Interactions of the Root Knot Nematode Meloidogyne incognita and the Stubby Root Nematode Trichodorus christiei with Verticillium albo-atrum on Tomato at Controlled Inoculum Densities

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ABSTRACT

The effect of the root knot nematode *Meloidogyne incognita* and the stubby root nematode *Trichodorus christiei* on the incidence of infection of tomato cultivar Bonny Best by *Verticillium albo-atrum* was determined. Effects of the fungus + nematode interaction on nematode reproduction and on host root development were also studied. Environmental conditions and the inoculum densities of both the nematodes and the fungus were controlled. A change in the infection incidence by the fungus was the criterion of interaction. The infection incidence increased at most fungal inoculum densities when *T. christiei* was also present, however, no consistent increases occurred with *M. incognita*. The infection incidence by *V. albo-atrum* also increased as the inoculation density of *T. christiei* was increased. Infection by the fungus did not enhance reproduction of either nematode in or on the host roots; however, an increase in root wt occurred when both the fungus and *T. christiei* were present.

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Increases in infection of *Verticillium albo-atrum* Reinke & Berth. (microsclerotial form) and an increase in severity of symptom development in the presence of plant parasitic nematodes have been demonstrated with a number of different hosts (3, 17, 18, 19). The nematodes most commonly involved are species of the migratory endoparasitic genus *Pratylenchus,* especially *P. penetrans* (4, 13, 14) and *P. minyus* (6, 7). Increases in nematode reproduction in plants infected by *V. albo-atrum* have also been reported (6, 7), however, such effects were not observed by Bergeson (2) and Conroy et al. (4).

There is less agreement on the interaction of *V. albo-atrum* and nematode species of the sedentary endoparasitic genus *Meloidogyne.* In field studies, both a decrease in Verticillium wilt (16) and no change in disease incidence (12) were reported after soil fumigation with nematicidal dosages of ethylene dibromide (EDB). In greenhouse studies, Overman et al. (16) found the highest incidence and severity of Verticillium wilt of tomato when the fungus and root knot nematode *Meloidogyne* sp. were combined at 29 C. Later, they found no evidence of fungus-nematode interaction at soil temp of 22 C and 25 C (10). Khoury and Alcorn (11) reported an increase in infection and disease severity on cotton infected with both *V. albo-atrum* and *M. incognita acrita.*

Interactions of *V. albo-atrum* and species of ectoparasitic nematodes are less well known. Overman and Jones (15) found increases in both disease incidence and severity on tomato with *V. albo-atrum* and the stunt nematode, *Tylenchorhynchus capitatus.*

In consideration of factors which influence infection incidence by the fungus, the mode of nematode parasitism may be important because it influences changes in both root physiology and morphology. Also, infection incidence by *V. albo-atrum* and severity of host symptom development are apparently under different parameters of inoculum density (4), and infection incidence by the fungus can be more accurately quantified than symptoms (4).

The present study considered possible interactions of *V. albo-atrum* and the sedentary endoparasitic nematode *Meloidogyne incognita* and the ectoparasitic nematode *Trichodorus christiei.* Environmental conditions and inoculum densities of both the fungus and nematodes were controlled. Changes in infection incidence by the fungus was the criterion of nematode-fungus interaction. The effect of the infection complex on final populations of the nematodes and changes in root morphology were also determined.

MATERIALS AND METHODS.—The experimental procedures used involving host, isolate of the fungus pathogen, preparation of the fungus inoculum, soil assay methods, and environmental conditions were identical to those described by Conroy et al. (4) and Green (9). Fungal inoculum in the soil was microsclerotia (ms) of *V. albo-atrum* and actual inoculum densities did not vary more than ±5% from the indicated levels.

The inoculum density of nematodes in soil was based on nemas/kg soil (30% saturated) and the nematodes were added to the soil by pipetting the predetermined number of larvae into holes in the soil around the base of the test plant. The root knot nematode *M. incognita* was increased on roots of tomato (cultivar Bonny Best) for 6 wk at 30 C. The galled roots were then washed free of soil, placed in a mist chamber (8), and the emerging larvae were collected after 3 days. Inoculum was prepared by serial dilution of this suspension before adding larvae to the soil.

TABLE 1. Infection percentage of tomato cultivar Bonny Best by *Verticillium albo-atrum* alone and in combination with the root knot nematode *Meloidogyne incognita* at controlled inoculum densities

Verticillium albo-atrum		*V. albo-atrum* + *Meloidogyne incognita*		
Inoculum density[a]	Infection (%)	Inoculum densities of *M. incognita*		
		5000[b]	10,000	15,000
0	0	0	0	0
25	26.6	33.3	26.6	33.3
50	40.0	53.3	46.6	53.3
100	73.3	66.6	66.6	80.0
200	100.0	100.0	100.0	100.0

[a]Microsclerotia/g soil.
[b]Nemas/kg soil.

