Interaction of Verticillium albo-atrum and the Root Lesion Nematode, Pratylenchus penetrans, in Tomato Roots at Controlled Inoculum Densities

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ABSTRACT

The influence of the root lesion nematode, *Pratylenchus penetrans*, on infection levels by *Verticillium albo-atrum* at controlled inoculum densities of both organisms and under controlled environmental conditions was considered. Infection of tomato seedlings, cultivar Bonny Best, by *V. albo-atrum* was 100% at the inoculum density of 200 microsclerotia/g soil, and infection levels were progressively lower at 100, 75, 50, and 25 microsclerotia/g soil. Consistent increases in infection occurred at all inoculum densities when the nematode was also present. Infection incidence by *V. albo-atrum* also increased with increases in the nematode population. There was no evidence of a synergistic effect on nematode reproduction, since the number of nematodes extracted from roots of tomato also infected by *V. albo-atrum* was significantly lower than from roots with the nematode alone. The role of the nematode in increasing susceptibility of the host plant to *V. albo-atrum* was considered, using a split-root technique. There was no increase in susceptibility of tomato to the fungus pathogen when the nematode was on the same root system but in isolation from the fungus.

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Additional key words: inoculum density, fungus-nematode interaction.

Verticillium albo-atrum Reinke & Berth. attacks uninjured roots of plants; however, root damage may increase the incidence of infection and the severity of symptom development (7, 10, 11). The interaction of *V. albo-atrum* and the root lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev & Stekhoven has been demonstrated, suggesting that root damage by the nematode increases the incidence of Verticillium wilt (7, 8, 9).

Mountain & McKeen (7, 8) showed increases in the incidence of wilt symptoms in eggplant at 4 inoculum levels of *V. albo-atrum* in the presence of *P. penetrans*. Faulkner & Skotland (3) demonstrated that the incubation period for symptom expression of Verticillium wilt of peppermint was decreased in the presence of *Pratylenchus minyus*, and Bergeson (1) found that wilt symptoms appeared 2 weeks earlier on peppermint plants infected with *P. penetrans* prior to inoculation with *V. albo-atrum* than with the fungus alone.

Faulkner et al. (2) suggested that the effect of *Pratylenchus minyus* on the incidence of Verticillium wilt of peppermint was not limited to providing infection courts only, but also had a direct effect on the host plant which, in turn, affected susceptibility. Adventitious roots were induced on peppermint stems at the stem base and below the stem apex. Incidence and severity of Verticillium wilt increased, and the incubation period for symptom expression was reduced in the presence of the nematode, whether or not the fungus and nematode were present on the same or a separate root system on the same plant.

A synergistic effect on nematode reproduction has also been suggested between *P. minyus* and *V. dahliae* (3), with higher numbers of nematodes recovered when the fungus and the nematode were combined on the same root system than with the nematode alone. However, Bergeson (1) reported no significant differences in the number of *P. penetrans* nematodes recovered from roots in the presence or absence of *V. albo-atrum*.

Results reported by different investigators are difficult to compare because of different experimental methods. Symptom expression and disease severity, which are highly subjective criteria, were generally used to evaluate results. Also, most investigators agreed that the effects of nematodes on the incidence of Verticillium wilt of various hosts was reduced or negated at high inoculum densities, but little effort was made to control this variable.

This paper presents results of a study of the effects of the lesion nematode, *Pratylenchus penetrans*, on the incidence of infection of tomato by *V. albo-atrum* under controlled environmental conditions and at critically controlled inoculum densities of both the fungus and nematode. Consideration was also given to effects of the nematode on the host other than root wounding, and of possible synergistic effects of the nematode and fungus on the same root system.

MATERIALS AND METHODS.– *Lycopersicon esculentum* Mill. 'Bonny Best' tomato was the indicator host, and the isolate of the pathogen was a highly pathogenic microsclerotial form of *V. albo-atrum*. All tests were conducted in growth chambers with a 16-hr photoperiod and a constant soil temperature of 26 C. A nonsterile soil mix (clay-loam + sand + perlite: 4:2:1) was used, and each test was maintained for 6 weeks. Results were based on infection incidence by *V. albo-atrum* rather than incidence and/or severity of symptom expression.

Reisolation of the pathogen was the criterion of infection. After 6 weeks, all plants were harvested, and a section of the lower stem was surface-sterilized in 1% NaOCl and placed on Czapek-Dox agar + 50 ppm (ea) of streptomycin sulfate and aureomycin. Stem pieces were incubated for 10 days at 26 C for final readings.

