The Role of Pratylenchus penetrans in the Root Rot Complex of Canning Pea

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ABSTRACT

The effect of *Pratylenchus penetrans* on root rot of canning pea caused by *Aphanomyces euteiches* was determined in untreated silt loam and sandy loam soils and in soils pasteurized with aerated steam at 60 C for 30 min. *Pratylenchus penetrans* increased the severity of root rot in the two soils, but the effect of the nematode was most striking in the untreated silt loam soil with a threshold level of 25 oospores/g soil and with a water potential between -1/3 and -1 bar. The effect of the nematode was reduced in soils treated with aerated steam or in soils with a higher level of inoculum of *A. euteiches*, as disease due to the latter organism alone was severe under these conditions.

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Aphanomyces euteiches Drechs. is the major pathogen causing pea root rot in Wisconsin (4). There have been many reports of plant disease complexes involving fungi and nematodes, including some on peas (6, 7). *Meloidogyne hapla* was found to increase Rhizoctonia damping-off in pea seedlings (8). Haglund & King (3) reported that *Tylenchorhynchus claytoni* increased root rot of peas incited by *A. euteiches* only when a highly virulent isolate of the fungus was employed. The "early yellowing" of peas in Europe was believed to be a root disease complex caused by *Hoplolaimus uniformis* and *Fusarium oxysporum* f. sp. *pisi* race 3 (5). Davis & Jenkins (2) found that root knot nematodes caused early development of wilt symptoms in a wilt-susceptible pea cultivar, Pluperfect, while causing the resistance of cultivar Alaska to *F. oxysporum* f. sp. *pisi* to break down.

Plant-parasitic nematodes were often found in appreciable numbers in soils from Wisconsin pea fields that had been found to have a high severity index when assayed for *A. euteiches* (10). Peas grown in soil from one of these fields were heavily infected with *P. penetrans*; and fumigation of this soil with ethylene dibromide (EDB) or with 1,3-dichloropropene and 1,2-dichloropropane (D-D) reduced the pea root rot considerably. This investigation was undertaken to determine the extent to which *P. penetrans* can affect the development of pea root rot caused by *A. euteiches*.

MATERIALS AND METHODS.—A moderately virulent isolate of *A. euteiches* isolated from peas grown in a heavily infested field near Madison was used in all experiments. Known concentrations of oospores were employed as inocula in all experiments. These were produced by the fungus growing in corn extract broth for 4 weeks. To free the oospores from mycelium, the culture was triturated in a glass homogenizer, and sonified for 30 sec twice with intermittent cooling on ice. The number of oospores in the suspension was determined with a haemocytometer.

The isolate of *P. penetrans* was obtained from W. F. Mai, Cornell University, Ithaca, N.Y. The nematodes were propagated monoxenically on alfalfa (*Medicago sativa* L. 'Ranger') callus tissue as described by Krusberg (8). To obtain a clear suspension of the nematodes, the infected callus was transferred to cold water in a 125-ml beaker. After the content of the beaker had been stirred for about 2 min to disperse the soft infected callus, the suspension was placed on the Baermann funnel for 2 to 3 hr. The number of *P. penetrans* was determined in aliquots of the clean suspension placed in partitioned Syracuse dishes under the dissecting microscope.

Two soil types were employed for the tests. The first soil, a Dodge silt loam, had a pH of 5.8, and moisture contents of 25.6 and 10.4% at matric potentials of -1/3 and -10 bars, respectively. The second soil, a Plainfield loamy sand, had a pH of 5.8 and moisture contents of 13.8 and 4.4% at -1/3 and -10 bars, respectively. Neither soil had a detectable level of *A. euteiches* or *P. penetrans*. The levels of *F. solani* and *Pythium* spp. that were pathogenic to peas were also negligible. When it was necessary to eliminate all the normally occurring pathogens from these soils, we pasteurized them by heating for 30 min with aerated steam (60 to 65 C) or fumigated with EDB at a rate of 3.2 ml/30 liters of soil; otherwise, both soils were used without treatment to reduce their natural microfloras. Each soil was thoroughly mixed with the required amount of inoculum, and weighed amounts of infested soil were placed in 3-inch plastic pots. Four captan-treated pea seeds (*Pisum sativum* L. 'Wisconsin Perfection') were planted in each pot. The pots were then set in saucers of water until the top soil became moist. From this point on, the pots were weighed periodically, and when the soil reached a weight indicating a water

potential of either -1 bar or -10 bars (as required in each treatment), water was added to the soil to bring the potential again to -1/3 bar.

