OBSERVATIONS ON THE PARASITIC BEHAVIOR OF *PRATYLENCHUS MEDITERRANEUS* ON EXCISED POTATO ROOTS

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RESUMEN

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El nematodo lesionador *Pratylenchus mediterraneus* se cultivó monoxénicamente en raíces excisadas de papa cv. Nicola y el comportamiento parasítico se observó mediante el microscopio de luz o el electrónico de barrido. El nematodo atacó las raíces ectoparasíticamente en células apicales o epidermales o en forma endoparásita invadiendo las células corticales de la raíz. Las raíces infectadas mostraron puntas hinchadas, células epidérmicas anormalmente alargadas, pelos radicales y zonas necrosadas conteniendo diferentes estadios. El ciclo de vida se completó en 4 semanas tanto fuera como dentro de la raíz.

Palabras clave: ciclo de vida, patogénesis, *Pratylenchus mediterraneus, Solanum tuberosum.*

INTRODUCTION

Some species of the genus *Pratylenchus* are known to be involved in disease complexes with the fungus, *Verticillium* spp (9). *Pratylenchus mediterraneus*, formerly *P. thornei* (1), a migratory nematode found in the southern regions in Israel and whose distribution, summer survival and histopathology have been previously reported (2,6,7), also was found to be associated with the fungus, *V. dahliae*, in potato early dying disease (5). Little is known on the interaction of the nematode with potato root tissue and the fungus in the disease complex. The objective of this study was to obtain data on the parasitic behavior and mode of parasitism of *P. mediterraneus* on potato roots and structural changes it causes in the roots.

Excised potato (*Solanum tuberosum* cv. Nicola) root tips, 2-3 cm long, were obtained from plantlets grown under aseptic conditions (4). The excised roots were cultured on half strength Skoog, Tsui and Whites' medium (8) and kept in the dark at a constant temperature of 20°C. Populations of *P. mediterraneus* were maintained on *Trifolium* callus on Skoog, Tsui and Whites' medium enriched with 2 ppm 2,4-D. Eggs were obtained from 8-10 week-old cultures using micro Baermann funnels under aseptic conditions. Twenty-four hours after the transfer of the potato root tips, a drop of *P. mediterraneus* egg suspension containing 15+5 eggs was pipetted into each of 20 Petri dishes at a distance of 3 cm from the root segments.

Light microscopy observations of the nematode development, parasitic behavior and the reaction of the infected root were made daily. Root samples were processed for scanning electron microscopy (SEM) with the conventional fixation and dehydration by the critical point method (10) for external features and using the

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polyethyleneglycol embedding and sectioning method (3) for histological observations.

Second-stage juveniles (J2) emerged from the eggs 2-7 days following inoculation; however, 80% of the eggs hatched within 4 days following inoculation. Nematode traces (tracks) were observed all over the media surface, but higher densities of the traces were visible close to the roots. Some J2 concentrated around the root tips and fed on them as ectoparasites (Fig. 1A). After 2-4 days, infected root tips ceased growing and became swollen with large hypertrophied epidermal cells (Fig. 1B, 1C).

The nematodes also behaved as endoparasites. A large proportion of the infective individuals invaded the root cortical parenchyma. At the infected sites, the roots became dark and somewhat swollen (Fig. 1F). In SEM observations these root surfaces seemed to be coated with a slimy substance that may have leaked from the injured tissues (Fig. 1G). Quite a few roots were completely covered by extensive brown to black lesions harboring all the nematode developmental stages. Some roots adjacent to infected ones remained intact and were distinguished by their bright color (Fig. 1G). In lesion sections, individuals of *P. mediterraneus* were observed inside the cortical parenchymal cells. It appeared that the nematodes were moving from one cell to the neighboring one feeding on the cell content. These cells appeared empty and the cell-wall punctured (Fig. 1H).

By the third week following inoculation, juveniles that remained outside the roots ceased feeding and stayed motionless beside the roots. At this stage the juveniles underwent molting, probably three consecutive molts and became adult females. No males were observed in the cultures. At four weeks following inoculation, eggs were laid. Many eggs were observed along the roots by females completing their life cycle outside the roots, or by females migrating from root lesions. Many eggs also were laid within the infected lesions. Under the experimental conditions of this study, one life cycle of *P. mediterraneus* was completed in approximately 4 weeks.

The second-stage juvenile (J2) of *P. mediterraneus* hatched from the egg and in a matter of few days migrated toward the potato roots. The observation that the nematodes were not evenly distributed alongside and within the roots but were concentrated in certain sites (1A, 1F) suggests that these sites were more attractive to the nematodes. Since all the roots in a culture plate were derived from a single root tip and grown under the same controlled conditions, it is suggested that a root part that was randomly attacked by a nematode attracted more nematodes possibly due to signals derived from the substances leaking from the injured root.

