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Plant-Parasitic Nematodes and Fungi Associated with Root Rot of Peas on Prince Edward Island[1]

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Abstract: Eight commercial pea fields on Prince Edward Island were sampled in June and July over a 2-year period (1986–87) to determine soil population densities and the incidence of nematodes and fungi associated with root rot of peas. Root lesion nematodes (*Pratylenchus* spp.) were the dominant endoparasitic nematodes recovered from roots and soil. Low populations of the northern root-knot nematode (*Meloidogyne hapla*) were also present. *Tylenchorhynchus* spp. and *Paratylenchus* spp. were recovered frequently from soil in the root zone, and *Helicotylenchus* spp. were also frequent, but in low numbers. *Fusarium solani* was the most common fungal species isolated from the epicotyl and hypocotyl tissues of pea. *Fusarium oxysporum* was also isolated frequently, and both *Fusarium* species were found in soil from all fields. *Rhizoctonia solani* and *Verticillium albo-atrum* were common in hypocotyl tissue, but *V. dahliae* was isolated infrequently. Root rot was rated as severe in all fields and was positively and significantly correlated ($P \leq 0.05$) with densities of *Tylenchorhynchus* spp. in soil and with incidence of *F. solani* in pea tissue. The incidence of *F. solani* root infections was positively and significantly correlated with densities in soil of *Tylenchorhynchus* spp. ($P \leq 0.01$), *Helicotylenchus* spp. ($P \leq 0.01$), and *Paratylenchus* spp. ($P \leq 0.05$).

Key words: *Fusarium solani*, *Helicotylenchus* spp., *Meloidogyne hapla*, *Paratylenchus* spp., pea, *Pisum sativum*, *Pratylenchus* spp., root rot, *Tylenchorhynchus* spp.

Pea (*Pisum sativum* L.) cultivation on Prince Edward Island has declined over the past 18 years from 2,200 ha in 1969 (7) to 720 ha in 1987 (pers. obs.). Economic loss caused by root rot has contributed to this decrease. Previous surveys indicated that root lesion nematodes (primarily *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven) were prevalent in soil and roots from pea fields that exhibited symptoms of root rot and had lower than average yields (8). Other surveys indicated that fusarium root rot and ascochyta blight were the major root rot diseases of peas on Prince Edward Island (7,14). *Fusarium oxysporum* Schlecht was identified as a causal agent of root rot on Prince Edward Island (7), whereas *F. solani* (Mart.) Appel & Wollenweber f. sp. *pisi* (F. R. Jones) Synd. & Hans. was the predominant root rot organism of peas in Ontario (17,18).

Root rot of peas is thought to be incited by several different organisms, and nematodes, as well as fungi, may contribute to the disease complex (3). The objectives of this study were to determine the incidence and soil population densities of several nematode genera and fungal species and to determine their association with root rot severity of peas on Prince Edward Island.

Materials and Methods

A total of eight commercial pea fields in the potato (*Solanum tuberosum* L.) growing region of Prince Edward Island were selected randomly and monitored for incidence of soilborne organisms and root rot severity in 1986 and 1987. Soil type was generally a fine sandy loam (ca. 65% sand, 25% silt, 10% clay; pH 5.1–6.0, 2.5% organic matter). Average rainfall during the growing season was ca. 45 cm in 1986 and 40 cm in 1987. The previous crop in all fields was either wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.).

Thirty plants were taken from a 5 × 10-m plot established randomly in each field in mid-June and late July of each year. Soil was also sampled at the same time by in-

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serting a 25-mm-d soil probe 15 cm deep in the root zone every 2 m in a cross pattern.

Root rot was rated on all plants using a scale of 0 to 5, based on the extent of lesions on roots, hypocotyl, or epicotyl tissue, in which 0 = healthy, 1 = slight discoloration, 2 = a few small lesions, 3 = large lesions (> 1 cm long), 4 = large lesions girdling the hypocotyl and (or) epicotyl together with rotted roots, and 5 = plants dead. Each sample was given a root rot severity index rating (from 1 to 100):

$$\text{Root rot severity} = \frac{\sum \text{no. of plants in disease category} \times \text{numerical value of disease category}}{\text{total no. of plants} \times 5} \times 100$$

