The Influence of Pratylenchus penetrans on the Incidence and Severity of Verticillium Wilt of Potato[1]

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Abstract: The influence of *Pratylenchus penetrans* on the incidence and severity of Verticillium wilt was examined in the potato cultivars 'Kennebec', 'Katahdin', and 'Abnaki'. Single-stem plants were grown in soil maintained at a temperature of 22 ± 1 C. Axenically cultured nematodes were suspended in water and introduced to the soil, at a rate of ca 5,000/25.4-cm pot, through holes made around each stem. Ten days after infestation with nematodes, conidial suspensions of *Verticillium albo-atrum* were introduced into the soil at a rate of ca 1,000,000/pot. Among Katahdin plants, the severity of foliar symptoms was increased in the presence of both pathogens 2 and 3 weeks after soil infestation. During the remaining 5 weeks, severity of foliar symptoms was not different between plants infected by both pathogens and those infected by *Verticillium* alone. Within the wilt-susceptible cultivar Kennebec and the resistant cultivar Abnaki, no effects on foliar symptom severity were observed. When plant heights, shoot weights, and tuber yields were analyzed, a *Pratylenchus-Verticillium* interaction was not evident within any of the cultivars tested. Nematode populations in roots and rhizosphere were suppressed in Kennebec and Katahdin plants in the presence of *Verticillium*. *Key Words:* Lesion nematode, nematode-fungus interaction, *Solanum tuberosum, Verticillium albo-atrum.*

Verticillium albo-atrum Reinke and Berthold may interact with other soil-borne microflora or fauna, including plant-parasitic nematodes. Associations between nematodes and *Verticillium* spp. in relation to the etiology of plant disease are well established (17, 18). Disease complexes involving the interactions of *Verticillium* spp. and *Pratylenchus* spp. have been reported on a number of hosts, including potato (12), eggplant (14), tomato (5), peppermint (7), and strawberry (1). The infestation of soil with both pathogens may result in a higher incidence of symptom expression than when either pathogen is present alone (2, 5, 11, 15). In addition, *Verticillium* infection may facilitate nematode population increases within plant roots (2, 11, 14).

Reports of a lower incidence of Verticillium wilt in potato fields treated with nematicides have provided supportive evidence for the existence of a *Pratylenchus-Verticillium* interaction (3, 12). In a greenhouse experiment, Morsink (13) recorded lower tuber yields from plants infected with *V. albo-atrum* and *P. penetrans* in comparison with yields from plants infected with either pathogen alone. However, nematode infection had no effect on foliar wilt symptoms and the number of nematodes extracted from roots was not affected by the presence or absence of the fungus.

This report describes the effects of *P. penetrans* on the incidence and severity of Verticillium wilt in three cultivars of potato under controlled environmental conditions. Emphasis was placed on the determination of wilt severity, tuber yield, and nematode population densities.

MATERIALS AND METHODS

In order to obtain measurements of plant growth and foliar symptom development, potatoes (*Solanum tuberosum* L. 'Katahdin', 'Kennebec', and 'Abnaki') were maintained as single-stemmed plants. These were produced by removing 3-cm plugs of individual axillary buds from tubers and planting them singly in 25.4-cm plastic-lined metal containers. The planting medium consisted of one part Hagerstown silt loam, one part peat, and one part perlite supplemented with 2.4 gm superphosphate, 1.6 gm dolomitic limestone, 0.8 gm magnesium sulfate, 1.2 gm gypsum, and 0.4 gm potassium nitrate/liter of medium. After 3 weeks of growth, 32 potted plants of each cultivar were selected for uniformity and placed in Esco temperature tanks in a randomized, complete block design. A soil temperature of 22 ± 1 C was maintained. The average greenhouse temperature was 31.8 C during the day and 26.3 C at night.

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The experiment was conducted during the months of June and July, and no artificial lighting was used.

Pratylenchus penetrans was cultured on callus tissue under axenic conditions (19). Alfalfa seeds (*Medicago sativa* L. 'DuPuit') were soaked in a water bath at 61 C for 10 min, surface sterilized in 0.5% Na hypochlorite for 15 min, and placed on potato-dextrose-agar (PDA) in sterile petri dishes. After 4 days, the seedlings were transferred to slants of modified White's medium (9). After a significant amount of callus had formed, portions of previously existing cultures of *P. penetrans* were transferred to the slants. The cultures were maintained in the dark at 25 C. Portions of the cultures were periodically transferred to fresh callus tissue. After ca 90 days of incubation, a suspension of *P. penetrans* was obtained by incubating the callus tissue and medium in Baermann funnels from 6 to 9 days at 22 C. The suspension was adjusted to a concentration of 1 x 10^3 nematodes/ml. Nematodes were introduced into the soil by making two holes with a glass rod, ca 4 cm from each stem, and adding 5 ml of suspension.