TABLE 2. The effect of infection by *Verticillium albo-atrum* on final root population, root weight, and root galling by root knot nematode *Meloidogyne incognita* of tomato cultivar Bonny Best

Fungus ms/g[a]	Nemas/kg soil	Nemas recovered/g root	Root wt/g	Root galling
0	0	0	2.32[b]	0[c]
0	10,000	157,573[d]	2.54	5.0
50	10,000	80,961	1.74	5.0
100	10,000	62,820	1.72	3.4
200	10,000	100,695	2.21	4.8

[a]Ms/g = microsclerotia/g soil.
[b]Mean root wt/replication (air-dry). No statistictical difference was observed between treatments.
[c]Mean root gall rating scale 0-6; 0 = no galling, 6 = severe galling. No statistical difference between treatments.
[d]Mean nemas recovered/g root. No statistical difference was observed between treatments.

The stubby root nematode *T. christiei* was increased on roots of dent corn seedlings grown for 6 wk at 30 C. The seedlings were lifted, the soil sifted and thoroughly mixed and then soil samples taken to determine soil populations of the nematode using the Baermann funnel technique (20). After determining the concn of larvae/g soil, this stock was used to establish inoculum densities of the nematode by appropriate dilution with noninfested soil.

Tomato seedlings were transplanted (three plants/pot) to containers with one kg soil infested with *V. albo-atrum* at inoculum densities of 0, 25, 50, 100, and 200 microsclerotia (ms)/g soil (30% saturated). After 3 to 5 days, root knot nematodes were added at rates of 0, 5,000, 10,000, and 15,000 larvae/kg soil. All treatments were replicated five times and maintained for 6 wk in growth chambers at a soil temp of 28 C ± 0.5 C. At the conclusion of the test, the lower stem of each plant was excised and stem sections were plated on Czapek's agar + 50 ppm each of streptomycin sulfate and aureomycin.

The effect of infection by the fungal pathogen on root colonization by *M. incognita,* and changes in final root populations in the presence and absence of the fungus, was also determined. A similar series of treatments as described above were used with fungal inoculum at 0, 50, 100, and 200 ms/g soil and nematode populations of 0 and 10,000 larvae/kg soil. Each treatment was replicated five times and all treatments incubated in controlled-climate chambers under the same conditions described previously. After 6 wk, the tomato roots were washed and nematode larvae recovered using the mist chamber (8). Galling of roots was rated on a scale of 0 to 6 (6 = severe galling) and, after rating, the roots were air-dried and weighed.

A similar series of experiments were conducted with the ectoparasitic nematode *Trichodorus christiei.* Soil fungal inoculum was 0, 25, 50, 100, and 200 ms/g soil (30% saturated) and the nematodes were added at rates of 0, 3,000, 5,000, and 10,000 larvae/kg soil. Each treatment was replicated five times and all treated plants incubated in controlled-climate chambers as described previously. After 6 wk, the infection incidence by *V. albo-atrum* was determined. The influence of fungus infection on final nematode populations and the influence of the fungus, nematode, and the fungus-nematode complex on root morphology and surface area was also determined. In the second series of tests, the fungus inoculum was 0, 50, 100, and 200 ms/g soil and the initial nematode populations were 0 and 10,000 larvae/kg soil. After 6 wk, the final nematode populations were determined and root wt and root surface area calculated. The root surface area was determined by a root titration technique (1).

TABLE 3. Infection percentage of tomato cultivar Bonny Best by *Verticillium albo-atrum* alone and in combination with the stubby root nematode *Trichodorus christiei* at controlled inoculum densities

Verticillium albo-atrum		*V. albo-atrum* + *T. christiei*		
Inoculum	Infection	Inoculum densities of *T. christiei*		
density[a]	(%)	5,000[b]	10,000	15,000
0	0	0	0	0
25	26.6	33.3	33.3	40.0
50	40.0	60.0	53.3	73.3
100	73.3	86.6	93.3	100.0
200	100.0	100.0	100.0	100.0

[a]Microsclerotia/g soil.
[b]Nematodes/kg soil.

TABLE 4. The effect of infection by *Verticillium albo-atrum* on final soil population of stubby root nematode, *Trichodorus christiei,* and weight and surface area of roots of tomato cultivar Bonny Best

Treat-	ms/g	Nemas/kg soil		Root wt.	Root surface
ment	fungus[a]	Start	Recovered	g/rep	area/rep
1	0	0	0	1.53[b c]	43.8[d]
2	0	10,000	29,416	2.43	32.1
3	50	10,000	40,271	2.56	32.6
4	100	10,000	31,285	2.90	32.8
5	200	10,000	36,685	2.86	38.0

[a]Ms/g = microsclerotia/g soil.
[b]Mean root wt, g/replicate (three plants) air-dried.
[c]Increases in root weights in treatments 3, 4, and 5 are statistically significant ($P = 0.05$) from treatment 1; difference between Treatments 1 and 2 is not statistically significant.
[d]Expressed as ml of titrated base. No significant differences between treatments.

