescens* prior to inoculation with *V. dahliae* resulted in positive cross protection (Table 1). Cross protection of cotton by mild strains of *V. albo-atrum* Reinke & Berth. against virulent strains of *V. albo-atrum* has been reported (1, 6, 8).

Cross protection was observed first in peppermint plants inoculated with *V. nigrescens* 2 days prior to inoculation with *V. dahliae*, and maximum protection was obtained when inoculation with *V. nigrescens* preceded inoculation with *V. dahliae* by 5 or 7 days (Table 1, Fig. 1). No reduction of wilt symptoms and severity was observed when inoculation with *V. dahliae* preceded inoculation with *V. nigrescens* by 2, 5, 7, and 9 days.

There was no appreciable difference in the uptake of *V.*

TABLE 1. Disease severity and plant height of cross-protected Mitcham peppermint and Scotch spearmint 4 weeks after inoculation with a challenger isolate of *Verticillium dahliae*. Mint shoot-tip cuttings were inoculated with *V. nigrescens* prior to being inoculated with *V. dahliae*

Inoculation schedule	Percentage of plants in disease severity classes					Plant height <sup>z</sup> (cm)
	None	Light	Moderate	Severe	Dead	
Mitcham peppermint						
<i>V. nigrescens</i> only	79	21	0	0	0	13.0 b
<i>V. dahliae</i> only	0	0	0	69	31	3.8 d
0 <sup>y</sup>	0	0	0	93	7	3.3 d
2	0	33	47	7	13	6.4 c
5	59	7	27	7	0	11.7 b
7	86	14	0	0	0	15.9 a
9	83	17	0	0	0	16.9 a
Non-inoc. controls	100	0	0	0	0	11.9 b
Scotch spearmint						
<i>V. nigrescens</i> only	100	0	0	0	0	13.4 a
<i>V. dahliae</i> only	0	0	0	59	41	3.3 c
0 <sup>y</sup>	0	0	0	47	53	3.7 c
2	0	0	0	69	31	3.6 c
5	0	0	0	81	19	4.4 c
7	100	0	0	0	0	12.7 a
9	81	0	0	19	0	8.5 b
Non-inoc. controls	100	0	0	0	0	13.4 a

<sup>y</sup>Number of days inoculation with *V. nigrescens* preceded inoculation with *V. dahliae*.

<sup>z</sup>Data followed by the same letter are not significantly different,  $P = 0.05$ , by Duncan's multiple range test.

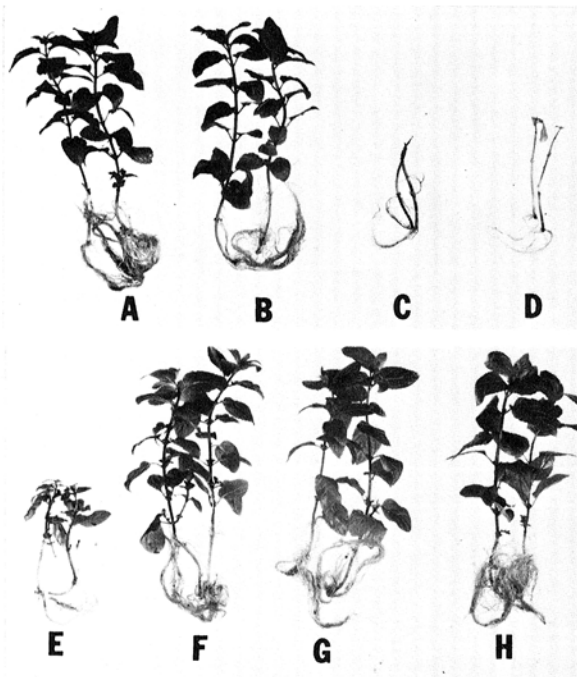


Fig. 1-(A to H). Mitcham peppermint plants 6 weeks after inoculation. A) Noninoculated control, B) Inoculated with *Verticillium nigrescens* only, C) Inoculated with *V. dahliae* only, and D to H) Inoculated with *V. nigrescens* 0, 2, 5, 7, and 9 days, respectively, prior to being inoculated with *V. dahliae*.

*dahliae* conidiospores between tip cuttings of peppermint cross-protected with *V. nigrescens* and the nonprotected plants, as determined by assaying fungus propagules on the selective medium. In both cross-protected and

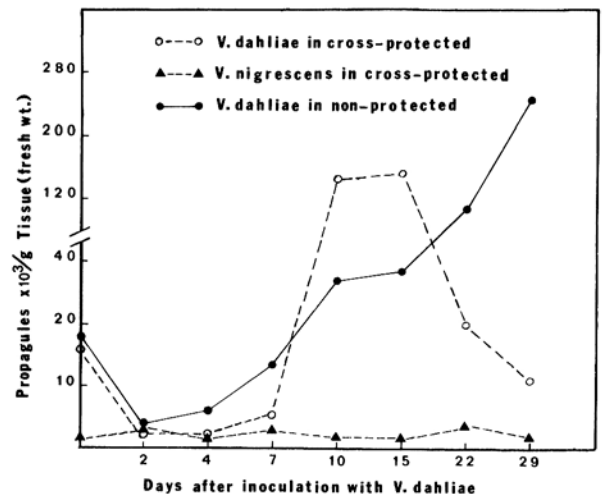


Fig. 2. Propagules of *Verticillium* in stems of cross-protected and nonprotected peppermint. Plants were cross-protected by inoculation of tip cuttings with *V. nigrescens* 7 days prior to inoculation with a challenger isolate of *V. dahliae*.

nonprotected peppermint, propagules of *V. dahliae* in stems of inoculated plants remained low up to 7 days, then rapidly increased 10 and 15 days after inoculation (Fig. 2). However, in cross-protected peppermint, *V. dahliae* propagules decreased substantially 22 and 29 days after inoculation, while propagules in nonprotected plants continued to increase. Four weeks after inoculation the number of propagules of *V. dahliae* in stems of cross-protected peppermint plants were one-fifteenth to one-twentieth of those of nonprotected control plants.

Propagule counts of *V. nigrescens* in peppermint tip

cuttings decreased by 80%, 7 days after inoculation. However, propagules of *V. nigriscens* remained stable, but at a low level, in plants which were also inoculated with *V. dahliae* (Fig. 2). The presence of a low population of *V. nigriscens* in stems of peppermint was apparently sufficient to maintain cross protection against *V. dahliae*. Colonies of *V. nigriscens* and *V. dahliae* were easily distinguished on the selective medium. *V. nigriscens* colonies were almost round and black with a smooth margin, while *V. dahliae* colonies were irregular in shape with a ragged margin.

Schnathorst and Mathre (6) cross protected cotton against severe Verticillium wilt, by inoculation with a mild strain of *V. albo-atrum* 7 days prior to inoculation with a challenger strain. Zaki et al. (8) suggested that cross protection of cotton to a severe isolate of *V. albo-atrum* was due to accumulation in the xylem of antifungal compounds (phytoalexins) in response to inoculation with a mild strain of *V. albo-atrum*. Our data on the populations of *V. nigriscens* and *V. dahliae* in cross-protected peppermint indicate that the protecting effect of *V. nigriscens* was not due to physical competition with *V. dahliae*, because the propagule counts of *V. nigriscens* were relatively stable during the test. This is indirect evidence for either the presence of inhibitory compound(s) in the plant, or for an alteration in the metabolism of the host in response to infection by *V. nigriscens* even at a relatively low level (Fig. 2). The cross-protection phenomenon reported here and by others (1, 6,

8) is a form of biological control, and may have application in control of wilt diseases in plants.

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