Interaction of Verticillium dahliae and Pratylenchus minyus in Verticillium Wilt of Peppermint: Influence of the Nematode as Determined by a Double Root Technique

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ABSTRACT

A technique was developed to determine if *Pratylenchus minyus* influences *Verticillium* wilt of peppermint solely by providing readily accessible infection courts to fungus hyphae. Detached peppermint stems were rooted at two locations to provide plants with two root systems. The root systems of these plants were placed in soil in separate plastic pots and inoculated with various combinations of *Verticillium dahliae* and/or *P. minyus*. The nematodes influenced the length of the incubation period and the incidence and severity of wilt even when each pathogen parasitized separated root systems of the same plant. Phytopathology 60:100-103.

Synergism has been demonstrated in several plant disease complexes involving interaction of nematodes and fungi (1, 5, 6, 7, 8). The role that nematodes play in such complexes has not been adequately elucidated. Nematodes, during penetration and feeding, create potential avenues of entrance for fungus hyphae. In the past, this mechanical role of nematodes has been suggested by nematologists and plant pathologists (1, 2, 6, 7, 8).

Evidence indicating that *Pratylenchus minyus* Sher & Allen might induce physiological changes in the host, resulting in increased susceptibility to *Verticillium dahliae* (Kleb.) f. *menthae* Nelson, was discussed in a previous paper (5). Soil temperatures favorable to the nematode had a greater influence on the development of *Verticillium* wilt than did temperatures favorable to the fungus.

The present study was undertaken to determine if *P. minyus* alters the susceptibility of peppermint to *Verticillium* wilt by means which are not solely of a mechanical nature.

Materials and Methods.—Pure populations of *P. minyus* Sher & Allen were isolated and maintained in a growth room by methods described previously (4). *Verticillium* inoculum consisted of microsclerotia obtained from infected peppermint stems. The stems were air-dried and ground to pass an 80-mesh screen in a Wiley Mill. The ground tissues contained approximately 50,000 *V. dahliae* (Kleb.) f. *menthae* Nelson propagules per g.

Two experiments were conducted in a greenhouse where, during the first experiment (July through August), average day air temperatures were 24 C ($\pm$ 4 C), and 18 C ($\pm$ 3 C) at night. Day-night air temperatures for the second experiment (December-March) were 21 C ($\pm$ 2 C) and 15 C ($\pm$2 C), respectively. Supplemental light (experiment 2) was provided by banks of Sylvania Gro-Lux fluorescent tubes with a photoperiod of 16 hr. New 10.1-cm round plastic pots were used in all experiments. The plants were grown in fumigated potting mixture (334 g methyl bromide/m^3) containing six parts sand, two parts Sagemoor fine sandy loam, and one part peatmoss. The plants were irrigated with tap water. A commercial fertilizer (Valgreen) containing 22% N, 16% P, 16% K with 0.12% Cu, 0.42% Mn, 0.11% Fe, 0.21% Zn, and 0.26% B was applied as required.

A technique was developed which allowed *P. minyus* and *V. dahliae* f. *menthae* to each parasitize separate root systems of peppermint plants. Peppermint stems ($>$ 20 cm long) were stripped of all but their terminal leaves, threaded through arched sections of black plastic tubing (15.0 cm long $\times$ 1.0 cm ID), and rooted beneath the stem apex and at the stem base in fumigated sand (334 g methyl bromide/m^3). Small holes (1.59 mm diam) were drilled through the lower side of the tubing to allow gas exchange and prevent accumulation of free moisture within. Foliage was allowed to develop only at the ends of stem sections which constituted the apical portion before removal. After 2 weeks, those plants with well-developed roots on both ends of the stem sections were transferred to the fumigated potting mixture in plastic pots. Each root system was placed in a separate pot.

The terms terminal and basal are henceforth used to designate location of root systems on doubly rooted plants. The root system located directly under the shoot is termed terminal, whereas the root system formed at the stem base and lacking its own vegetative shoot is termed basal. Also, P, V, and O are used to designate treatments with *P. minyus*, *V. dahliae* f. *menthae*, and no inoculum, respectively. Their respective locations (terminal/basal) are shown on either side of the slash.

After placing the bare roots in 2.0-cm $\times$ 5.0-cm depressions in the potting mixture, inoculations were made by adding nematode and/or *Verticillium* inoculum and covering the roots with potting mixture. Water was added to prevent drying. The inoculum contained either 1,000 root-lesion nematodes in 5 ml of water or 40 mg of the ground *Verticillium* inoculum (2,000 propagules), or both.

