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Effect of *Ditylenchus dipsaci* and *Pratylenchus penetrans* on Verticillium Wilt of Alfalfa

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Abstract: *Verticillium albo-atrum* wilt symptoms appeared faster and were significantly more severe in the presence of *Ditylenchus dipsaci* in Vernal, a wilt-susceptible cultivar, than in Maris Kabul, a wilt-resistant cultivar. Winter kill in the field was not affected by the nematode during the first winter, but 50% of plants were killed in the second winter. Forage yield from nematode-infected plants was significantly reduced the second year. Interaction with *V. albo-atrum* did not significantly reduce forage yields below that of *D. dipsaci* alone. *Pratylenchus penetrans* did not increase the severity of wilt symptoms in the presence of *V. albo-atrum*, nor did it affect forage yield in the greenhouse. It did, however, reduce alfalfa yields in presence of *V. albo-atrum* under field conditions. *D. dipsaci* and *P. penetrans* reproduced faster in Vernal than in Maris Kabul when the fungus was present.
Key words: stem and bulb nematode, root lesion nematode, resistance, interaction, winter survival, *Medicago sativa,* Canada, yield.

Verticillium wilt, caused by *Verticillium albo-atrum* Reinke and Berthold, has recently spread and become an important disease of alfalfa, *Medicago sativa* L., in Western Canada (2,11). The root lesion nematode, *Pratylenchus penetrans* Cobb, is widespread in alfalfa and can cause significant yield losses (15,17). The stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, was found in 1980 in many alfalfa growing areas of British Columbia (16) just before the discovery of the spread of *Verticillium albo-atrum. D. dipsaci* affects yield and increases winterkill, apparently by interfering with the cold hardening process, in alfalfa plants (4). The possible interactions of *D. dipsaci* and *Verticillium albo-atrum* on alfalfa and their effect on alfalfa yield have not been studied.

The interactions of *P. penetrans* and *Verticillium albo-atrum* have been studied in strawberry (1), peppermint (3), potato (5), tomato (7), and eggplant (13), where the nematode increased the severity of wilt symptoms and the wilt symptom increase was usually a function of the number of nematodes present. The resistance status of the host to the nematode, measured as the rate of multiplication of the nematode in the plant, changed in presence of *V. albo-atrum. P. penetrans* multiplied faster in eggplant (13) but slower in tomato (7) and potato (5) in the presence of *V. albo-atrum.*

The objective of this study was to examine the interactions of *D. dipsaci, P. penetrans,* and *V. albo-atrum* in alfalfa, including the severity of wilt symptoms in a resistant and a susceptible cultivar, and their effects on yield and overwintering.

MATERIALS AND METHODS

The verticillium wilt-resistant and susceptible cultivars of alfalfa used were Maris Kabul and Vernal, respectively. Both cultivars are susceptible to *D. dipsaci* infections, although Maris Kabul is less susceptible than Vernal (Vrain, unpubl.). The host status of these cultivars to *P. penetrans* is not known.

Nematode and fungus inocula: The *D. dipsaci* inoculum was obtained from dried infected alfalfa leaf and stem material collected from several fields in southern British Columbia. The plant material was placed in a mist chamber at 24 C, and emerging nematodes were collected every 24 hours for 3 days, stored at 6 C, and calibrated. The *P. penetrans* inoculum was from a pure culture on greenhouse grown raspberry plants. Pieces of roots were placed in a mist chamber, and nematodes were collected and stored in the same manner as the *D. dipsaci* inoculum.

The *V. albo-atrum* conidiospore inoculum was obtained from V8 juice agar cultures of several isolates of medium virulence col-

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TABLE 1. Verticillium wilt ratings on two alfalfa cultivars infected or not infected with *Ditylenchus dipsaci.*

	Verticillium wilt rating index			
	Vernal		Maris Kabul	
Days after *V. albo-atrum* inoculation	Without nematodes	With nematodes	Without nematodes	With nematodes
17	1.73 b	2.00 c	1.48 a	1.43 a
25	1.88 b	2.31 c	1.64 ab	1.61 a
31	1.99 b	2.39 c	1.70 a	1.71 a
38	2.11 a	2.77 b	1.78 a	1.83 a

Verticillium rating scale: 1 = no symptoms; 2 = one to three leaflets of one shoot wilted, chlorotic, or necrotic; 3 = most leaflets of one shoot with symptoms; 4 = more than one shoot showing symptoms; 5 = plant dead.

Numbers in rows followed by the same letter do not differ significantly from each other according to Duncan's multiple-range test ($P < 0.05$).

lected in southern British Columbia. After 14 days at 25 C, spores were collected in distilled water and calibrated.

