

Reproductive Potential of the Root-Lesion Nematode *Pratylenchus penetrans* (Cobb, 1917) Sher and Allen, 1953 (Nematoda: Pratylenchidae) in Monoxenic Cultures

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Abstract

Pratylenchus penetrans reproduced monoxenically on carrot disc and on *Agrobacterium rhizogenes* transformed potato root cultures. The life cycle was completed in 40 days at 24 °C. An initial inoculum level of 54 individuals provided three times more nematodes than ten or twenty individuals. In all tests most nematodes were found in the egg stage and only a low proportion of the population reached the adult stages.

Key words: Root-lesion nematode, *Pratylenchus penetrans*, reproduction, monoxenic culture.

1. INTRODUCTION

Lesion or root-lesion nematodes (*Pratylenchus* spp.) are the most common nematodes attacking mint and cause substantial damage. The common name of these nematodes is derived from the often conspicuous necrotic lesions they cause on host roots (Siddiqi, 1977).

Lesion nematodes are migratory endoparasites that enter the host root for feeding and reproduction and move freely through or out of the root tissue. They do not become sedentary in the roots, as do the cyst or root-knot nematodes. Feeding is restricted almost entirely to the cortex of the root (Ecevit and Akyazi, 2010).

Lesion nematodes are essentially worldwide in distribution. Five of the more than 40 species of *Pratylenchus* that have been described all over the world. *Pratylenchus penetrans* (Cobb) Sher and Allen, *Pratylenchus thornei* Sher and Allen, *Pratylenchus neglectus* (Rensch) Filipjev and Schuurmans Stekhoven, *Pratylenchus scribneri* Steiner and *Pratylenchus alleni* Ferris. Lesion nematodes may exist as a single species at a given site, or as a complex of two or more species (Aytan-Ediz, 1978; Kagoda *et al.*, 2010; Mudiope, 2004).

P. penetrans is most often found in nurseries, orchards and strawberry fields. This lesion nematode has been responsible for severe decline and for replant failure in many cherry, apple and peach orchards. Because of its importance, this species has been studied more than any other lesion nematode (Perry and Moens, 2006; De Waele and Elsen, 2002; Luc, 1987).

The life cycle of a lesion nematode is rather simple. Sexual reproduction occurs in *P. penetrans*. After mating, the female lays its eggs singly or in small groups in the host root or in the soil near the root surface (Perry, 2002). The first larval stage and molt occur within the egg. The egg hatches within 1 to 3 weeks, depending on the soil temperature. The second-stage larva emerges from the egg and undergoes three more molts before becoming an adult. All life stages outside the egg are infective lesion nematodes appear to be attracted to host roots, especially to the region of root hair production and to the root tips. Most penetration, however, occurs behind the region of elongation. The nematode may feed ectoparasitically from the root surface for a brief period before entering the root, eventually penetrating the root by forcing its way between or through the epidermal cells. Entrance is aided by the mechanical action of the nematode's feeding structure, the stylet, and by the cell wall degrading enzymes secreted through oesophageal glands. Once inside the roots, the nematode feeds on cortical cells, creating cavities as tissue is destroyed (Zunke, 1990).

When an infected root becomes highly necrotic, dies, or otherwise becomes unfavourable for feeding and reproduction, the nematode migrates through the cortex to a healthier area or into the soil to another root. Lesion nematodes do not attack the root stele, as do cyst and root-knot nematodes (Siddiqi, 1977).

Lesion nematodes overwinter as eggs, larvae, or adults in roots or soil. The length of the life cycle depends on the species and the soil temperature. For example, the optimum temperature for population development on soybeans for *P. neglectus* is 30 °C while that for *P. penetrans* is 25°C (Prasad, 1999).

Lesion nematodes remain inactive when soil temperatures are below 15 °C; except for *P. penetrans*, there is little activity until temperatures rise above 20 °C. *P. penetrans* completes its life cycle in 30 days at 30 °C, 35 days at 24 °C and 86 days at 15 °C (Siddiqi, 1977; Ecevit and Akyazı, 2010).

