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Penetration and Feeding Behavior of *Pratylenchus penetrans* (Nematoda: Pratylenchidae) In Red Radish Roots

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Abstract: In this study, *Pratylenchus penetrans* which is known the root-lesion nematode were inoculated onto red radish seedlings growing in agar on microscope glass slides. The feeding and penetration behaviour of nematodes were observed for three weeks. Patterns of behaviour identical to those described for other nematodes were recognized. Most nematodes migrated to the root hair zone and selected an epidermal cell by rubbing the cell surfaces with lips and stylet. When feeding stylet thrusting, salivation, predigestion and ingestion phases were recognized. Penetration through cells, fed upon or not, occurred after the nematodes bore a series of holes in the cell wall and forced their way through. Penetration of roots via the root hairs is reported.

Keywords: *Pratylenchus penetrans*, the root-lesion nematode, penetration, feeding behaviour

Kırmızı Turp Köklerinde *Pratylenchus penetrans* (Nematoda: Pratylenchidae)'ın Beslenme Davranışı ve Penetrasyonu

Özet: Bu çalışmada, kök-lezyon nematodu olarak bilinen *Pratylenchus penetrans*, mikroskop lamları üzerinde agarda yetiştirilen kırmızı turp fidelerine aşılandı. Nematodların penetrasyon ve beslenme davranışı üç hafta boyunca gözlendi. Bu davranış şekillerinin, diğer nematodlar için tarif edilenlerle aynı olduğu anlaşıldı. Birçok nematod kök tüyü bölgesine yerleşip, dudakları ve iğnesi ile hücre yüzeylerine zarar vererek, bir epidermal hücre seçti. Beslenme anında iğne saplamaları, salgı yapma, ön sindirim ve sindirim kanalına alma safhaları kaydedildi. Hücrelere olan penetrasyonda, beslenme olsun ya da olmasın, hücre duvarında bir seri delik oluşturuldu. Bu faaliyet baştan sona oldukça zor gerçekleşti. Kök penetrasyonunun, kök tüyleri aracılığı ile olduğu kaydedildi.

Anahtar kelimeler: *Pratylenchus penetrans*, kök-lezyon nematodu, penetrasyon, beslenme davranışı

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Introduction

Root-lesion nematodes are migratory endoparasites. Reproduction is sexual. Females lay eggs singly in roots or in soil. Second-stage juveniles hatch from eggs, feed, and undergo three molts to the adult stage. The complete life cycle takes 30 to 86 days, depending on temperature, and is shortest at 30 C. Adults, J2, J3 and J4 can all invade roots. If conditions in the root become unfavorable any juveniles stage or the adult nematodes may leave the root and invade other nearby roots. Invasion usually takes place in the region of elongation (Doncaster 1971, Siddiqi 1975).

Root-lesion nematodes feed upon cells in the root cortex. Cells are killed and in many instances small roots are killed. The migratory parasitism of this nematode opens up roots to secondary invasion by other soil microorganisms such as fungi and bacteria. In some hosts such as walnut, and stone fruits the nematodes may establish colonies in larger roots and large necrotic lesions are formed. Plants infested with root-lesion nematodes grow poorly, the foliage is frequently chlorotic and sparse and the terminal growth of branches may be suppressed. Crop yields are reduced. Seedlings planted in infested soil frequently fail to grow to normal size and may never produce satisfactory trees or vines (Siddiqi 1975).