The soil inoculum was prepared as follows: A 1-ml spore-mycelial suspension was flooded over sterilized discs of cellophane (cellulose xanthate) placed on Czapek-Dox agar in large petri plates. After 21 to 28 days, the cellophane was stripped from the medium and macerated in a food blender. To remove mycelial fragments and spores, the suspension was washed through a 100-, 200-, 340-mesh series of screens. Microsclerotia were collected from the 200- and 340-mesh screen and mixed into nonsterile soil, using a Patterson-Kelly twin shell blender with intensifier. Soil moisture was adjusted to 30-40% saturation, and the soil was stored in plastic bags at room temperature for several weeks.

Inoculum density was determined by a direct assay of the stock soil using the soil extract agar method described by Green & Papavizas (5). The final inoculum density of the soil was determined by the soil assay method previously described.

The infection incidence of tomato seedlings at inoculum densities of 0, 25, 50, 75, 100, 200, and 500 microsclerotia of *V. albo-atrum*/g soil (30% saturation) was determined. The range of inoculum densities used was based on findings by Green (4), who determined that 100 microsclerotia/g were necessary for a 100% infection under similar conditions.

Pratylenchus penetrans was increased on alfalfa seedling callus grown on a modification of Krusberg's tissue culture medium containing 2 mg/liter of 2,4-dichlorophenoxyacetic acid (2,4-D) to enhance callusing of tissue (6). Cultivar DuPuits alfalfa seeds were surface-sterilized by soaking for 4 min in 70% ethyl alcohol, followed by a 5-min soak in commercial bleach (5.2% NaOCl) and 2 rinses with sterile water. The seeds were germinated on water agar for 2-3 days in the dark at room temperature, and 3 to 4 germinated seedlings were placed in each culture tube and allowed to callus for 2 to 3 weeks. The nematodes were subcultured by transferring small pieces of callus tissue from older tubes. After 75 to 90 days, nematodes were extracted from both the callus and the medium, using the Baermann funnel method.

Inoculum density of the nematodes in soil was based on nematodes per kilogram soil (30% saturation). Nematodes were added to the soil by first making holes in the soil around the plant roots with a glass rod, then pipetting the desired number of nematodes.

After the inoculum density for a given infection incidence by *V. albo-atrum* was determined, the effect of the root lesion nematode, *Pratylenchus penetrans*, on infection levels could be evaluated. Two tests were conducted: the first consisted of inoculum densities of *V. albo-atrum* of 0, 10, 25, 75, and 200 microsclerotia/g alone and in combination with 5,000 nematodes/kg soil. The second trial consisted of inoculum densities of 0, 10, 25, and 75 microsclerotia/g alone and in combination with 10,000 nematodes/kg soil. The nematode population was increased to 20,000 nematodes/kg soil at one inoculum density (75 microsclerotia/g). Every treatment consisted of five pots, each containing 1 kg of soil and three plants.

The effect of *V. albo-atrum* at various inoculum densities on reproduction of *P. penetrans* in tomato roots was determined. *Verticillium albo-atrum*, at 0, 75, and 200 microsclerotia/g soil, and 10,000 nematodes were added to each of the five pots containing 1 kg of soil and three plants in each treatment. After 6 weeks, the nematodes in soil were recovered by washing and screening through 100- and 200-mesh screens. The tomato roots from each pot were aerated in water for 1 week to recover the nematodes. The roots were then air-dried and weighed. As expected, recovery of *P. penetrans* from the soil was very low, so the final population of *P. penetrans* was based upon the number of nematodes recovered from the tomato roots.

The influence of *P. penetrans* on the incidence of infection of tomato seedlings by *V. albo-atrum* at controlled inoculum densities when these two organisms were in isolation of each other was considered using a split-root technique. The taproot and the basal stem section of tomato seedlings (6 to 8 inches in height) were split lengthwise, and pieces of aluminum foil were placed between the two root sections to prevent callus reunion. These plants were placed in moist, sterile vermiculite for 48 hr to allow healing of the wounded surfaces.

Double plastic pots were used, and half the split-plant root system was placed into each half of the double pot. Care was taken to prevent contamination with soil between the two sections of the double pot. The split-root treatments were P/V, VP/O, V/O, V/V, P/O, O/O; where V = *V. albo-atrum*, P = *P. penetrans*, and O = noninoculated soil. The inoculum densities of *V. albo-atrum* were 25, 75, and 200 microsclerotia/g soil. Nematodes were added at a rate equivalent to 10,000/kg soil, and each double pot contained one plant with 10 replications/treatment.

A parallel experiment was conducted under similar conditions in which the root systems were not split and both organisms were combined in the same pot for comparison with results obtained from the split-root test. The inoculum densities of *V. albo-atrum* and *P. penetrans* were the same as in the split-root test with five replications of treatments VP, V, P, and O.