RESULTS.—There was little evidence of nematode-induced changes in the infection incidence of tomato by *V. albo-atrum* in treatments with *M. incognita* + fungus compared to treatments with the fungus alone (Table 1). At the fungal inoculum of 50 ms/g soil, infection was higher in all treatments with the nematode + fungus than with the fungus alone, but this is not considered significant because no increase occurred in any other treatment where the nematode and fungus were included. The fungus infection incidence was 100% when the inoculum density was increased to 200 ms/g soil, regardless of other treatments. Also, under the conditions of these experiments, test plants did not consistently exhibit symptoms despite infection levels of from 27% to 100%.

Final populations of *M. incognita* were lower by from 30% to more than 50% in treatments with the nematode + fungus compared to the nematode alone (Table 2). In addition, mean root weights (air-dried) and root galling were reduced in the nematode + fungus treatments compared to the nematode alone. However, none of these differences was statistically significant.

Trichodorus christiei increased the infection incidence by *V. albo-atrum* in all treatments compared to the fungus alone (Table 3). The infection increases were greatest at fungal inoculum densities of 50 and 100 ms/g soil + 10,000 nemas/kg soil, but increases occurred with all nematode + fungus treatments compared to the fungus alone. Final populations of *T. christiei* in soil were similar in treatments with the nematode alone compared to the nematode + fungus (Table 4). Root weights (air-dried) were significantly higher in the nematode + fungus treatments than with the fungus alone or the control, but no increase in root surface area was measured. In all treatments which included the nematode, visual comparisons indicated that roots were much thickened and produced few fine, lateral secondary roots.

DISCUSSION.—These results agree with earlier reports that infection incidence by *V. albo-atrum* is increased in the presence of certain nematodes (2, 4, 7, 13, 15). Data reported here and elsewhere (4) indicate that nematodes which cause damage on or at the root surface, as do the ectoparasitic and endomigratory forms, increase infection incidence when the inoculum density of the fungus is limiting. By contrast, the sedentary endoparasites, which cause little disruption of root surfaces during penetration, appear to have little effect on the infection incidence under these conditions. This appears to be true despite the fact that the ultimate effect on the host root morphology and physiology may be much greater with this latter group.

The results reported here and earlier (4) do not suggest any direct influence of infection by the fungus on nematode reproduction in or on the host roots. There was considerable variation in final nematode populations between treatments with *M. incognita* + fungus (Table 2), with a strong indication that nematode reproduction was reduced in plant roots also infected by *V. albo-atrum.*

However, these results were not significant. The final populations of *T. christiei* in soil were very consistent, regardless of treatment (Table 4). The fact that the soil was thoroughly mixed before samples were taken may have been a factor in these results, however.

The significant increases in root wt without an increase in root surface area in treatments with *T. christiei* + fungus is unexplained. The alterations in root morphology noted occurred in all treatments with the nematode, but the significant changes in root wt occurred only in treatments with the nematode + fungus.

Root leakage and/or increases in root exudates caused by nematodes may explain the observed differences in infection incidence by the fungus, since germination of microsclerotia of *V. albo-atrum* in soil is nutrient-dependent (5). Under controlled inoculum density of both the fungus and nematode and controlled environmental conditions, the implications are that as root leakage and/or exudations increase into the rhizosphere following introduction of the ectoparasitic or endomigratory nematodes, germination of microsclerotia is increased and thus infection incidence inereases. Conversely, with the sedentary endoparasitic nematode, such as root knot nematode, there is apparently little change in the nutrient state in the rhizosphere during nematode penetration, and thus no change in infection incidence by the fungus.

Our results with the interactions of *V. albo-atrum* and *M. incognita* differ from those of Khoury and Alcorn (11), who found an increase in both infection incidence and symptom severity in concomitant infection of cotton seedlings by this fungus and the root knot nematode species *M. incognita acrita*. These differences may be the result of different hosts, but may also be due to different experimental conditions, especially fungal inoculum. In our studies, the inoculum was limited to microsclerotia of uniform size, uniformly distributed in the soil, and at precisely controlled inoculum densities. By contrast, most other investigators have used inoculum consisting of mixtures of mycelium, spores, and microsclerotia added to soil after the test plants are established and quantified only as a proportion of a predetermined volume. Green (9) showed that the inoculum potential of mycelium/spore mixtures from *V. albo-atrum* is variable and this inoculum is ephemeral in soil. We also demonstrated earlier (4) that infection incidence and symptom development and severity are under different parameters of fungus inoculum density. These differences in experimental procedures may explain differences in results.

Our results also suggest that, in all cases of the fungus-nematode interactions, careful consideration should be given to whether a synergistic effect occurs, or whether observed differences are additive effects of the two pathogens. If, in fact, the fungal infection incidence is 100% at inoculum densities well below that where differences in symptom development or severity can be accurately measured, then the observed differences in symptoms may be an additive effect of the two pathogens rather than a synergistic effect.

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