The fungal inoculum was prepared as a singled-spored isolate of *V. albo-atrum* (the dark mycelial form) cultured on PDA slants in the dark at 25 C. After 21 to 28 days, the slants were flooded with 10 ml of sterile distilled water and agitated. The resulting suspension was passed through a double layer of cheesecloth to remove mycelial fragments and adjusted to a concentration of 1 x 10^5 conidia/ml. Ten ml of the conidial suspension were added to the soil 10 days after nematode inoculations by means of the technique previously described.

At weekly intervals after Verticillium inoculations, plant heights were measured. The number of petioles formed, and the number of necrotic and abscised petioles were recorded.

A symptom and senility index, modified from McClean and Walker (10) by Isaac and Harrison (9), was used to distinguish between natural senescence and foliar symptoms resulting from *Verticillium* infection. At weekly intervals, stems were measured and divided into four sections of equal lengths. Ratings of 1-4 were assigned to each plant if foliar chlorosis or necrosis was observed in the basal, second, third, or apical quarters, respectively.

Seven weeks after *Verticillium* infestation, the plants were harvested. The stems and leaves were dried at 90 C for 24 h and weighed. Tuber yields and weights were recorded. Nematodes were extracted from root and soil samples by incubation.

Isolation of *V. albo-atrum* was attempted by surface sterilizing lower stem and tuber sections with 0.5% sodium hypochlorite and placing them on straw agar containing 50 μg/ml streptomycin sulfate.

Data were analyzed by using analysis of variance and means were separated by use of Duncan's modified (Bayesian) least significant difference test (6).

RESULTS

Two weeks after the infestation of soil with *V. albo-atrum,* incipient wilting and chlorosis of the basal leaves was evident within the cultivars Kennebec and Katahdin. Foliar symptoms were not observed among similarly treated Abnaki plants.

At the end of the second and third weeks, the foliar symptoms of Katahdin potatoes, growing in soil infested with *V. albo-atrum* and *P. penetrans,* were more advanced than those observed among plants in association with either pathogen alone (Table 1). However, during subsequent weeks, no differences in foliar wilt severity were recorded among *Verticillium*-infected plants within the cultivars tested.

Three weeks after infestation, the stunting of 'Katahdin' and 'Kennebec' plants subjected to *Verticillium* inoculum was reflected by height (Fig. 1). Within the cultivar Abnaki, treatment differences were not significant.

In relation to the number of petioles formed, there were no significant differences between treatments until the seventh week after *Verticillium* infestation. At that time, *Verticillium*-treated plants, within the cultivars Katahdin and Kennebec, had fewer new petioles than plants treated with *P. penetrans* alone or nontreated controls. Nematode infection had no effect upon the number of petioles formed.

Within the cultivars Katahdin and Kennebec, fungal infection resulted in

TABLE 1. Foliar response of potatoes to infestation of soil with *Verticillium albo-atrum* and *Pratylenchus penetrans*.

Treatment	Cultivar	Mean symptom and senility indices 2nd week[z]	3rd week[z]
P. penetrans	Katahdin	1.0 a	1.1 a
	Kennebec	1.0 a	1.1 a
	Abnaki	1.0 a	1.0 a
V. albo-atrum	Katahdin	1.1 a	1.3 a
	Kennebec	1.8 b	2.1 b
	Abnaki	1.0 a	1.0 a
P. penetrans + *V. albo-atrum*	Katahdin	2.0 b	2.2 b
	Kennebec	1.8 b	2.1 b
	Abnaki	1.0 a	1.0 a
Control	Katahdin	1.0 a	1.1 a
	Kennebec	1.1 a	1.5 a
	Abnaki	1.0 a	1.0 a

[z]Within a column, indices followed by the same letter do not differ significantly from each other according to Duncan's Bayesian LSD test ($P = 0.05$).

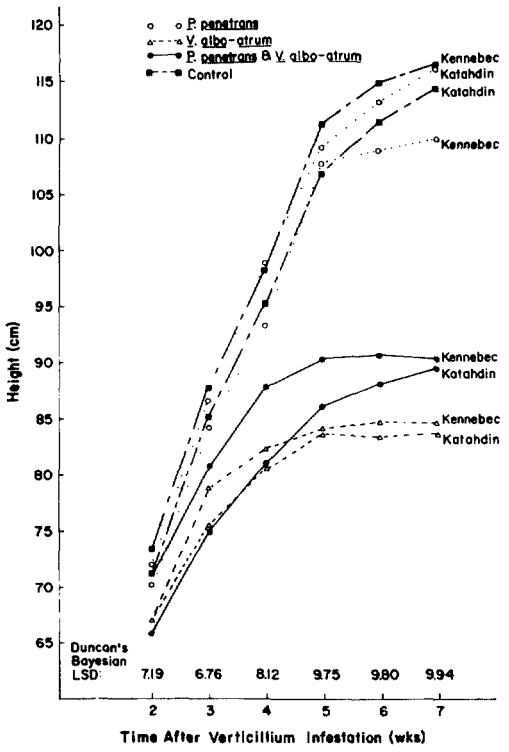

FIG. 1. Height of Kennebec and Katahdin plants grown in soil infested with *Pratylenchus penetrans* and *Verticillium albo-atrum*.

suppression of root and shoot growth (Table 2). In the presence of both pathogens, Kennebec and Katahdin plants exhibited a greater mean shoot weight than plants subjected only to fungal inoculum. The numbers of nematodes in roots and surrounding soil were lowest in the presence of *V. albo-atrum*. No differences were observed within the cultivar Abnaki. Nematodes alone had no effect on root or shoot weights within the cultivars tested.