The feeding behaviour of migratory endoparasitic nematodes is poorly documented relatively to that of ectoparasites (Doncaster 1971, Wyss 1981. Yu and Potter 1988, Wyss and Grundler 1992). Ectoparasitic species of phytoparasitic nematodes of the families *Paratylenchidae, Tylenchidae, Belonolaimidae* and *Trichodoridae* are known to feed on root hairs and the effects vary. For example, feeding on root hairs by *Tylenchorhynchus dubius* eventually caused cell death (Wyss 1973, 1987) but the effect was less rapid than that caused by *Trichodorus spp*., where the death of attacked root hairs probably resulted from the destruction of the tonoplast at the onset of ingestion (Wyss 1982). Doncaster and Seymour (1973) analyzed the behaviour of eleven species of *Tylenchida*. They systematized the different stages of exploration, penetration and feeding and presented a set of assumptions to explain the observed behaviour of these nematodes. Whereas the movements of *Pratylenchus* species are difficult to follow inside of roots, their histopathology, namely the formation of lesions, is easily seen and well documented (Townshend 1963a, 1963b, Troll and Rohde 1966, Mamiya 1970, Acedo and Rohde 1971, Corbett 1972, Pinochet 1978, Hussey 1989, Lee 2002).

Pratylenchus penetrans (Cobb 1917) Filipjev and Schuurmans-Stekhoven, 1941 is an obligate plant parasitic nematodes with a simple life cycle. It prefers to enter roots in the region of root hair development, although the zone of elongation can also be invaded (Troll and Rohde 1966; Townshend 1978; Tsai and Van Gundy 1990). *P. penetrans* feeds mainly on the cortical cells, where cavities are formed when the tissue collapses. In most plant host roots these cavities can be seen a few hours to a few days after penetration as discrete brownish lesions of varying sizes (Townshend 1963b, Acedo and Rohde 1971, Everts et al. 2004) but not in cereals (Troll and Rohde 1966).

The object of this study was to observe the penetration and feeding processes of *P. penetrans* in red radish roots.

Material and Methods

Small red radish *Raphanus sativus* L. cv. Radicula (Kadirli-Turkey, 37° 22.2' N; 36° 04.2' E) plantlets were soaked for 1 minute in 0.5 % sodium hypochlorite solution and rinsed four times in sterile water. Thin layers of 0.8 % water agar containing two drops of Hoagland solution were poured over glass slides (25 x 82 mm) and covered partially with a cover slip (22 x 40 mm). One plantlet was placed onto the agar at the uncovered end of each glass slide. The plantlets on the slides were kept in a petri dish on a moist filter paper at 20 °C, under fluorescent tubes with daylight of 16 hours. The slides were set vertically so that the roots grew underneath the coverslip.

All stages of *Pratylenchus penetrans*, extracted from infected red radish roots, were used for inoculum. The nematodes were axenized in 72 hours in a 4 ppm solution of methoxy ethyl mercury chloride with sterile air bubbling through the solution. When the red radish plantlets had grown four or five roots, 1.5 cm in length, twenty five sterile nematodes were handpicked and placed near the roots. Red radish plantlets were also grown in sandy loam in 20 ml polystyrene microbeakers and inoculated to study histopathology.

For the behavioural study, 32-35 nematodes on or inside of roots were observed for three weeks at 400 or 1000 x magnification without precautions to keep the preparations sterile. For the ultrastructural study, the slides with plants and agar, and pieces of roots from the inoculated plants growing in sandy loam, were placed in liquid nitrogen. The frozen roots were placed in plastic tubes 13 mm long, 8 mm diameters, closed at the bottom with 15 µm pores nylon sieve. The roots were fixed in 4 % glutaraldehyde in 0.05 M cacodylate buffer (pH: 6.8) for 12 hours. The roots growing in agar were placed in warm 0.05 M cacodylate buffer to remove the agar. All roots were post fixed in 2 % osmium tetroxide for two hours and dehydrated in an ethanol series starting with 20 % ethanol. When in 70 % ethanol the roots were examined under a stereomicroscope and pieces 2 – 2.5 mm long were selected for further dehydration and embedding in Epon. After this treatment, roots critically point dried for SEM. The embedded material was cut into 0.5 to 1.5 µm thick sections, with an ultramicrotome and stained with a polychrome stain (Van Reempts and Borgers 1975).

