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Population Densities of Five Migratory Endoparasitic Nematodes in Carrot Disk Cultures

S. Verdejo-Lucas and J. Pinochet[1]

Abstract: Numbers of nematodes recovered per culture varied greatly among five species cultured on carrot disks. *Radopholus similis* and *Pratylenchus vulnus* showed the highest population densities, with 23,400-fold and 16,600-fold increases, respectively, in 90 days. Final populations of *P. thornei* and *Zygotylenchus guevarai* were similar but lower than those of *R. similis* and *P. vulnus*. The population of *P. neglectus* increased 74 times. Species with the greatest reproduction in this study reproduce sexually.

Key words: burrowing nematode, lesion nematode, monoxenic culture, nematode, *Pratylenchus neglectus, P. thornei, P. vulnus, Radopholus similis,* reproduction, *Zygotylenchus guevarai.*

Nematodes of the family Pratylenchidae, such as *Pratylenchus, Radopholus,* and *Zygotylenchus,* are migratory endoparasites that destroy host cells, forming cavities and causing necrosis. Species belonging to these genera show different pathogenicity and host preferences. For example, *P. vulnus* Allen & Jensen is considered a major pest in perennial crops (6), *P. thornei* Sher & Allen is parasitic primarily on cereal crops (3), and *R. similis* (Cobb) Thorne is an important root pathogen of ornamentals, bananas, and citrus in tropical and subtropical environments (10).

The ovary of a *Pratylenchus* female is single, whereas *R. similis* and *Z. guevarai* (Tobar Jimenez) Braun & Loof females have ovaries that are paired, outstretched, and opposed. Reproduction occurs sexually in *P. vulnus* (11) and *R. similis* (10), but *P. neglectus* (Rensch) Filipjev & Schuurmans Stekhoven reproduces by mitotic parthenogenesis (11). The pattern of reproduction in *Z. guevarai* is unknown but is thought to occur sexually, because males are abundant. In contrast, males are rare in *P. thornei.* The presence of one or two female ovaries and different reproductive mechanisms may have implications in nematode multiplication and pathogenicity.

The use of carrot disks for culturing migratory endoparasitic nematodes under laboratory conditions is a tool generally used and accepted in nematology (5). Large numbers of *R. similis, P. vulnus,* and *Z. guevarai* have been obtained using such cultures (4,7,9,14). *Pratylenchus thornei* and *P. neglectus* have been cultured on alfalfa callus and root explants of corn, tobacco, and red clover (1,8), but no report has been found on the culture of these nematodes on carrot disks.

The objective of this study was to compare final population densities of five migratory endoparasitic nematodes—*P. vulnus, P. neglectus, P. thornei, R. similis,* and *Z. guevarai*—using monoxenic carrot (*Daucus carota* L.) cultures as a substrate (5).

Materials and Methods

Pratylenchus vulnus was isolated from the rhizosphere of rose (*Rosa multiflora* L.) in Cabrils, Barcelona, Spain. *Pratylenchus neglectus* was associated with peach (*Prunus persica* (L.) Batsch) in Artesa, Lerida, Spain. The identification of these two *Pratylenchus* species was confirmed by the Commonwealth Institute of Parasitology, St Albans, United Kingdom. The population of *P. thornei* was provided by Rothamsted Experimental Station, Harpenden, United Kingdom, where it had been maintained on excised corn (*Zea mays* L.) root cultures for many years. *Zygotylenchus gue-*

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[1] Research Nematologists, Departamento de Patología Vegetal, IRTA, Crta de Cabrils s/n, 08348 Cabrils, Barcelona, Spain.

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varai was isolated from the rhizosphere of almond trees (*Prunus amygdalus* Batsch) in Reus, Tarragona, Spain. Carrot disk cultures of *R. similis* were provided by the Centre de Coopération Internationale en Recherche Agronomique pour le Développment (CIRAD), Montpellier, France. This population was isolated originally from banana (*Musa* AAA) in Talamanca, Limón, Costa Rica.

The five nematode species have been maintained on carrot disk cultures for approximately 2 years in the laboratory. Carrot disks (ca. 2 g fresh weight) of the cultivar Nantesa were prepared (7) and were placed singly in 5-cm-d sterile petri dishes. Nematodes from stock cultures were recovered by adding 5 ml sterile water to the cultures. After 24 hours, nematodes that had migrated to the water were collected on a 0.025-mm-pore (500-mesh) screen by washing the culture plate. Nematodes were surface disinfected in a solution of 0.01% mercuric chloride and 1% streptomycin sulfate for 5 minutes and then rinsed in sterile water. Drops of the nematode suspensions were pipetted to 1% water-agar plates, and 10 mature females of each nematode species were hand picked individually and deposited in a drop of sterile water next to a fresh carrot disk. Carrot disks were placed over the droplet of nematode suspension at the end of each nematode inoculation. Ten cultures were prepared for each nematode species and maintained at 26 ± 1 C in the dark for 90 days. The experiment was repeated once.