Data were collected on the rate of development, incidence, and severity of *Verticillium* wilt and on populations of root lesion nematodes. Disease severity was evaluated and tabulated by assigning index numbers

TABLE 1. Influence of *P. minyus* on number of plants expressing *Verticillium* wilt symptoms at various time intervals following inoculation and on disease severity after 60 days

Inoculum treatment (16 plants each)	No. plants expressing symptoms, days after					Avg disease index[c] (60 days)
	35	40	45	50	55	
PV/PV[a,b]	4	11	16	16	16	3.6
V/V[b]	2	4	4	9	11	1.8
P/P[b]	0	0	0	0	0	
O/PV	1	7	9	13	14	2.8
PV/O	0	6	10	13	14	2.0
P/V	0	2	5	8	11	1.5
V/P	0	0	3	5	10	1.3
V/O	0	0	1	4	6	0.6
O/V	0	0	1	2	2	0.3
P/O	0	0	0	0	0	
O/P	0	0	0	0	0	
O/O	0	0	0	0	0	

[a] In the treatment designations, O = no inoculum; P = *P. minyus*; and V = *V. dahliae*; their respective locations (terminal/basal) are shown on either side of the slash.

[b] Plants received standard inoculum dosages on both root systems, and these results are not directly comparable with the other treatments.

[c] Based on symptom index where 0 = no symptoms and 5 = plants dead.

based on the relative expression of symptoms as follows: 0 = no symptoms; 1 = light; 2 = moderate; 3 = heavy; 4 = severe; and 5 = plants dead. Nematode populations were determined by extracting nematodes from roots by a modification of the spraying method of Chapman (3). The nematodes were suspended in a known volume of water, and counts were made from 1-ml portions thereof.

Attempts to isolate *V. dahliae* f. *menthae* from the terminal and basal ends of stem bridges were made by surface-disinfesting mint tissues with 25% sodium hypochlorite solution for 3 min and plating sections of tissue on ethyl alcohol-streptomycin agar (9).

RESULTS.—In the first of two experiments, 16 each of the doubly rooted peppermint plants received treatments designated as follows: PV/PV, V/V, P/P, O/PV, PV/O, P/V, V/P, V/O, O/V, P/O, O/P, and O/O. Treatments PV/PV, V/V, and P/P received twice normal dosages of inoculum (standard dosages were applied to each root system) and are not directly comparable with the other inoculum treatments. Nonetheless, in all cases where both the nematode and the fungus were present, the development of *Verticillium* wilt was either similar to or exceeded that observed when plants received the standard amount of *Verticillium* inoculum on both root systems (Table 1).

In the directly comparable treatments (O/PV, PV/O, P/V, V/P, V/O, and O/V), the incubation period for development of wilt symptoms was reduced by *P. minyus,* and the disease was increased in both incidence and severity whether or not the fungus was present on the same or a separate root system of a plant.

As shown by standard fungus isolation and nematode extraction procedures, neither pathogen traversed the stem bridge between pots.

In the second experiment, 45 each of the doubly rooted plants were given selected treatments as follows: O/VP, P/V, O/V, V/P, V/O, P/O, O/P, and O/O. The numbers of plants expressing wilt symptoms after various time intervals, and the severity of wilt

TABLE 2. Influence of *P. minyus* on number of plants expressing *Verticillium* wilt symptoms at various time intervals following inoculation and on disease severity after 90 days

Inoculum treatment	No. plants expressing symptoms												Avg disease index (90 days)
	Total/treatment, days after						Average/replication, days after						
	40	50	60	70	80	90	40	50	60	70	80	90	
O/VP[a]	4	15	27	30	33	33	0.8	3.0	5.4	6.0	6.6	6.6	2.6[b]
P/V	1	7	14	17	21	27	0.2	1.4	2.8	3.4	4.2	5.4	1.6
V/P	2	4	9	16	21	25	0.4	0.8	1.8	3.2	4.2	5.0	1.3
O/V	0	1	3	10	15	21		0.2	0.6	2.0	3.0	4.2	0.8
V/O	1	1	4	5	6	15	0.2	0.2	0.8	1.0	1.2	3.0	0.6
O/O[c]	0	0	0	0	0	0							
P/O[c]	0	0	0	0	0	0							
O/O[c]	0	0	0	0	0	0							
LSD = .05							NS	0.97	1.20	1.37	0.54	0.35	0.56
= .01							NS	1.42	1.78	2.02	0.80	0.55	0.82

[a] In the treatment designations, O = no inoculum; V = *V. dahliae*; and P = *P. minyus*; their respective locations (terminal/basal) are shown on either side of the slash.

[b] Based on symptom index where 0 = no symptoms and 5 = plants dead.

[c] Treatments O/P, P/O, and O/O were not included in the data analyzed, since wilt symptoms were lacking and could not be expected.

symptoms after 90 days, are shown in Table 2. Symptoms were first observed 33 days after the inoculations were made, but significant differences between treatments did not occur until 50 days had elapsed. From 70 days to termination of the experiment after 90 days, highly significant differences in the number of plants expressing wilt symptoms occurred between inoculation treatments, including both pathogens and those where only the fungus was present. Significant differences in the severity of symptom expression were also recorded.

After 90 days, the various treatments yielded average *P. minyus* populations as follows: P/V—9,866; V/P—6,959; P/O—6,721; O/P—4,403; O/VP—4,256; V/O—0; O/V—0; and O/O—0. The differences observed among treatments containing nematodes in the inoculum were not statistically significant (LSD at 5% level = 8,191).