Results and Discussion

Most nematodes moved to the root hair region within two hours of being transferred to the agar. They explored the root by touching the surface of epidermal cells with their lips, and protracting their stylet enough to touch but not to pierce the cell walls. Some nematodes explored root hairs along their entire length. Very seldom were they seen exploring a root tip or cell elongation zone and these areas were never fed upon or penetrated.

surfaces Following local exploration of cell the area beside an intercellular wall would be pierced, and the stylet thrusted several times into the cell. The epidermis cell walls appeared very elastic and difficult to puncture as many nematodes had to repeatedly thrust their stylet to get through. For their initial feeding, before penetration, some nematodes did not puncture an epidermal cell wall but instead pushed their stylet intercellularly to the cortical cell beyond. Traces of feeding on epidermal cells, for example the minute holes made through the cell walls, were difficult to recognize with certainty in scanning electron microscopy (SEM) or semi-thin sections.

Root hairs were sometimes penetrated but never fed upon. The nematodes would pierce the cell walls six to eight times along a line, then tear a hole (Fig. 1) by pressing their head and waving it vigorously until it was inside the root hair. Sometimes they would drawback and open another hole on the opposite side of the root hair. The cytoplasm of these cells circulated vigorously during and after nematode penetration, then the cells would collapse rapidly. Several nematodes penetrated the roots via the root hairs, through the basal epidermal cells to the cortical tissue.

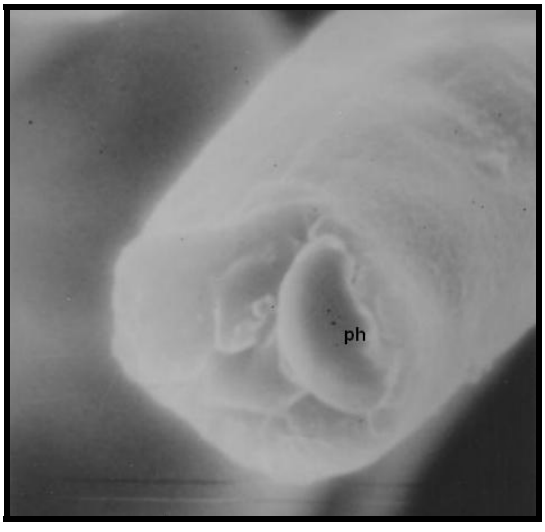

Figure 1. Penetration hole (ph) in root hair of red radish after *P. penetrans* entered the root. (SEM, x 40.000)

Once their stylet was inside an epidermal or a cortical cell, the nematodes stopped moving. During salivation the stylet was sometimes reorientated within the cell. Their median bulb pulsated only three to five times in 8 or 10 seconds, a globular secretion was seen passing from the stylet tip into the cell, and the movement of the cytoplasm became more rapid. The nematodes were inactive for 4 to 6 minutes before ingestion began. Their median bulb contracted a few times in the next 4 minutes, and then pulsated at several contractions per second for 6 to 8 minutes. There was only one salivation and one ingestion period in the epidermal cells, before the nematodes penetrated (Fig. 2).

Several nematodes would often penetrate neighboring epidermal cells or even go through the same hole in an epidermal cell. The nematodes moved through epidermal cells to cortical cells centripetally, and once in the cortex they migrated along the root length, moving slowly from cell to cell, making every time a row of holes and pushing their head through into the adjacent cells. The cavities made by the collapse of cells fed upon and the penetration of the nematodes were quickly colonized by bacteria (Fig. 3).