Mean tuber numbers and weights of Katahdin and Kennebec plants grown in *Verticillium*-infested soil were lower than those from plants grown in soil infested with *P. penetrans alone* (Table 3). Fungal infection did not influence tuber numbers or weights within the cultivar Abnaki. The presence of *P. penetrans,* alone and in combination with *V. albo-atrum,* had no effect on tuber numbers. Vascular browning was observed only within stems of Katahdin. *V. albo-atrum* could not be isolated from thin sections taken from the stem end of tubers.

DISCUSSION

During the second and third weeks of the experiment, the increased incidence and severity of foliar symptoms observed in Katahdin potatoes infected with both pathogens was indicative of a *Pratylenchus-Verticillium* interaction. However, in relation to other parameters, i.e., shoot weight, plant height, tuber numbers, and weight, nematode infection had no effect on wilt severity. Morsink (12) obtained similar results except he found infection by both pathogens to cause greater growth suppression of tubers in comparison with growth suppression in plants infected with the fungus alone. However, his results were not reproducible when microsclerotia were used as fungus inoculum and the fungus and nematode inoculum levels were varied.

Although high numbers of nematodes were isolated from potato roots, pathogenesis was not evident. In fact, root infection by *P. penetrans* resulted in a slight stimulation of plant growth. Faulkner and Skotland (8) found that low levels of infection by *P. minyus* enhanced the growth of peppermint plants. Although unexplained, similar host reactions have been

TABLE 2. Mean fresh shoot and root weights and nematode yields from potato plants grown in soil infested with *Verticillium albo-atrum* and *Pratylenchus penetrans*.[x]

Treatment	Cultivar	Shoot wt. (gm)[y]	Root wt. (gm)[z]	Nematodes/gm soil	Nematodes/gm root
P. penetrans	Katahdin	5.3 a	5.5 a	25 a	261 a
	Kennebec	5.1 a	5.3 a	24 a	237 a
	Abnaki	5.0 ab	4.7 a	23 a	185 a
V. albo-atrum	Katahdin	2.6 d	1.9 b		
	Kennebec	2.8 d	1.4 b		
	Abnaki	4.4 bc	4.4 a		
P. penetrans + *V. albo-atrum*	Katahdin	3.6 cd	1.7 b	4 b	108 b
	Kennebec	4.0 bc	2.0 b	4 b	103 b
	Abnaki	4.7 ab	5.5 a	18 a	223 a
Control	Katahdin	5.7 a	6.1 a		
	Kennebec	5.4 a	5.1 a		
	Abnaki	5.0 ab	5.6 a		

[x]Readings 7 weeks after inoculation with fungus and 8 weeks, 3 days after addition of nematode. Within a column, numbers followed by the same letter do not differ significantly from each other according to Duncan's Bayesian LSD test.
[y]Oven dry weight.
[z]Fresh weight.

reported for other nematode species (4, 16).

The data demonstrating that root infection by *V. albo-atrum* results in a decrease in nematode population densities is supported by similar reports on tomato (5) and eggplant (14). One suggestion is that root deterioration, resulting from fungal infection, produces a less favorable site for nematode reproduction. However, with *V. dahliae* and *P. penetrans* in tomato, higher numbers of nematodes occurred in the roots of plants inoculated with the fungus and nematode than in those plants in soil with nematodes alone (14). One must conclude that a general statement concerning the effect of an interacting fungus on a nematode cannot be made. Each combination of fungus nematode and host must be tested under optimum conditions.

TABLE 3. Mean tuber yields and weights from potato plants grown in soil infested with *Verticillium albo-atrum* and *Pratylenchus penetrans*.[y]

Treatment	Cultivar	Number of tubers per plant	Mean tuber wt. (gm)[z]
P. penetrans	Katahdin	1.2 a	13.9 a
	Kennebec	1.5 a	12.6 a
	Abnaki	1.8 a	12.5 a
V. albo-atrum	Katahdin	0.4 b	3.1 b
	Kennebec	0.1 b	0.3 c
	Abnaki	1.6 a	12.8 a
P. penetrans + *V. albo-atrum*	Katahdin	0.5 b	2.6 b
	Kennebec	0.1 b	0.2 c
	Abnaki	1.2 a	11.2 a
Control	Katahdin	1.5 a	15.0 a
	Kennebec	1.2 a	13.4 a
	Abnaki	1.6 a	11.5 a

[y]Reading made 7 weeks after inoculation with fungus and 8 weeks, 3 days after addition of nematode. Within a column, numbers followed by the same letter do not differ significantly from each other according to Duncan's Bayesian LSD test.
[z]Fresh weight.

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