Nematodes were extracted by placing a 50-g subsample of soil and 5 g fresh washed roots from each sample in a modified Baermann funnel (16) and a mist chamber (5), respectively. After 7 days at 20–25 C, nematodes that had separated from soil or roots were identified and counted with a stereomicroscope. Hypocotyl, epicotyl, and root tissue segments (1 cm long) were excised from each plant, surface sterilized in 0.06% NaOCl for 1 minute, and rinsed in distilled water for 1 minute. Sixty segments of hypocotyl and epicotyl tissue and 180 segments of root tissue from each field in both years were placed on isolation media. *Rhizoctonia solani* Kuhn and *Verticillium albo-atrum* Reinke & Berthier were isolated on medium described by Ko and Hora (10), *Verticillium dahliae* Kleb. was isolated on medium described by Huisman and Ashworth (6), and *Fusarium* spp. were isolated on potato dextrose agar (PDA) containing 0.61 g PCNB/liter. Tissue samples on all media were incubated at 20 C for 7–20 days before fungal identifications were attempted. Colonies showing characteristics of *Fusarium* spp. were transferred to PDA, incubated for 5 days, and illuminated with UV light for 5 days prior to identification of species.

Rhizoctonia solani populations were estimated from 50-g subsamples of soil on Ko and Hora (10) medium using the screening procedure of Weinhold (19). *Verticillium dahliae* population levels were estimated from a 15-g subsample of soil using the method outlined by Huisman and Ashworth (6). *Verticillium albo-atrum* populations were estimated by spreading 10 cm^3 of a suspension of soil : 0.1% water agar (1:1,000) on each of 10 petri dishes containing medium described by Christen (1). *Fusarium* spp. were estimated in the same manner using a modified PCNB medium (12). Colonies of soilborne fungi were counted, and *Fusarium* spp. were transferred to PDA and illuminated with UV light as described above.

Nematode data were transformed to $\log_{10}$, and fungal data were expressed as percent incidence and transformed using arcsine prior to statistical analysis. Analysis of variance and mean separation utilizing protected least significant differences (LSD) were performed on data to determine differences in the incidence of fungi in different tissues. Correlation and multiple linear regression analysis was used to determine the association between fungi and nematode levels and root rot severity.

Results and Discussion

Root lesion nematodes, primarily *Pratylenchus penetrans,* were the most abundant endoparasitic nematodes recovered from roots and were also very prevalent in soil (Table 1). Previous studies have shown that *P. penetrans* accounted for 80–97% of the root lesion nematode populations in various crops grown on Prince Edward Island, with *P. crenatus* Loof accounting for the remainder (8,9). The northern root-knot nematode, *Meloidogyne hapla* Chitwood, also was recovered from roots and soil, but at lower levels than *Pratylenchus* spp. These nematodes have been isolated previously from soil and pea roots on Prince Edward Island (8) and have been associated with root rot and reductions of pea yields in Europe and the United States (2,11,15). Stunt nematodes, identified as mostly *Tylenchorhynchus dubius* (Butschli) Filipjev, pin nematodes (*Paratylenchus* spp.), and to a

TABLE 1. Root and soil populations of plant-parasitic nematodes in peas grown on Prince Edward Island.

	No. per g dry root		No. per kg dry soil	
	June	July	June	July
Pratylenchus spp.	2,880 (1,042) a	2,100 (944) a	3,700 (1,880) a	2,150 (1,437) a
Meloidogyne hapla	0 (0) b	35 (4) b	660 (186) b	630 (144) b
Helicotylenchus spp.			0 (0) b	100 (45) b
Paratylenchus spp.			95 (110) b	660 (270) b
Tylenchorhynchus spp.			220 (99) b	2,240 (1,498) a

Figures in columns followed by the same letter are not significantly different ($P \leq 0.05$) using protected least significant differences. Data are arithmetic means from four fields per year, collected over 2 years, but the statistical analyses were performed on log-transformed data. Anti-log of data presented in parentheses.

lesser extent, spiral nematodes (*Helicotylenchus* spp.), were common ectoparasites in soil around roots of peas. Sanwal (13) also recovered stunt and pin nematodes frequently in a survey of pea fields in Ontario and suggested the possibility of a relationship between these nematode genera and fusarium wilt.

Fusarium solani was the most common fungus isolated from hypocotyl and epicotyl tissue, and *F. oxysporum* was prevalent in epicotyl tissue (Table 2). These two species were detected in soil from every field. *Rhizoctonia solani* and *V. albo-atrum* also were recovered, particularly from hypocotyl tissue. *Verticillium dahliae* was not common in pea tissue. Ascochyta blight was not found in this survey, although earlier studies indicated it was an important disease of peas on Prince Edward Island (7,14).

