Suppression of Alfalfa Growth by Concommitant Populations of Pratylenchus penetrans and two Fusarium species[1]

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Abstract: Growth of alfalfa (*Medicago sativa* cv. Vernal) seedlings was compared after inoculation with combinations of either *Pratylenchus penetrans* and *Fusarium solani* or *P. penetrans* and *F. oxysporum* f. sp. *medicaginis*. A synergistic disease interaction occurred in alfalfa when *F. oxysporum* and *P. penetrans* were added simultaneously to the soil. Alfalfa growth was suppressed at all inoculum levels of *P. penetrans* and *F. oxysporum*, but not with *F. solani*. Seedlings inoculated with the nematode alone gave lower yields than when inoculated with either *Fusarium* species alone. *Fusarium oxysporum*, but not *F. solani*, was pathogenic to alfalfa under similar experimental conditions. *Fusarium oxysporum* did not alter the populations of *P. penetrans* in alfalfa roots, whereas the presence of *F. solani* was associated with a diminished number of *P. penetrans* in the roots. *Key words:* alfalfa, decreased yield, synergistic interaction, *Fusarium oxysporum* f. sp. *medicaginis*, *F. solani*, *Pratylenchus penetrans*.

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The root lesion nematode, *Pratylenchus penetrans* (Cobb, 1917) Chitwood and Oteifa, 1952, is known to decrease alfalfa (*Medicago sativa* L.) yields (1,16). *Fusarium* species, which are frequently isolated from alfalfa roots, have variable effects on yields (2,14,15); *F. solani* is commonly isolated from alfalfa roots and *F. oxysporum* f. sp. *medicaginis* is the causal organism of *Fusarium* wilt of alfalfa. The objective of this study was to determine the effects on alfalfa growth of simultaneous infections of *P. penetrans* and *F. solani* (Mart.) Sacc. or *P. penetrans* and *F. oxysporum* f.sp. *medicaginis* (Weimer) Snyder and Hansen.

MATERIALS AND METHODS

Alfalfa cv. Vernal was seeded into 7.5-cm-d pots of a sterilized 3:1:2, loam:sand:peat mixture (two seeds/pot) and inoculated 10–15 days after seeding. An aqueous suspension of *P. penetrans* was obtained by extracting the nematodes from red clover (*Trifolium pratense* L.) roots on a Burrell wrist-action shaker. Both species of *Fusarium* were cultured in Tochinai broth which produced aqueous suspensions of chlamydospores and conidia. The suspensions of fungal propagules and/or nematodes were pipetted into 3-cm-deep holes in the soil adjacent to the seedlings. Separate experiments were established for each of the two *Fusarium* species-nematode combinations, and the pots were arranged in a completely randomized design in the greenhouse. Four inoculum levels of *P. penetrans* (0, 200, 400, and 800 nematodes/pot) and four inoculum levels of each *Fusarium* species (0, 1×10^5, 1×10^6, and 1×10^7 *F. solani* spores/pot and 0, 5×10^5, 5×10^6, and 5×10^7 *F. oxysporum* spores/pot) were combined in 16 treatments for each nematode-fungus combination and each replicated five times. The experiments were done in a greenhouse at about 21 C with supplemental illumination giving a 14:10-h light:dark ratio. Every 3 wk after planting, a commercial 20-20-20 water-soluble fertilizer was added to each pot.

At the termination of the experiments, the plants were lifted and the roots and tops were weighed. Estimates were made of the nematode population in either cotton blue/lactophenol stained small roots or aqueous extracts of large roots that had been on a Burrell wrist action shaker for 4 days (8,13). Estimates of the fungus populations in the roots and soil were made based on the technique of Nash and Snyder (9). The data were subjected to an analysis of variance and the treatments ranked using a Student-ized range test or according to Fisher's modified least-significant differences.

RESULTS

Alfalfa seedings infected with *F. oxysporum* f. sp. *medicaginis* commonly showed

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a reddish discoloration of the lower leaves. Older alfalfa roots infected with *F. oxysporum* appeared only slightly discolored on the surface, but when cut longitudinally showed a reddish brown discoloration of the vascular system. No visible symptoms developed on plants inoculated with *F. solani*. Severe stunting, but little discoloration, of the foliage was observed in plants infected with *P. penetrans*. Root systems infected with *P. penetrans* were smaller than those of the controls and showed many dark brown to black lesions.