In this paper, the monoxenic culture (living within a single host during a parasite's life cycle) of *P. penetrans* in both carrot and transformed potato root is reported.

2. MATERIALS AND METHODS

The nematode was isolated from soil around apple trees in Eğirdir, Isparta (37° 52.2'N; 30° 51.0'E). Adult individuals recovered from soil were hand picked and surface disinfected in a 100 ppm mercuric chloride and 1000 ppm streptomycin sulphate solution for 5 minutes. For identification of specimens, Siddiqi (1977) and Southey (1986) were used. Nematodes were then pipetted to carrot disc cultures prepared as indicated by O'Bannon and Taylor (1968), Lawn and Noel (1986). Such cultures were used as the source of inoculum for the reproduction studies. Transformed potato roots were grown on solid Gamborg's B5 medium plus vitamins (1976) and incubated for 7 days at 24 °C before nematode inoculation.

To compare nematode reproduction on differentiated and non-differentiated tissues, 15 ± 1 surface disinfected females were added to each of 20 cultures containing either transformed potato roots or carrot discs (disc / per culture). The number of *P. penetrans* added per culture was determined after nematode inoculation. Cultures were maintained at 24 °C in the dark for 60 days. Nematodes were recovered from carrot disc cultures by adding 5 ml distilled water per culture. Nematodes that migrated from the disk to the

clear water were collected 24 hours later. The carrot disc was then weighted and blended in a blender for a total of 40 seconds given as 10 second periods. Both suspensions were mixed and nematodes counted determining the number of eggs, juvenile stages (J2, J3 and J4), males and females. To recover *P. penetrans* from carrot cultures, the agar was melted in a microwave oven for 1-2 minutes, roots blotted dry and weighted. Nematodes remaining in the agar plate and in the roots were counted as indicated above.

The reproduction rate of the nematode was studied on carrot disc cultures inoculated with 10 ± 1 surface disinfected females. Nematode reproduction was assessed, as described previously, in ten cultures at 40, 80 and 120 days respectively.

The effect of the initial inoculum level on nematode multiplication was determined after 120 days by adding 12, 24 and 54 surface disinfected a mixture of all life cycle stages of nematodes to 250 ml flasks containing five carrot discs. Each inoculum level was replicated three times.

3. RESULTS AND DISCUSSION

Pratylenchus penetrans was established in monoxenic cultures on both carrot discs and *Agrobacterium rhizogenes* (Riker) Conn transformed roots (Table 1). The population increased 86 times on carrot discs in 2 months. Clusters of nematodes were observed in the plate outside the carrot disc (Figure 1). Although the nematode was able to complete its life cycle on transformed potato roots, as indicated by the presence of males, these cultures were apparently a poor host for the nematode. Nematodes were found in the agar but no nematode was observed into the root tissue. The nematode completed its life cycle in 40 days which seems to be the minimum time required to undergo development from adult to adult at 24 °C (Table 2). The reproduction rate of *P. penetrans* was 82 and 1203 fold after 80 and 120 days, respectively.

An initial inoculum level of ten or twenty individuals provided similar numbers of nematodes per culture after 97 days (Table 3). However, cultures inoculated with 54 individuals yielded three times more nematodes. The egg stage was predominant in carrot disc cultures in the three experiments except when the reproduction rate was assessed at 40 days. At this time, the population was composed mainly by females (57 %).

Carrot disc cultures of *Radopholus similes* (Cobb) Thorne and *Pratylenchus brachyurus* (Goldfrey) Filipjev and Schuurmans Stekhoven yielded large number of eggs (O'Bannon and Taylor, 1968). High numbers of eggs were also found in cultures of *P. brachyurus* on carrot callus tissue; however, those cultures of *Pratylenchus agilis* Thorne and Malek, *Pratylenchus scribneri*, *Radopholus citrophilus* Huettel, Dickson, Kaplan and *R. similis*, yielded low numbers of eggs (Gamborg *et al.*, 1976; Brown and Vessey, 1985). Only a low percentage of the population reached the adult stage, the proportion of males to females never being higher than 8 and 11 % of the population respectively.