The rates of disease development in the two experiments, as affected by *V. dahliae* f. *menthae* (± *P. minyus*), are shown in Fig. 1-A and B) (experiments 1 and 2, respectively). Although these experiments were conducted under different environmental conditions, similar trends were observed among treatments included in both (e.g., O/PV, P/V, V/P, O/V, and V/O).

Discussion.—The role of *P. minyus* in the fungus-nematode complex associated with peppermint wilt is not limited to the opening of infection courts for *V. dahliae*. Presence of the nematode increased both incidence and severity of peppermint wilt even when the interacting organisms were held in isolation on separate root systems of the same plant. To do this, *P. minyus* must induce changes in host tissues which make them either more attractive to the fungus, less tolerant of the fungus, or both.

Although treatments P/V and V/P yielded final nematode populations roughly 1.5 times greater than those of their respective counterparts (P/O and O/P), these differences were not statistically significant. We previously reported similar increases in nematode reproduction when *P. minyus* was placed in association with *V. dahliae* (5, 6). Nematode yields were highly variable regardless of the inoculum treatment, but variation has been greatest each time the pathogens were placed on the same plant. In the present study, variation was up to four times greater among treatment replications inoculated with both pathogens than it was among those receiving only the nematode. Since our data have consistently indicated that *V. dahliae* increases the reproductive rate of *P. minyus,* we believe its influence is real even though the high degree of variation may preclude proof by statistical methods.

The double root technique has several inherent difficulties. Excessive numbers of cuttings must be started to procure sufficient plants with suitable root systems for experimentation. Care must be taken that stem connections between root systems do not rot apart. If rotting goes undetected early in an experiment, the final results may be skewed. Also, root systems developing at older nodes were only 25% to 50% the size of those developing at younger nodes.

Nonetheless, the technique does have several advantages. Effects of one root parasite on another, even when in separate root systems of the plant, can be measured. It has a distinct advantage over the split root method in that translocation is not strictly dependent upon lateral movement in stem tissues, but may occur freely from one root system to the other through relatively undisturbed vascular tissues.

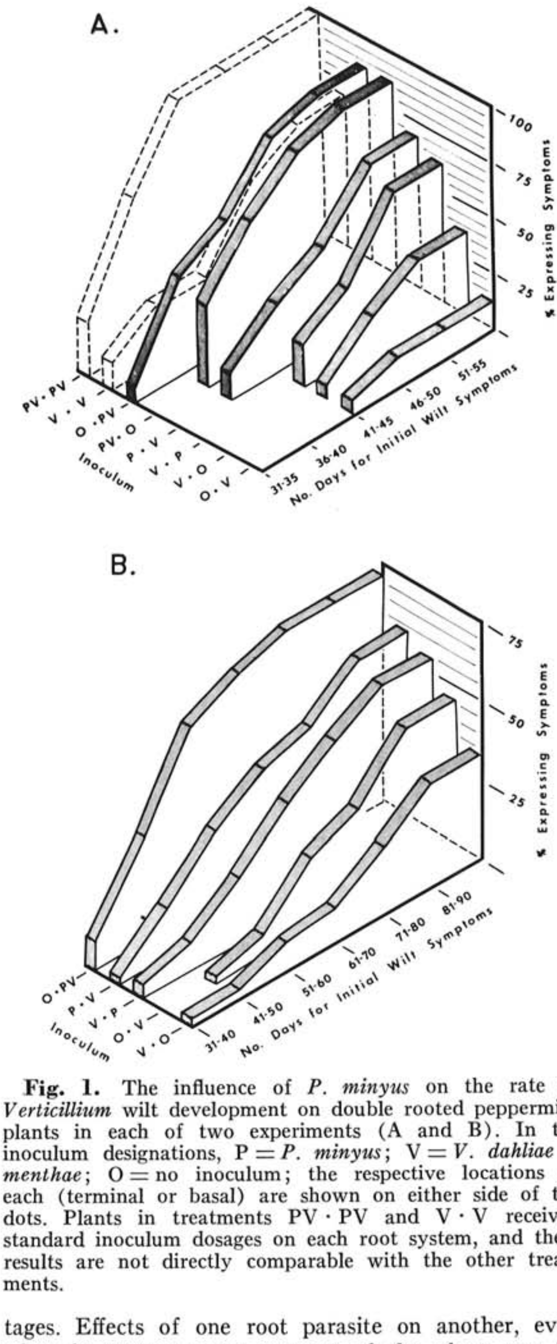

Fig. 1. The influence of *P. minyus* on the rate of *Verticillium* wilt development on double rooted peppermint plants in each of two experiments (A and B). In the inoculum designations, P = *P. minyus*; V = *V. dahliae* f. *menthae*; O = no inoculum; the respective locations of each (terminal or basal) are shown on either side of the dots. Plants in treatments PV · PV and V · V received standard inoculum dosages on each root system, and these results are not directly comparable with the other treatments.

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