Top growth was suppressed by *P. penetrans* and *F. oxysporum* f. sp. *medicaginis* either alone or in combination (Table 1). Top weights were decreased by 31 and 28%, respectively, when plants were inoculated with the highest levels of the fungus (N0F3) and nematode (N3F0) alone. The highest inoculum density of both pathogens together (N3F3) significantly reduced the top weight by 69%. Reductions in dry weight of tops were similar to those for fresh weight. Root growth was significantly retarded at most levels of inoculum of *P. penetrans* and *F. oxysporum* and was suppressed most by the combined high inoculum (N3F3). The nematode generally suppressed root growth significantly more than did the fungus. The inoculum density of one pathogen did not significantly affect the final population density of the other pathogen (Table 1).

Fusarium solani alone at any inoculum level did not significantly decrease the fresh weight of roots (Table 2). However, *P. penetrans* alone reduced root weight by 50 and 73% at the lowest and highest inocula densities, respectively. Top weights were diminished significantly by *P. penetrans*, but not by *F. solani*. Simultaneous inoculation with *F. solani* and *P. penetrans* did not suppress top growth more than did *P. penetrans* alone. In all treatments where *P. penetrans* was inoculated together with *F. solani*, the number of *P. penetrans* extracted from the roots was less than when *P. penetrans* was inoculated alone, but only when both pathogens were inoculated at the highest level (N3F3 in Table 2) was this decrease significant ($P < 0.05$). The number of propagules of *F. solani* recovered from the soil was not significantly changed by the presence of *P. penetrans* in the roots (Table 2).

Table 1. Effects of inoculation of *Pratylenchus penetrans* (N) and *Fusarium oxysporum* f. sp. *medicaginis* (F) alone and in combination on the growth of Vernal alfalfa and on the final pathogen densities. (Mean of five replicates.)

	Tops		Roots			
Treatment*	Fresh weight (g)	% decrease	Fresh weight (g)	% decrease	*P. penetrans* per g root	*F. oxysporum* per g dried soil
N0F0	1.18	0	1.73	0	0	0
F1	1.00	15.3	1.07	38.2	0	348.5
F2	0.81	31.4	0.77	55.5	0	772.0
F3	0.81	31.4	1.08	37.6	0	4,098.0
N1F0	0.71	39.8	0.86	50.3	715.4	37.0
F1	0.93	21.3	1.09	58.9	469.6	379.0
F2	0.48	59.3	1.43	17.4	469.8	436.9
F3	0.45	41.9	1.04	39.9	630.3	4,167.0
N2F0	0.87	26.3	1.28	26.0	1,400.4	48.9
F1	0.96	18.6	1.02	41.1	816.0	378.0
F2	1.05	11.0	1.62	6.4	1,068.4	784.1
F3	0.44	42.7	1.07	38.2	1,998.0	4,636.0
N3F0	0.85	28.0	0.90	48.0	2,053.0	32.9
F1	0.40	66.1	1.29	25.4	2,422.0	369.3
F2	0.53	55.1	1.44	16.8	1,950.6	2,130.0
F3	0.37	68.6	0.62	64.2	2,002.7	6,459.0
LSD at $P < 0.05$	0.33		0.39			

*Pathogen densities/7.5-cm pot:
Number of nematodes: N0 = 0, N1 = 200, N2 = 400, N3 = 800.
Number of fungal propagules; F0 = 0, F1 = 5×10^5, F2 = 5×10^6, F3 = 5×10^7.

Table 2. Effects of inoculation of *Pratylenchus penetrans* (N) and *Fusarium solani* (F) alone and in combination on the growth of Vernal alfalfa and on the final pathogen densities. (Mean of five replicates.)