All the experiments were conducted in a controlled-environment plant growth chamber with a light intensity of 800 to 1,000 ft-c supplied by incandescent and cool-white fluorescent bulbs, with photoperiod of 15 hr. The light period temperature was 28 C; the dark period temperature, 13.3 C. Soil temperature in 3-inch plastic pots, filled with Dodge silt loam soil and kept at a potential of -1/3 bar, was 27 C during the day and 16 C at night. The relative humidity in the chamber was 60 ± 5%. These conditions were selected to simulate those prevailing in the fields during most of the pea-growing season in Wisconsin.

The treatments used included (i) no pathogen; (ii) *P. penetrans* alone; (iii) *A. euteiches* alone; and (iv) *P. penetrans* + *A. euteiches*. The amount of root rot disease developing during the experiment was expressed as per cent infection based on the number of plants with typical foliage symptoms of the disease that could be noted without uprooting the plants. At the end of the experiment, the disease severity index based on the severity of root damage was determined as described by Sherwood & Hagedorn (9). In the latter method, the disease index varied from 100 with all the plants dead, to 0 in which all plants were healthy and had clean, nonwater-soaked roots. Fields with a disease index of 75 or above are considered unsafe for growing peas.

RESULTS.—In preliminary tests using an untreated silt loam soil, the amount of inoculum of each pathogen required to incite detectable symptoms in 50% of the plants (threshold level) was determined. With *A. euteiches*, the inoculum required under the conditions of the experiment consisted of 25 oospores/g soil. Few plants were infected at 5 oospores/g soil, and all plants were infected when the dosage was 125 oospores/g soil. With *P. penetrans*, infection occurred at all levels, but a significant (.05) reduction in root dry weight during 5 weeks' growth only occurred at inoculum levels of 2,000 nematodes/pot. This was used as a standard nematode dosage unless otherwise noted.

The effect of *P. penetrans* on Aphanomyces root rot of canning peas was most evident in soils that had received no prior treatment to reduce their natural microfloras. The incidence and the severity of Aphanomyces root rot disease increased significantly (0.05) both in untreated Dodge silt loam and Plainfield loamy sandy soils when they were infested with *P. penetrans* (Tables 1, 2). The root rot was generally more severe in the loamy sand than in the silt loam (Fig. 1.), and in soils with water potential cycling between -1/3 and -1 bar than in those with water potential cycling between -1/3 and -10 bars (Tables 3, 4).

The inoculum level of *A. euteiches* was critical, as illustrated in an experiment in which the two untreated soils were infested with 1, 5, 25, or 125 oospores/g soil alone or together with the standard nematode dosage. There was severe pea root rot in both soils with 125 oospores/g soil whether they contained nematodes or not. Root rot disease was low in both soils, with 1 oospore/g soil regardless of the presence or absence of *P. penetrans*. The nematodes caused root rot to increase significantly (.05) in the silt loam infested with 5 and 25 oospores/g soil. Pea root rot by *A. euteiches* alone was sufficiently high in the loam sand at 5 and 25 oospores/g soils for the effect of the nematode to be nonsignificant (.05).

In soils pasteurized with aerated steam at 60 C, the effect of *P. penetrans* on the severity of Aphanomyces root rot of peas was significant (.05) despite the fact that the disease caused by *A. euteiches* alone was very high (Table 3).

The effect of *P. penetrans* on the pea root rot in soil fumigated with EDB before reinfestation with *A. euteiches* was not striking (Table 4). Increased pathogenic activity of *A. euteiches* in treated soils

TABLE 1. Rate of development of Aphanomyces root rot of peas grown in untreated Dodge silt loam and Plainfield loamy sand in the presence and absence of *Pratylenchus penetrans* under two soil moisture regimes

Soil type	Days after inoculation	Per cent of emerged plants killed: *Aphanomyces euteiches* alone		*A. euteiches* + *P. penetrans*	
		SDC[a]	LDC[b]	SDC	LDC
Dodge silt loam					
	17	0[c]	0	37	0
	21	13	6	94*	6
Plainfield loamy sand					
	17	85	35	94	54*
	21	100	46	100	75*

[a] Short drying cycle (water potential -1/3 to -1 bar).