RESULTS.—The controlled inoculum densities of *Verticillium albo-atrum* were correlated with levels of infection of test plants. The per cent infection was determined by the number of plants infected compared to the total number of plants in each treatment. In these experiments, infection levels of 100% occurred at the inoculum density of 200 microsclerotia/g soil or above. The per cent infection at inoculum densities of 25, 50, 75, and 100

microsclerotia/g was 26, 46, 60, and 80%, respectively. These results, which represent an average of three experiments, proved highly reliable in further experiments to measure the effect of *P. penetrans* and other factors on the incidence of infection.

The effect of *P. penetrans* on infection levels by the wilt fungus at controlled inoculum densities was determined. In these experiments, both the inoculum density of the fungus and the population of the nematode were varied to assess the effect of both variables on the infection level of the tomato indicator host.

The data presented in Table 1, Experiment A, clearly show the consistent increases in infection of tomato seedlings by *V. albo-atrum* at all inoculum densities in the presence of the nematode when compared to infection incidence with the fungus alone. In these experiments, the nematode population was constant (5,000/kg soil), and inoculum densities of *V. albo-atrum* were varied. The effect of the nematode on infection incidence was masked at 200 microsclerotia/g when infection was 100% in the presence or absence of the nematode.

When the nematode population was increased to 10,000 nematodes/kg soil (Table 1, Experiment B), there was a further increase in infection levels at all inoculum densities of *V. albo-atrum* as compared to populations of 5,000 nematodes/kg soil. Increases in infection levels varied from 19% (10 microsclerotia/g) to 34% (75 microsclerotia/g) which were more than double the infection increases that occurred with 5,000 nematodes/kg soil. When the population of *P. penetrans* was increased to 20,000/kg soil at the inoculum density of 75 microsclerotia/g soil, there was a further increase in infection incidence.

The effect of infection of tomato plants by *V. albo-atrum* on final root populations of *P. penetrans* was studied at inoculum densities of 75 and 200 microsclerotia/g soil. In previous tests, at the inoculum density of 75 microsclerotia/g the infection level of tomato seedlings was 59% in the absence of the nematode, and 93% in the presence of 10,000 nematodes/kg soil. At 200 microsclerotia/g, the infection level was 100% in the presence or absence of the nematode. The initial population of *P. penetrans* in this test was 10,000/kg soil at each inoculum density of *V. albo-atrum* and the noninoculated control. There were three plants in each replication of each treatment.

The average number of nematodes recovered from the roots of tomato seedlings at inoculum densities of *V. albo-atrum* of 75 and 200 microsclerotia/g was 1,350 and 1,050, respectively, compared to 2,750 nematodes recovered from the roots in the absence of the fungus (Table 2). This decrease was statistically significant at the 1% level.

The decrease in the number of nematodes recovered from the tomato roots at the two inoculum densities of *V. albo-atrum*, when compared to the treatment with the nematodes alone, was not due to a reduction in root mass. The results (Table 2) show that the average root weight of plants grown at the inoculum density of 75 microsclerotia/g soil was slightly higher than the noninoculated plants, but the nemas per gram of root recovered was significantly lower.

Statistical analyses of the results presented in Table 2 show that the decrease in average root weight in treatment 3 (200 microsclerotia/g soil) was significant at the 1% level when compared to treatments 1 and 2. Infection by *V. albo-atrum* was 100% at 200 microsclerotia/g soil in the presence or absence of the nematode, and there was an obvious decrease in root development at the higher infection level. The decreases in the average number of nematodes recovered per gram of root in treatments 2 and 3, compared to treatment 1, were also statistically significant at the 1% level.

The influence of *P. penetrans* on infection by *V. albo-atrum* at controlled inoculum densities when these two organisms were separated from each other but on the same plant was determined, using a split-root technique. For comparison, a parallel experiment was conducted in which both organisms were combined on the whole root system. The initial nematode population was 10,000/kg soil, and inoculum densities of *V. albo-atrum* were 25, 75, and 200 microsclerotia/g soil. The results are presented in Table 3.

With the split-root technique, the infection level of *V. albo-atrum* at the inoculum density of 25 microsclerotia/g soil was slightly higher (50%) when the fungus and nematode were combined on the same side of the split-root system (VP/O), compared to

TABLE 1. Influence of soil inoculum density of *Verticillium albo-atrum* alone and in combination with the lesion nematode *Pratylenchus penetrans* on infection of tomato cultivar Bonny Best

	Experiment A		Experiment B		
Inoculum ms/g[a]	Fungus alone	Fungus + 5,000 nemas/kg soil	Fungus alone	Fungus + 10,000 nemas/kg soil	Fungus + 20,000 nemas/kg soil
0	0	0	0	0	0
10	19[b]	36	31	50	
25	36	44	45	78	
75	38	53	59	93	100
200	100	100			

[a] ms/g = microsclerotia per g soil (30% saturation).
[b] Infection per cent, based on total number of plants infected out of the 15 plants used for each treatment.