Viable axenic nematodes obtained after soaking and migration in streptomycin sulphate and, mercuric chlorur were injected into fresh carrot discs and incubated at 29 °C. At 70 days, population increases resulting from axenic cultures were not significantly different (Lawn and Noel, 1986).

Sandstedt and Schuster (1965) observed greater callus growth at the radical end of carrot discs when inoculated with *Meloidogyne incognita* (Kofoid and White), Chitwood and suggested that this was partly due to enhanced nematode nutrition at the radical end. A similar growth pattern of callus tissue was

observed in this study, but discs were placed randomly in the flakers resulting in both apical and radical end of discs being inoculated, which may explain partly why nematode increases varied between replications. Variability in the life stages of the initial nematode inoculum also may account for the differences.

I have maintained monoxenic cultures of *P. penetrans* for 3 years. The techniques developed and described in this paper have provided large numbers of axenic nematodes for other studies, and should provide researchers studying other *Pratylenchus* species a method for producing monoxenic cultures.

Table 1. Reproduction of *Pratylenchus penetrans* on carrot disc and *Agrobacterium rhizogenes* transformed potato roots cultures inoculated with 15 ± 1 females after 60 days at 24 °C (Mean of five replications)

Substrate	Tissue weight	No eggs/culture	No juveniles/culture	No females/culture	No males/culture	Total nematode	Reproduction rate
Carrot discs	3.0	756	412	66	55	1289	86
Potato roots	0.9	3	18	6	4	31	2

Table 2. Reproduction of *Pratylenchus penetrans* on carrot discs inoculated with 10 ± 1 females after 40, 80 and 120 days at 24 °C (Mean of five replications)

Days after inoculation	Carrot tissue weight (g)	No eggs/culture	No juveniles/culture	No females/culture	No males/culture	Total nematode	Reproduction rate
40	3.1	7	24	44	2	77	8
80	3.0	326	333	93	70	822	82
120	3.1	6410	4126	981	512	12029	1203

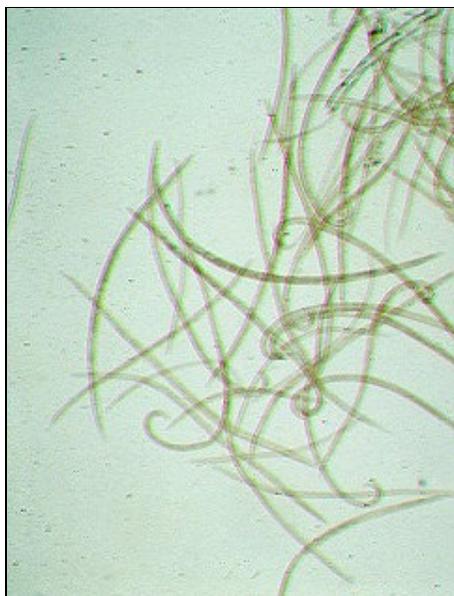


Figure 1. Monoxenic culture of *Pratylenchus penetrans*. Clusters of *P. penetrans* observed in carrot disc cultures incubated with 15 ± 1 females after 60 days at 24°C . (x400)

Table 3. Reproduction of *Pratylenchus penetrans* on carrot discs inoculated with 12, 24 and 54 nematodes after 97 days at 24°C (Mean of five replications)

Inoculation level	No eggs/culture	No juveniles/culture	No females/culture	No males/culture	Total nematode	Reproduction rate
12	5012	4116	384	162	9674	806
24	6981	3046	252	156	10435	435
54	19796	10685	1208	765	32454	601

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