Although severity varied over this 2-year study, severe root rot symptoms were observed in all fields by the end of July in both 1986 and 1987 (average severity index = 64.9). Root rot severity was positively correlated with incidence of *F. solani* in subterranean pea tissue ($P \leq 0.01$) and with density of *Tylenchorhynchus* spp. in soil around roots ($P \leq 0.05$), but not with densities of *Pratylenchus* spp. in soil or roots (Table 3). Multiple linear regression analyses indicated that 88.7% of the variation in root rot severity was explained by the incidence of *F. solani* and *V. albo-atrum* in epicotyl tissue and *F. oxysporum* in roots. Incidence of root infection by *F. solani* was correlated positively with soil population densities of *Tylenchorhynchus* spp. ($P \leq 0.01$), *Helicotylenchus* spp. ($P \leq 0.01$), and *Paratylenchus* spp. ($P \leq 0.05$), but not with *Pratylenchus* spp. or *M. hapla* in roots or soil (Table 4).

The results indicated that *F. solani* was an important component of the root rot

TABLE 2. Incidence in hypocotyl, epicotyl, and root tissue and population levels in soil of fungi associated with peas grown on Prince Edward Island.

Sample location	*R. solani*	*V. albo-atrum*	*V. dahliae*	*F. oxysporum*	*F. solani*
			Percent incidence†		
Hypocotyl	17.5 (22.4) a	18.1 (20.8) a	1.3 (2.3) a	15.0 (19.2) ab	40.0 (38.6) a
Epicotyl	11.3 (13.0) b	13.1 (16.3) a	1.3 (1.7) a	27.5 (28.4) a	41.9 (37.0) a
Roots	2.4 (4.3) c	7.5 (11.0) a	0.4 (0.9) a	10.0 (13.3) b	10.2 (14.0) b
			Colony forming units‡		
Soil	6.4	175.0	0.6	2,621.0	1,230.0

Figures in columns for plant tissue followed by the same letter are not significantly different ($P \leq 0.05$) using protected least significant differences. Data are arithmetic means of two sample dates per field, four fields per year collected over 2 years, but the statistical analyses were performed on arcsine-transformed data; means shown in parentheses.

† Percent incidence = $\frac{\text{No. of samples with fungus}}{\text{Total no. of samples}} \times 100$.

‡ Colony forming units per gram dry soil, except for *R. solani* where 50 g was used and *V. dahliae* where 15 g was used.

TABLE 3. Correlation coefficients between root rot severity of peas and incidence of fungi in plant tissue, or between root rot severity and population densities of nematodes in roots and soil on Prince Edward Island.

Organism	Correlation coefficient			
	Hypocotyl	Epicotyl	Root	Soil
Rhizoctonia solani	0.38	0.38	0.46	—
Verticillium albo-atrum	0.13	0.36	0.18	—
V. dahliae	0.05	0.13	0.05	—
Fusarium oxysporum	−0.14	0.19	0.39	—
F. solani	0.73**	0.87**	0.76**	—
Pratylenchus spp.	—	—	0.05	−0.30
Meloidogyne hapla	—	—	0.31	−0.06
Helicotylenchus spp.	—	—	—	0.41
Paratylenchus spp.	—	—	—	0.45
Tylenchorhynchus spp.	—	—	—	0.59*

* and ** indicate significant correlations at $P \leq 0.05$ and $P \leq 0.01$, respectively (14 df). Correlation analyses performed on arcsine (fungi) and log-transformed (nematode) data from two sample dates per field, four fields per year collected over 2 years. Dashes (—) indicate organism not sampled from this location.

complex of peas grown on Prince Edward Island. The association of *Tylenchorhynchus* spp. with root rot severity tended to agree with greenhouse studies (4) on the relationship in peas between *T. martini* (Fielding) and *Aphanomyces euteiches* (Drechs.). In our study, however, the role of *Tylenchorhynchus* spp. as well as *Pratylenchus* spp. and *Helicotylenchus* spp. in the root rot complex of peas cannot be ascertained. Three species of *Tylenchorhynchus,* four species of *Paratylenchus,* and two species of *Helicotylenchus* have been identified in cereals in Prince Edward Island (9), and it is likely that these species were present also in succeeding pea crops. Although the relationships between the various ectoparasitic nematode species and fungal species have yet to be determined, the significant positive correlations indicate the possibility of a biological interaction in root rot of peas grown under field conditions on Prince Edward Island.

TABLE 4. Correlation coefficients between incidence of *F. solani* in roots and population densities of nematodes in soil and roots of peas grown on Prince Edward Island.

	Source	Correlation coefficient
Pratylenchus spp.	Roots	0.07
	Soil	−0.12
Meloidogyne hapla	Roots	0.26
	Soil	−0.04
Helicotylenchus spp.	Soil	0.64**
Paratylenchus spp.	Soil	0.51*
Tylenchorhynchus spp.	Soil	0.70**

* and ** indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively (14 df). Correlation analyses performed on arcsine (*F. solani*) and log-transformed (nematode) data of two sample dates per field, four fields per year collected over 2 years.

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