TABLE 2. The effect of *Verticillium albo-atrum* on populations of the lesion nematode *Pratylenchus penetrans* in tomato cultivar Bonny Best roots

Inoculum			Nemas			
Treatment	ms/g[a]	Replications[b]	Nemas/kg soil	Avg/replications	Root wt (g)	Nemas/g root
1	0	10	10,000	2,750	5.6	583.0
2	75	5	10,000	1,350[c]	5.7	263.8
3	200	5	10,000	1,050	3.2	320.0

[a] ms/g = microsclerotia per g soil (30% saturation).
[b] Replications = three plants/pot.
[c] Any two means not connected by vertical lines are significantly different at the 1% level.

TABLE 3. Influence of *Pratylenchus penetrans* on infection of tomato cultivar Bonny Best by *Verticillium albo-atrum* as determined by comparing a split-root to a whole root system

	Inoculum densities, % infection		
Treatment	25 ms/g[c]	75 ms/g	200 ms/g
V/P[a,b]	33	60	90
VP/O	50	70	100
V/O	25	40	80
P/O	0	0	0
V/V	40	50	100
O/O	0	0	0
VP	60	80	100
V	40	60	100
P	0	0	0
O	0	0	0

[a] P = *Pratylenchus penetrans* at 10,000/kg soil equivalent. V = *Verticillium albo-atrum*. O = Noninoculated.
[b] Letters separated by a slash (/) are split-root treatments.
[c] ms/g = Microsclerotia per gram soil (30% saturation).

infection levels (33%) when the fungus and nematode were on opposite sides of the split-root (P/V). Both treatments increased the infection level over the fungus alone (V/O) on one side of the split-root (25%).

Similar results occurred at the inoculum density of 75 microsclerotia/g soil, with a slight increase in infection when the fungus and the nematode were combined on the same side of the split-root system (VP/O) as compared to infection when they were on opposite sides of the split-root (V/P). Infection in both treatments was higher than with the fungus alone on one side of the split-root (V/O).

When results of the whole root system at inoculum densities of 25 microsclerotia/g and 75 microsclerotia/g soil are compared to those from the split-root system, it is apparent that the infection levels with the fungus-nematode combination (VP) are comparable on the whole root and on one side of the split-root system (VP/O), and that when the fungus and the nematode are separated (V/O), the infection level is somewhat lower. Both treatments show an increase in infection when compared to treatments with the fungus alone (V/O and V).

From previous experiments, it was assumed that with 200 microsclerotia/g soil, infection levels of 100% would occur, regardless of treatments. When the whole root system was inoculated, the infection level was 100% in the presence or absence of the nematode. However, with the split-root system, the 100% infection level was recorded only when the fungus and nematode were combined on the same side (VP/O) of the split-root system. When the fungus and nematode were separated (V/P), infection was reduced somewhat (90%), and with the fungus alone (V/O), infection was reduced to 80%.

DISCUSSION.–These results are in agreement with those of other investigators who demonstrated that root lesion nematodes, *Pratylenchus* sp., increase infection incidence by *V. albo-atrum* at given inoculum densities. Infection by *V. albo-atrum* also increased with increases in the nematode population. Control of inoculum density of the fungus was important in the assessment of the effect of the nematode at different population levels.

Our results differed from those of Faulkner & Skotland (3), who found that populations of *P. minyus* increased in root tissues of peppermint also infected by *V. dahliae* as compared to infection by the nematode alone. In our studies, populations of *P. penetrans* decreased when tomato seedlings were also infected by *V. albo-atrum*, with highly significant differences (1% level) when compared either on a total root or root weight basis. Likewise, the suggestion that the nematode may increase susceptibility of the indicator host species to infection by *V. albo-atrum* when the two organisms were isolated on different parts of the same root system could not be fully substantiated. When the effect of the nematode on infection incidence by the fungus was considered on a true split-root system, there was only a slight increase in infection incidence when the fungus and nematode were separated (V/P), as compared to the fungus alone (V/O) on one side of the split-root system. However, infection was less than the infection incidence when the fungus-nematode were on the same side of the split-root (PV/O), or the fungus alone on both sides of the split-root (V/V). Thus, the effect of the nematode in this fungus-nematode complex appears to be limited to providing more favorable infection courts or other similar changes in the root system which favored colonization by the fungus. There was no evidence of a change in the susceptibility of the host plant induced by the nematode when the fungus

and nematode were isolated on different parts of the same root system.

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