Nematodes outside the carrot disks were recovered by adding 5 ml distilled water to the culture, and nematodes remaining in the disks were extracted by maceration in a commercial blender (14). Numbers of eggs, juveniles, males, and females were determined from a 1 ml aliquot. Data were transformed to log (x + 1) and subjected to analysis of variance, and means were compared by the Tukey Test ($P = 0.01$).

Results and Discussion

All five migratory endoparasitic nematodes completed their life cycles and multiplied on carrot tissue, but the total numbers recovered per culture varied ($P = 0.01$) depending on the nematode species (Table 1). *Radopholus similis* showed the highest population density, with a 23,400-fold increase in 3 months. Numbers of *R. similis* recovered per culture, however, did not differ ($P = 0.01$) from those of *P. vulnus*. Masses of nematodes swarming on the plate outside the carrot disk were observed in all *R. similis* cultures. This nematode apparently used up the carrot substrate in less time than the other species, because carrot tissue breakdown had occurred and disks were brown in color and looked water soaked. Among the *Pratylenchus* species, *P. vulnus* reached the highest population density. Nematodes also were observed outside of the carrot disks, and the decay of carrot disks had occurred in some of the cultures. Numbers of nematodes recovered from cultures inoculated with *P. thornei* and *Z. guevarai* did not differ and were lower than those of *R. similis* and *P.*

Table 1. Nematodes recovered from carrot disk cultures inoculated with 10 mature females of five migratory endoparasitic nematode species after 90 days at 26 C.

	Eggs/ culture	Juveniles/ culture	Females/ culture	Males/ culture	Total nematodes/ culture
Radopholus similis	24,560 (10)	138,430 (59)	46,380 (20)	24,760 (11)	234,130 a
Pratylenchus vulnus	38,400 (23)	87,561 (53)	20,978 (13)	19,022 (11)	165,961 a
Zygotylenchus guevarai	6,720 (33)	9,180 (46)	3,070 (15)	1,230 (6)	20,201 b
Pratylenchus thornei	8,350 (57)	3,330 (23)	2,940 (20)	0 (0)	14,620 b
Pratylenchus neglectus	111 (15)	233 (31)	400 (54)	0 (0)	744 c

Values in parentheses are percentages of the total population. Numbers followed by the same letter are not significantly different according to the Tukey Test ($P = 0.01$).

vulnus. Although *P. neglectus* showed the lowest population density of the five nematodes tested, the population increased 74-fold in 90 days. Small clusters of nematodes were observed in *P. thornei, Z. guevarai,* and *P. neglectus* cultures, but tissue breakdown did not occur in those cultures.

The majority of the final population consisted of juveniles in cultures inoculated with *R. similis, P. vulnus,* and *Z. guevarai* (59, 53, and 56% of the population, respectively), whereas the egg stage was dominant in *P. thornei* cultures (55%). The adult female was the dominant stage in *P. neglectus* cultures (54%).

Several factors may influence the length of the life cycle and rate of development of species and populations of migratory endoparasitic nematodes. Development is completed between 20 and 50 days, depending on species, infectivity of nematode inoculum, temperature, and host suitability (12). A common host was used in this study; thus, differences found in population densities can be largely attributed to differences in nematode development and, therefore, in life cycle. The temperature (26 C) was within the range in which most *Pratylenchus* species reproduce successfully (12) and has been reported as the optimum for *P. vulnus* reproduction on carrot cultures (2,13). Data on the optimum temperature for reproduction of *Z. guevarai* and *P. thornei* in monoxenic cultures are not available, although 45 days were required by *Z. guevarai* to undergo development from adult to adult on carrot cultures at 25 C (14). Reproduction of *P. neglectus* might have been affected by suboptimal temperatures, thereby resulting in lower numbers of nematodes. Excised corn, tobacco, and red clover root cultures infected with *P. neglectus* yielded high numbers of nematodes when incubated at 30 C (8).

After 3 months, carrot tissue breakdown occurred only in cultures with high population levels, which suggests that high nematode densities may trigger the proliferation of intrinsic microflora within the carrot tissue (7). Carrot disks are maintained, apparently free of contamination, in our laboratory for up to 8 months before nematode inoculations (pers. obs.).

The presence of one or two ovaries in *Pratylenchus* species *R. similis,* and *Z. guevarai* does not appear to affect total population development. Species showing the greatest reproduction in this study were those that reproduced sexually.

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