Greenhouse experiment: Seeds of alfalfa *Medicago sativa* cv. Vernal or Maris Kabul were germinated in tap water. After 24 hours, 10 seeds per pot were planted in pasteurized greenhouse soil mix (loamy sand and peat 3:1) in 15-cm-d plastic pots; after 6 days seedlings were thinned to four per pot. A suspension of *Rhizobium meliloti* in 2 ml water was added in each seed hole. Each germinating seed of appropriate treatments was inoculated with 30 *D. dipsaci* of all stages in 0.1 ml water at planting (16). Plants were inoculated with *V. albo-atrum* 3 weeks after the nematodes were added, by cutting off two trifoliate leaves with scissors dipped in a suspension of 1.0×10^8 spores/ml.

The plants were grown in a greenhouse at 19–24 C under fluorescent tubes at 160 $\mu E\ m^{-2}\ s^{-1}$ flux and a 16-hour photoperiod. Pots were arranged in a completely randomized design with six replications of each of the eight treatments. Fertilizer and insecticides were applied uniformly as required. Symptoms of wilt were rated 17, 25, 31, and 38 days after fungal inoculations in the greenhouse, using a rating scale from 1 to 5 as follows: 1 = no symptoms; 2 = one to three leaflets of one shoot wilted, chlorotic or necrotic; 3 = most leaflets of one shoot with symptoms; 4 = more than one shoot with symptoms; 5 = plant dead. Fresh weight of stem and foliage were recorded at harvest 110 days after planting. The nematodes were extracted from 5 g of fresh leaves and stem tissue in the same manner as the inoculum and their density recorded.

A similar experiment was done with *P. penetrans* inoculation treatments. Each seed of the appropriate treatments was inoculated with 540 *Pratylenchus penetrans* suspended in 2 ml water in the seed hole. Wilt symptoms were rated with the same rating scale as in the previous experiment, but only once, 28 days after *V. albo-atrum* inoculation. The number of surviving plants and their foliage and root weight were recorded 115 days after planting. Nematodes were extracted from leaf tissue and roots and recorded in the same manner as in the previous experiment.

Field experiments: In the first field experiment (1982–83) in a sandy loam soil, germinated seeds were planted 10 cm apart, in holes 2 cm deep, on 5 May 1982. One ml of a suspension of *Rhizobium meliloti* was added on each seed. Each seed hole was filled with sand and appropriate treatments were inoculated with 30 *D. dipsaci* of all stages in 0.1 ml of water. Plots, 0.2 m^2 with 20 seeds each, were arranged in a randomized complete block design with eight replications. *Verticillium albo-atrum* was inoculated to the field seedlings in the same manner as in the greenhouse experiments. The field was hand weeded and overhead irrigated as needed during the summer. Foliage was harvested three times each year (22 July, 1 September, 12 October 1982; 12 May, 21 June, and 4 August 1983). At each harvest the number of plants per plot was recorded, foliage was cut approximately 20 cm above ground, and fresh and dry weights were taken. *P. penetrans* densities in roots and soil were determined at the end of each experiment, from 2 g of feeder roots placed in a mist chamber or from 50 cm^3 of rhizosphere soil placed in Baermann pans for 7 days at 23 C.

The following year another experiment

TABLE 2. Yields of two alfalfa cultivars inoculated with *Verticillium albo-atrum* alone or with *Pratylenchus penetrans*.

	Forage yields (g per plot)									
	Second greenhouse experiment		First field experiment				Second field experiment			
			Vernal		Maris Kabul		With *Pratylenchus*		Without *Pratylenchus*	
	Vernal	Maris Kabul	1982	1983	1982	1983	1983	1984	1983	1984
Control	16.8 a	17.8 a	602 a	1,877 b	692 a	3,239 c	678 ab	1,328 c	524 a	1,420 c
Verticillium	13.4 b	18.4 a	593 a	991 a	644 a	2,980 c	684 ab	1,280 c	482 a	906 ab

Numbers in each experiment followed by the same letter are not different according to Newman-Keuls multiple-range test ($P < 0.05$).
† $P < 0.1$ for second field experiment with Vernal only.

(1983–84) was set up in the same field with the cultivar Vernal. The plots, 1.2 m^2 in area, were seeded on 9 June 1983 with 10 seeds 20 cm apart. *D. dipsaci* and *V. albo-atrum* were inoculated in the same way as in the first field experiment. *P. penetrans* was inoculated at planting, 1,000 nematodes in each seed hole of appropriate treatments. Cultural practices and irrigation were identical as in the previous field experiment. Plants were harvested, and the number of yielding plants and fresh and dry weight of foliage were recorded on 7 September 1983 and on 8 May 1984.

For all experiments nematode data were log transformed, and plant and nematode data were analyzed by analysis of variance, with multiple-range tests to separate the treatment means.

RESULTS AND DISCUSSION

Greenhouse studies: In the first greenhouse experiment regardless of the presence of *D. dipsaci*, Vernal showed significantly more severe symptoms than Maris Kabul (Table 1) and nematode infection significantly increased the severity of wilt symptoms in Vernal but not in Maris Kabul. This increase of wilt symptoms in Vernal was also observed in the *D. dipsaci* + *V. albo-atrum*-infected plants in the second greenhouse experiment. The way the nematode increases the severity of wilt symptoms is not known. We know that it does not carry *Verticillium* conidiospores on its cuticle (Vrain, unpubl.) as it does with the bacterial wilt agent (10).