Treatment*	Tops		Roots		*P. penetrans* per g root (mean of 3)	*F. solani* per g dried soil
	Fresh weight (g)	% decrease	Fresh weight (g)	% decrease		
N0F0	0.44	0	0.26	0	0	23.8
F1	0.51	(15.9)	0.35	(34.6)	0	146.7
F2	0.35	20.5	0.23	11.6	0	2,820.0
F3	0.50	(13.6)	0.26	0	0	3,010.0
N1F0	0.23	47.7	0.13	50.0	2,812	0
F1	0.29	34.1	0.19	26.9	1,522	161.0
F2	0.27	38.6	0.17	34.6	1,280	1,789.0
F3	0.29	34.1	0.23	11.5	1,929	12,800.0
N2F0	0.23	47.7	0.13	50.0	8,577	5.0
F1	0.16	63.6	0.09	65.4	5,519	965.0
F2	0.24	45.5	0.12	58.6	2,941	2,028.0
F3	0.20	54.5	0.14	46.2	5,804	4,903.0
N3F0	0.12	72.7	0.07	73.1	14,830	131.9
F1	0.14	68.2	0.07	73.1	4,700	213.5
F2	0.12	72.7	0.07	73.1	4,685	2,694.0
F3	0.09	79.5	0.05	80.8	4,041	4,526.0
LSD at $P < 0.05$	0.14		0.12			

*Pathogen densities/7.5-cm pot:
Number of nematodes: N0 = 0, N1 = 200, N2 = 300, N4 = 800.
Number of fungal propagules: F0 = 0, F1 = 1×10^5, F2 = 1×10^6, F3 = 1×10^7.

DISCUSSION

Under field conditions, necrosis of alfalfa roots probably is caused by a complex of organisms (7). Nevertheless, *P. penetrans* is a primary pathogen, and in these experiments this pathogen alone caused lesions and poor growth of alfalfa seedlings. The threshhold of visible seedling damage by *P. penetrans* was reached at an inoculum level of 200 nematodes/pot, and often growth was more severely retarded by higher inoculum levels. In previous experiments (4) with alfalfa, a negative linear relationship was shown between the number of *P. penetrans* invading the roots and inoculum densities greater than 200 nematodes/plant. At densities below 200, the percentage entering increased as the inoculum level increased.

Growth of alfalfa is retarded more by *P. penetrans* than by *Fusarium* species, and *F. oxysporum* retards growth more than does *F. solani*. *Fusarium oxysporum* and *P. penetrans* decreased the top weight more than did infection by either pathogen alone which suggests a synergistic interaction. This response parallels that of *Meloidogyne incognita acrita* which is reported (11) to increase the tissue decay of alfalfa caused by *F. roseum* and *F. oxysporum* f. sp. *batatus*. Further, simultaneous inoculation with *P. penetrans* and *Trichoderma viride* Pers. ex Fr. retards alfalfa growth to a much greater extent than with either pathogen alone (7). A synergistic interaction was identified in the wilt-resistant 'Wisconsin Perfection' pea (10) where the resistance to wilt caused by *F. oxysporum* f. sp. *pisi* was broken down by *P. penetrans*.

Although all three organisms colonize alfalfa roots, only *F. oxysporum* and *P. penetrans* caused measurable damage. Further, the pathogenicity of *F. solani* was not demonstrated and was not induced by the presence of *P. penetrans*. Alfalfa produces antifungal substances (5) and *F. solani* may be inhibited by a phytoalexin response in the roots, whereas *F. oxysporum* may be resistant to such a response. *Fusarium oxysporum* on the other hand is more specialized and may more readily overcome such inhibitory effects of the plant response and of soil micro-organisms.

Plant growth differs in the two experiments, as can be seen by comparing the

controls for each experiment. Such growth differences were due largely to the different experimental conditions because the experiments were done at different times of the year. Light intensity and temperature may have affected plant growth, and the differences in *P. penetrans* population in the two experiments may be partly due to the temperature effect (6).

Edmunds and Mai (3) reported that alfalfa roots infected with *F. oxysporum* were more attractive to *P. penetrans* than noninoculated roots. In our work, the number of *P. penetrans* recovered from roots inoculated simultaneously with the nematode and *F. oxysporum* was not significantly different from the number found in roots inoculated with the nematode alone. However, the number of *P. penetrans* in the roots is diminished in the presence of *F. solani*. This may be because *F. solani* causes the roots to become partially resistant to *P. penetrans* or because *F. solani* directly affects nematode development and subsequent reproduction within the roots. Seinhorst and Kuniyasu (12) showed that the rate of multiplication of *P. penetrans* on peas decreased in the presence of *F. oxysporum* f. sp. *pisi*.

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