Some of the nematodes behaved as ectoparasites feeding on the root tips and on epidermal cells which reacted by swelling both in the apex region and along the root. Although ectoparasitic feeding of *P. penetrans* has been already described by Zunke (11,12), such a host response has not been reported.

Typical to species of the genus *Pratylenchus*, individuals of *P. mediterraneus* invaded the potato roots, fed and completed their life cycle within the tissues of the root cortex as endoparasites causing the appearance of lesions. These observations indicate cell content leaking from the lesions may serve as a rich medium for the development and reproduction of soil microorganisms. Indeed, an association between the nematode and bacteria was demonstrated in *P. mediterraneus* infected common vetch root (7). It is possible, therefore, that the interaction between *P.*

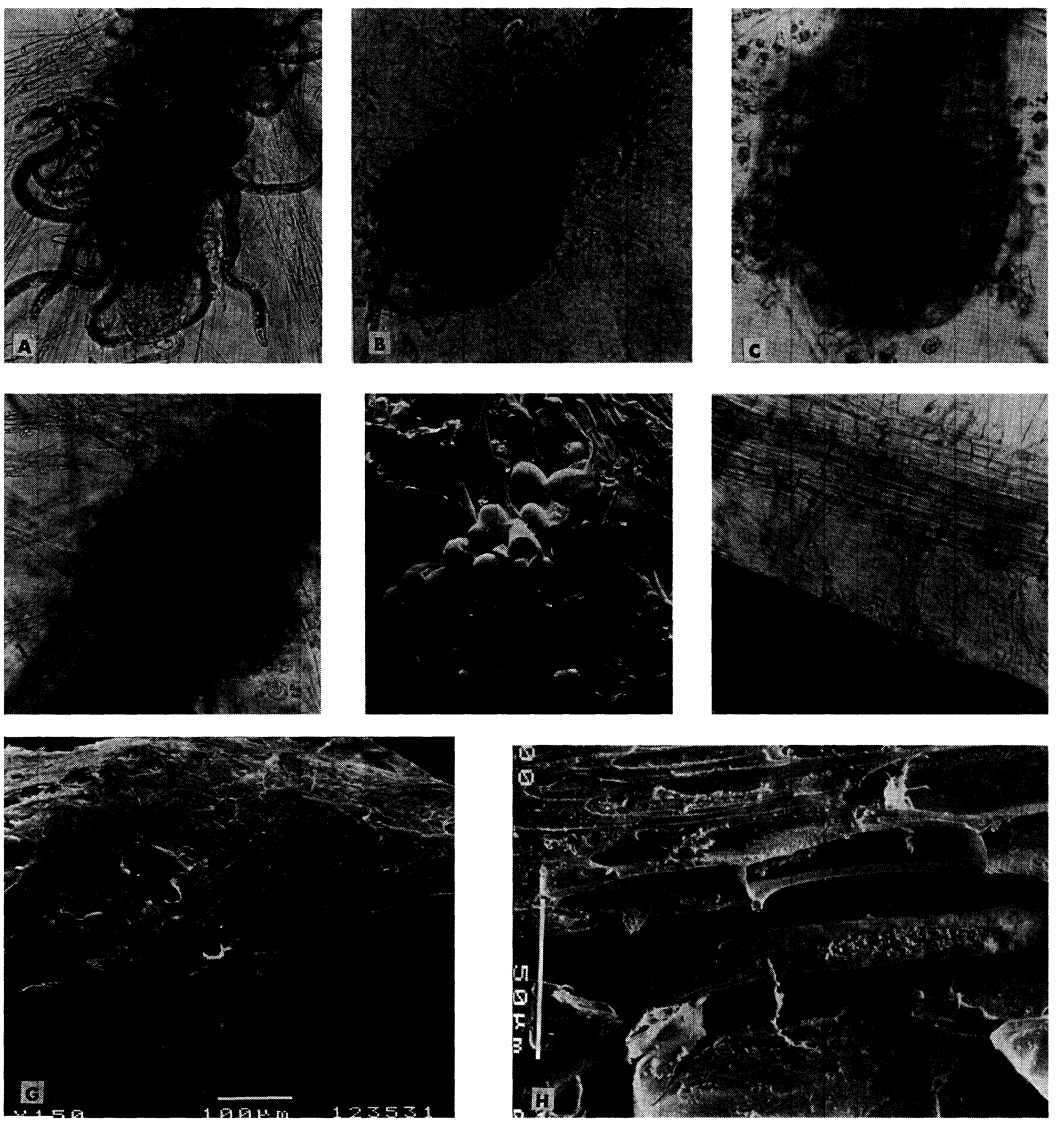
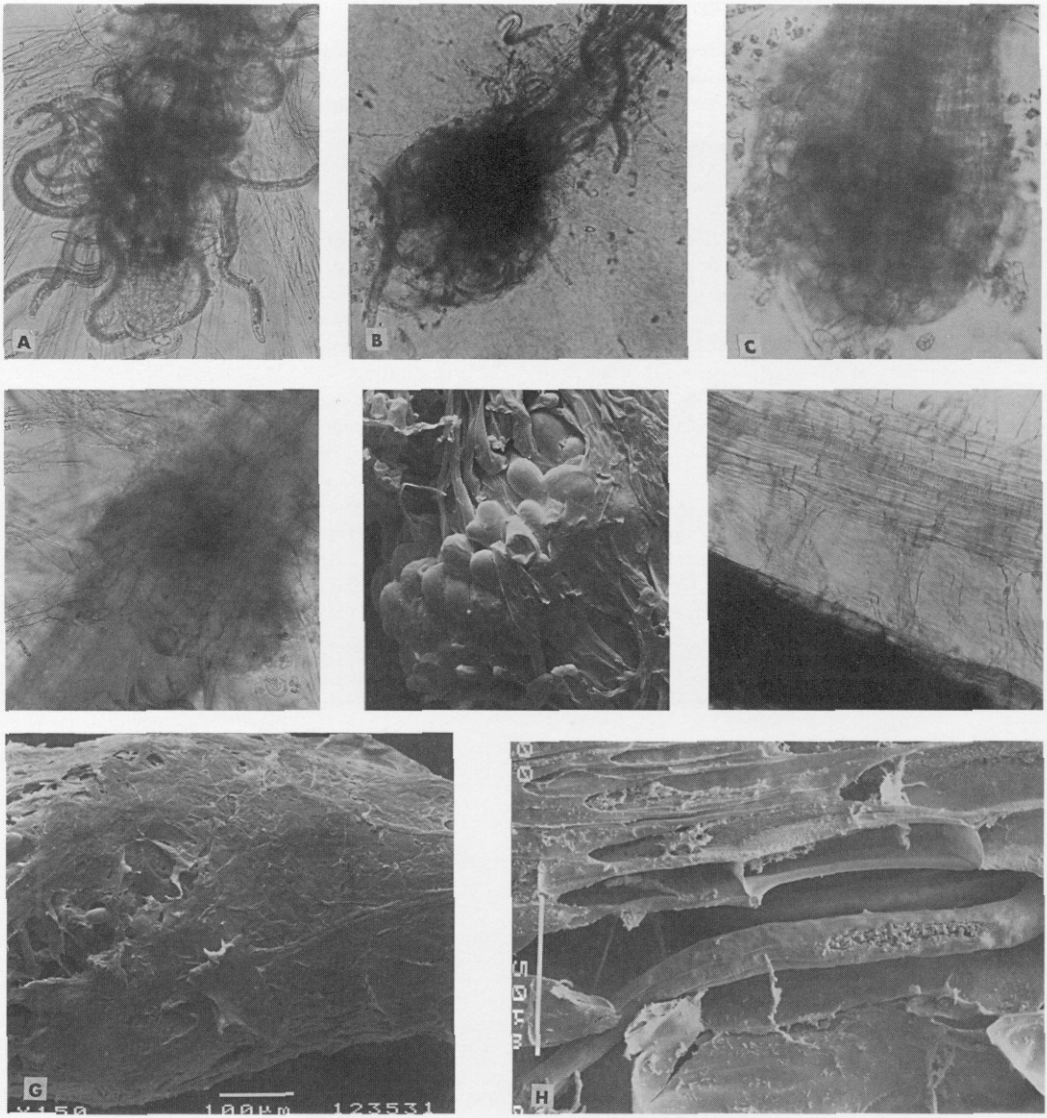


Fig. 1. Response of excised potato roots to *Pratylenchus mediterraneus* infection. A. Second-stage juveniles aggregating and feeding on potato root tip (×63). B. A swollen root tip 2-3 days following the nematodes attack (×63). C. Large cells on the surface of swollen root tip (×63). D. Nematodes feeding on root epidermal cells (×63). E. Scanning electron micrograph of root showing feeding by the nematodes. Note the large epidermal cells. F. A discolored root infected with the nematodes and an adjacent intact root (×250). G. Scanning electron micrograph of a swollen lesion. Note the cracks in the epidermis and the coating on the lesion surface. H. Scanning electron micrograph of a nematode moving within the root parenchymal cells in a longitudinal section of a lesion.

mediterraneus and *V. dahliae* in potato early dying disease takes place by enhancing the growth of the fungus saprophytic stage on the lesion surface, or in its immediate vicinity, thus amplifying the fungus inoculation potential.



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