[b] Long drying cycle (water potential -1/3 to -10 bars).

[c] Mean of four replicates; asterisk indicates means in which the presence of *P. penetrans* increased Aphanomyces root rot significantly (.05), using Duncan's new multiple range test.

TABLE 2. Influence of *Pratylenchus penetrans* on the Aphanomyces root rot of peas using two soil moisture regimes in untreated silt loam and Plainfield loamy sand soils 21 days after planting

	No pathogen added		*P. penetrans* alone		*Aphanomyces euteiches* alone		*P. penetrans* + *A. euteiches*	
	SDC[a]	LDC[b]	SDC	LDC	SDC	LDC	SDC	LDC
Dodge silt loam								
Root rot disease index	12[c]	0	12	12	56	38	88*	36
Emerged plants killed, %	0	0	0	0	6	6	73*	6
Plainfield loamy sand								
Root rot disease index	12	0	25	12	96	66	98	84*
Emerged plants killed, %	0	0	6	8	100	58	100	54

[a] Short drying cycle (water potential -1/3 to -1 bar).
[b] Long drying cycle (water potential -1/3 to -10 bars).
[c] Mean of four replicates; asterisk indicates means significantly different (.05) from those with *A. euteiches* alone, using Duncan's new multiple range test. Disease index of zero = healthy plants; 100 = all plants dead.