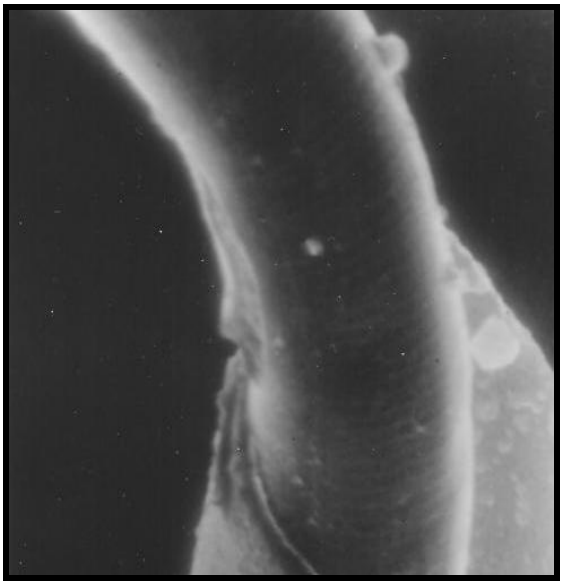

Figure 2. Nematode penetrating epidermal tissue (et). (SEM, x 20.000)

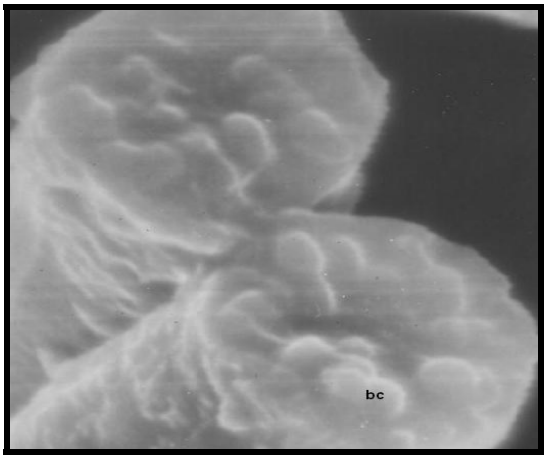

Figure 3. Penetration hole in epidermal cell after *P. penetrans* entered the the root, numerous bacterium colonies (bc) are found near the opening. (SEM, x 60.000)

All the nematodes under observation remained inside the roots for the duration of the experiments and several eggs were laid in the cortex. In the injured cortical cells the intercellular areas were enlarged and intensely colored in light blue in the polychrome stained semi-thin sections, whereas uninvaded areas of the cortex did not take any stain. In the three weeks of the experiment the nematodes did not penetrate the endodermis and did not migrate to the zone of differentiation, the root tip area, or the zone of initation of lateral roots.

The behaviour of *P. penetrans* follows the pattern recognized for most other nematodes (Doncaster 1971, Wyss 1981). The nematodes migrated almost exclusively to the root hair zone to feed and penetrate. Once in the feeding zone of the root they selected an epidermal cell or a root hair and probed the cell surface with lips and stylet to find a suitable penetration site. The nematodes were somewhat immobile while injecting their saliva which appeared as opaque globules similar similar to those described in other nematodes (Wyss 1981).

The role of the saliva is to lower the viscosity of the cytoplasmic material to be ingested, then the few slow contractions of the median bulb preceding the true ingestion period might indicate a monitoring of the external digestion process by the nematode. Whether the cell content was ingested or not a series of

stylet thrust punctured and weakened the cell wall along a line through which the nematodes forced its way into the cell, as described for *P. crenatus* (Klinkenberg 1963) and *Helicotylenchus dihystera* (Jones 1978; O'Bannon and Inserra 1989).

Temperature influences the speed of feeding and penetration behaviour (Townshend 1978; Pudosaini et al. 2008; Boag 1980). In my experiment the speed of stylet thrusting could not be analyzed accurately. The observations were not done at a constant temperature under the microscope and the data given are averages of many observations.

Penetration of roots via the root hairs has not been reported for other endoparasitic nematodes. However, although observed several times, it did not constitute a significant route to the cortex. Penetration through epidermal cells was more common. Feeding on the endodermis was not observed in the three weeks of my experiments, although other researchers have shown that *Pratylenchus* spp. will eventually migrate to and feed on this tissue (Mamiya 1970, Acedo and Rohde 1971, Corbett 1972, Pinochet 1978, John and Hague 1974, Bird 1977).

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