In the second greenhouse experiment, *P. penetrans* did not increase the appearance of wilt symptoms nor did it affect forage yield. *Verticillium albo-atrum* significantly ($P < 0.05$) suppressed the yield of Vernal plants in the second greenhouse experiment (16.8 g per inoculated plant vs. 13.4 g) but not the yield of Maris Kabul (17.8 vs. 18.4 g) (Table 2). Multiplication of *D. dipsaci* was similar in both cultivars (Table 3). When the plants were also infected with *Verticillium,* however, reproduction of *D. dipsaci* was greater in Vernal than in Maris Kabul, indicating a difference in physiological reaction between the

TABLE 3. Densities of *Ditylenchus dipsaci* and *Pratylenchus penetrans* on alfalfa inoculated with *Verticillium albo-atrum* and one or both nematodes.

	Number of *Ditylenchus dipsaci* per plant (×1,000)				*Pratylenchus penetrans*			
	Without *Pratylenchus*		With *Pratylenchus*		No. per g of roots		No. per plant (×1,000)	
	Vernal	Maris Kabul	Vernal	Maris Kabul	Vernal	Maris Kabul	Vernal	Maris Kabul
Control	144 ab	140 ab	361 ab	33 a	218.4 a	235.2 a	26.4 a	23.6 a
Verticillium	404 b	13 a	16 ab	273 ab	465.8 b	193.6 a	38.1 b	17.8 a

Numbers in paired columns followed by the same letter are not different according to Newman-Keuls multiple-range test ($P < 0.1$).

TABLE 4. Number of plants and forage yield from field grown alfalfa plantings infested with *Ditylenchus dipsaci.*

	First test				Second test			
	Plants†		Yield‡		Plants†		Yield‡	
	1982	1983	1982	1983	1983	1984	1983	1984
Control	10.5 b	9.5 a	672 a	2,530 c	9.2 a	9.0 a	681 b	1,887 c
Ditylenchus-infected	13.0 c	10.0 ab	642 a	1,918 b	8.2 b	4.1 c	504 a	580 a

Numbers in paired columns followed by the same letter are not significantly different according to Newman-Keuls multiple-range test ($P < 0.1$).
† Number of plants per plot.
‡ Grams of fresh weight per plot.

two cultivars. *P. penetrans* had an inhibitory effect on the reproduction of *D. dipsaci,* since the difference in susceptibility of the two cultivars to *D. dipsaci* in wilt-susceptible plants disappeared in the presence of *P. penetrans* (Table 3). Similar nematode–nematode interactions have been shown previously, although their study has involved only nematodes feeding on the roots. Chapman and Turner (6) showed that *Meloidogyne incognita* repressed the reproduction of *P. penetrans* in alfalfa, and split root experiments with the same nematodes in tomato roots suggested that translocatable inhibitory substances were involved.

The density of *P. penetrans* per gram of roots or per root system was significantly increased (Table 3) in *V. albo-atrum* infected plants in the wilt-susceptible Vernal but not the wilt-resistant Maris Kabul, but there was no effect of *D. dipsaci* on *P. penetrans* reproduction. The influence of *Verticillium* species on *P. penetrans* has been reported in some wilt-susceptible crops (14) but was not observed in others (3,7). The increased or decreased susceptibility to the nematode may be determined by the health status of the host; when the root system is badly damaged, by the nematode and (or) the fungus, the rate of nematode reproduction declines.

Field studies: Verticillium albo-atrum significantly suppressed the yield of Vernal plants in the field (Table 2), as it had done in the greenhouse, but not the yield of Maris Kabul. In the first field experiment, the *D. dipsaci* infection did not significantly reduce the number of plants even after the winter (Table 4). That winter the temperature dropped between 0 and −3 C for only 29 days, and the next year most of the infected plants were severely stunted and the yield of foliage was significantly reduced (Table 4). A significant decrease in yield was also observed in the second field experiment in the plots infected with *D. dipsaci,* and although the interaction with *Verticillium* was not significant, the number of overwintering *D. dipsaci*-infected plants was significantly reduced. During that second winter there were 31 days with freezing temperatures down to −12 C. Winter temperatures and the winterkill in the alfalfa growing areas of British Columbia would be expected to be more severe than those in Vancouver. Our results confirm those of Boelter et al. (4) who observed that severe winter temperature in Wyoming killed a large proportion of *D. dipsaci*-infected alfalfa plants. They suggested that *D. dipsaci* infection in Wyoming may impair the cold hardening process and therefore the overwintering of the plants.

In plants where the fungus was present with *P. penetrans,* the yield was significantly lowered the second year (Table 2). Other studies (12,13) have shown that wilt-susceptible crop plants become even more susceptible and the interaction of the two pathogens is usually additive or synergistic.

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