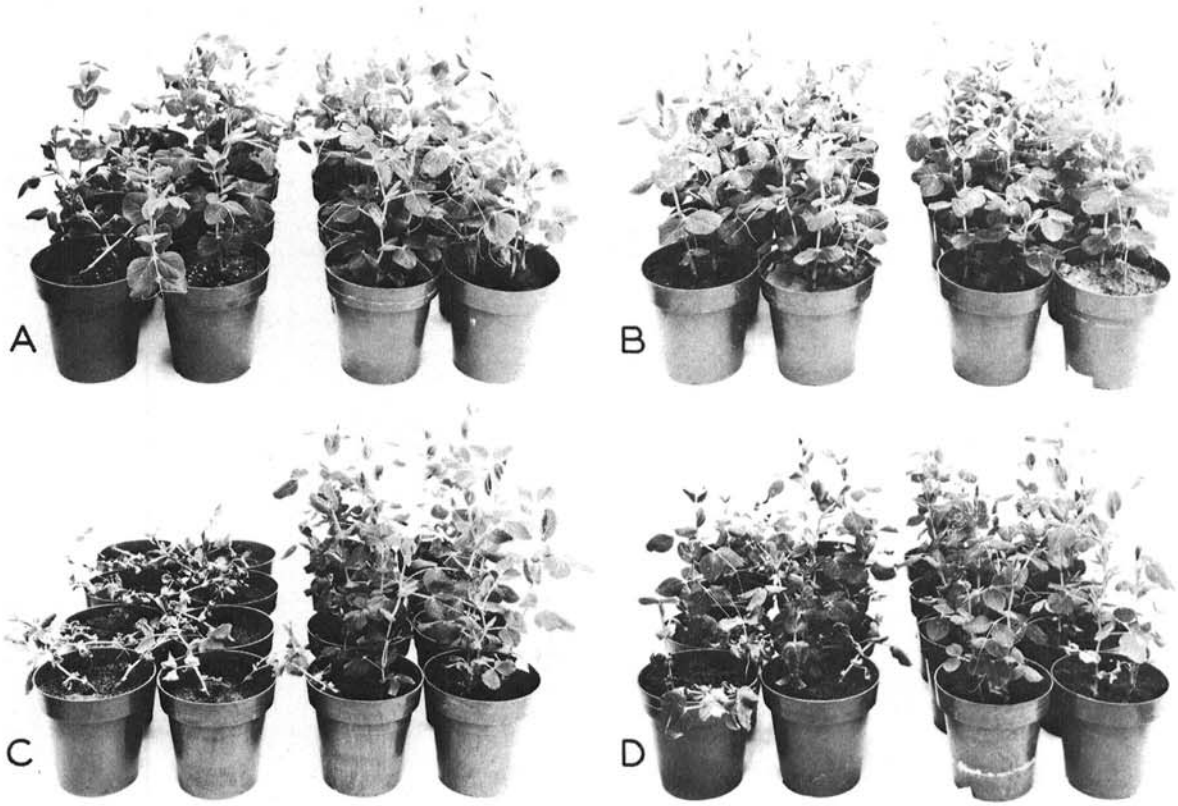

Fig. 1. Aphanomyces root rot of peas in untreated Dodge silt loam (A, B) and in Plainfield loamy sand (C, D) soils. **A, C)** Soils had matric potential cycled between -1/3 and -1 bar; **B, D)** between -1/3 and -10 bars. Treatments in each case are (right to left) no pathogens; *Pratylenchus penetrans* alone; *Aphanomyces euteiches* alone; and *P. penetrans* + *A. euteiches*.

TABLE 3. Influence of *Pratylenchus penetrans* on the severity of Aphanomyces root rot of peas grown for 4 weeks in soils infested with the pathogens after treatment with aerated steam at 60 C for 30 min

Inoculum	Root rot disease index[a]	
	Dodge silt loam	Plainfield loamy sand
None	5[b]	19
P. penetrans	13	25
Aphanomyces euteiches	63	74
P. penetrans + *A. euteiches*	79*	90*

[a] Disease index of 0 = all plants healthy; 100 = all plants dead.

[b] Mean of four replicates; asterisk indicates means differing significantly (.05) from those in *A. euteiches* alone, using Duncan's new multiple range test.

masked almost completely the influence of *P. penetrans* in enhancing the root rot disease. The effect of the presence of *P. penetrans* became less discernible with increasing period for disease development in the fumigated soils.

DISCUSSION.—Root lesion nematodes were commonly present in some Wisconsin soils that have been found to have a high root rot severity index (10), and large numbers were found within pea roots from one field where root rot was severe. From the results of the present studies, it is evident that *P. penetrans* may be a factor in increasing damage due to *A. euteiches* under certain conditions.

Aphanomyces euteiches is an aggressive pathogen when conditions are favorable for the development; and the presence of *P. penetrans* had little important effect when inoculum levels of *A. euteiches* were high, or where the inhibiting action of normal microbial population of the soil was reduced by prior treatment of soil with aerated steam or fumigants. Significant increases were noted with low levels of *A. euteiches* and normal soil microbial populations.

The simultaneous presence around a pea root of *A. euteiches* and *P. penetrans* can be expected to increase the probability of infection by the former. When crop rotations and soil conditions favor the maintenance or buildup of populations of *P. penetrans*, the probability of root rot due to *A. euteiches* may be greater than would otherwise be expected with a declining population of the latter.

The nature of the effect of *P. penetrans* on the root tissue suggests that the mechanism by which root rot enhancement occurs is similar to that demonstrated by Beute & Lockwood (1) for pea root rot enhancement in virus-infected plants. Increased loss of nutrients by affected roots stimulated increased root infection by both *Fusarium solani* f. sp. *pisi* and by *A. euteiches*.

It is concluded from this study that *P. penetrans* may be an important factor in predisposing the pea plant to infection by *A. euteiches* when nematodes are active around pea roots in the presence of threshold levels of inoculum of the root rot pathogen.

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TABLE 4. The effect of *Pratylenchus penetrans* and soil moisture content on the rate of root rot development in two soil types, both fumigated and then infested with identical levels of *Aphanomyces euteiches* and nematodes

Soil type	Days after planting peas in infested soil	% of emerged plants killed by			
		A. euteiches alone		*A. euteiches* + *P. penetrans*	
		SDC[a]	LDC[b]	SDC	LDC
Dodge silt loam					
	14	0[c]	0	0	0
	19	25	12	27	33
	21	64	50	73	54
	28	100	75	100	75
Plainfield loamy sand					
	14	75	71	74	94*
	16	92	75	100	100*
	17	100	82	100	100
	21	100	100	100	100

[a] Short drying cycle (water potential -1/3 to -1 bar).

[b] Long drying cycle (water potential -1/3 to -10 bars).

[c] Mean of four replicates; asterisk indicates means differing significantly (.05) from those with *A. euteiches* alone, using Duncan's new multiple range test.

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