Penetration and feeding behavior of *Pratylenchus penetrans* in strawberry roots

Sirpa KURPPA* and Thierry C. VRAIN

Agriculture Canada Research Station, 6660 N. W. Marine Drive, Vancouver, B. C., Canada V6T 1X2.

SUMMARY

Pratylenchus penetrans were inoculated onto strawberry seedlings growing in agar on microscope glass slides. The feeding and penetration behavior of nematodes were observed for two weeks. Patterns of behavior identical to those described for other nematodes were recognized. Most nematodes migrated to the root hair zone (widespread exploration), and selected an epidermal cell by rubbing the cell surfaces with lips and stylet (local exploration). 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.

RÉSUMÉ

Comportement de Pratylenchus penetrans *durant l'invasion des racines de fraisier et la prise de nourriture*

Des plantules de fraisier ont été cultivées sur lame gélosée, de manière que les racines puissent croître entre lame et lamelle. Le comportement de 25 individus du nématode *Pratylenchus penetrans,* inoculés près des racines, a été observé durant la prise de nourriture et l'invasion des racines pendant près de deux semaines. Des modes de comportement similaires à ceux décrits pour d'autres nématodes parasites ont été reconnus. La plupart des nématodes se sont déplacé vers la zone pilifère (attirance de loin) et ont choisi une cellule du rhizoderme ou de l'assise pilifère en explorant la surface de la racine par contact de la zone labiale ou du stylet (exploration locale). La prise de nourriture s'est effectuée par l'insertion répétée du stylet au travers de la paroi cellulaire, par l'injection de sécrétions digestives dans la cellule (salivation) et enfin par la prédigestion et l'ingestion du cytoplasme cellulaire. Les nématodes ont pénétré dans les cellules, dont ils s'étaient nourris ou non, en perçant avec leur stylet une série de trous alignés, affaiblissant ainsi une des parois cellulaires et y forçant un passage. Certains nématodes ont pénétré dans les racines en passant par les poils absorbants.

The feeding behavior of migratory endoparasitic nematodes is poorly documented relatively to that of ectoparasites (Doncaster, 1971; Wyss, 1981). Doncaster and Seymour (1973) analyzed the behavior 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 behavior 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 & Rohde, 1966; Mamiya, 1970; Acedo & Rohde, 1971; Corbett, 1972; Pinochet, 1978).

Pratylenchus penetrans (Cobb) Filip. & Sch. Stek. prefers to enter roots in the region of root hair development, although the zone of elongation can also be invaded (Troll & Rohde, 1966; Townshend, 1978). *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 à few hours to a few days after penetration as discrete brownish lesions of varying sizes (Townshend, 1963b; Acedo & Rohde, 1970), but not in cereals (Troll & Rohde, 1966).

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

Materials and methods

Small strawberry plantlets, cv. U. C. 11, were soaked for 1 min in 0.5 % sodium hypochlorite solution and rinsed three times in sterile water. Thin layers of 0.8 % water agar containing two drops of Hoagland solution were poured over glass slides (25 × 82 mm) and covered partially with a cover slip (22 × 40 mm). One plantlet

* Present address : Agricultural Research Center, 31600 - Jokioinen, Finland.

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 25°, under fluorescent tubes with daylight of 16 h and light intensity of 37 μ Einstein $m^{-2}s^{-1}$. The slides were set vertically so that the roots grew underneath the coverslip.

All stages of *Pratylenchus penetrans*, extracted from infected raspberry roots, were used for inoculum. The nematodes were axenized in 72 hours in a 4 ppm solution of methoxy ethyl mercury chloride (Aretan®), with sterile air bubbling through the solution (modified from Townshend, 1963a). When the strawberry plantlets had grown two to three roots, 1 cm in length, twenty sterile nematodes were handpicked and placed near the roots. Strawberry plantlets were also grown in sand in 20 ml polystyrene microbeakers and inoculated to study histopathology.

For the behavioral study, 25 nematodes on or inside of roots were observed for two weeks at 400 or 1,000 × 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 sand, were placed in liquid nitrogen. The frozen roots were placed in plastic tubes (BEEM® embedding capsules, size 00) 13 mm long, 8 mm diam., closed at the bottom with 15 μm pores nylon sieve. The roots were fixed in 4 % glutaraldehyde in 0.05 M sodium 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 serie starting with 20 % ethanol. When in 70 % ethanol the roots were examined under a stereomicroscope and pieces 1-2 mm long were selected for further dehydration and embedding in Epon 812® or, 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 & Borgers, 1975).

Observations and results

Most nematodes moved to the root hair region within three 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.

Following local exploration of cell surfaces 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, *i.e.* the minute holes made through the cell walls, were difficult to recognize with certainty in SEM or semi-thin sections.

Root hairs were sometimes penetrated but never fed upon. The nematodes would pierce the cell walls seven to ten times along a line, then tear a hole (Fig. 1A) 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 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.

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 two to three times in 5 or 6 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 2 to 5 mn before ingestion began. Their median bulb contracted a few times in the next 2 mn, and then pulsated at several contractions per second for 7 to 8 mn. There was only one salivation and one ingestion period in the epidermal cells, before the nematodes penetrated (Fig. 1B).

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. 1C and D).

All the nematodes under observation remained inside the roots for the duration of the experiments and many eggs were laid in the cortex. In the injured cortical cells the intercellular areas were enlarged and intensely colored in blue in the polychrome stained semi-thin sections, whereas uninvaded areas of the cortex did not take any stain. In the two 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.

Discussion

The behavior of *Pratylenchus penetrans* follows the pattern recognized for most other nematodes (Doncas-

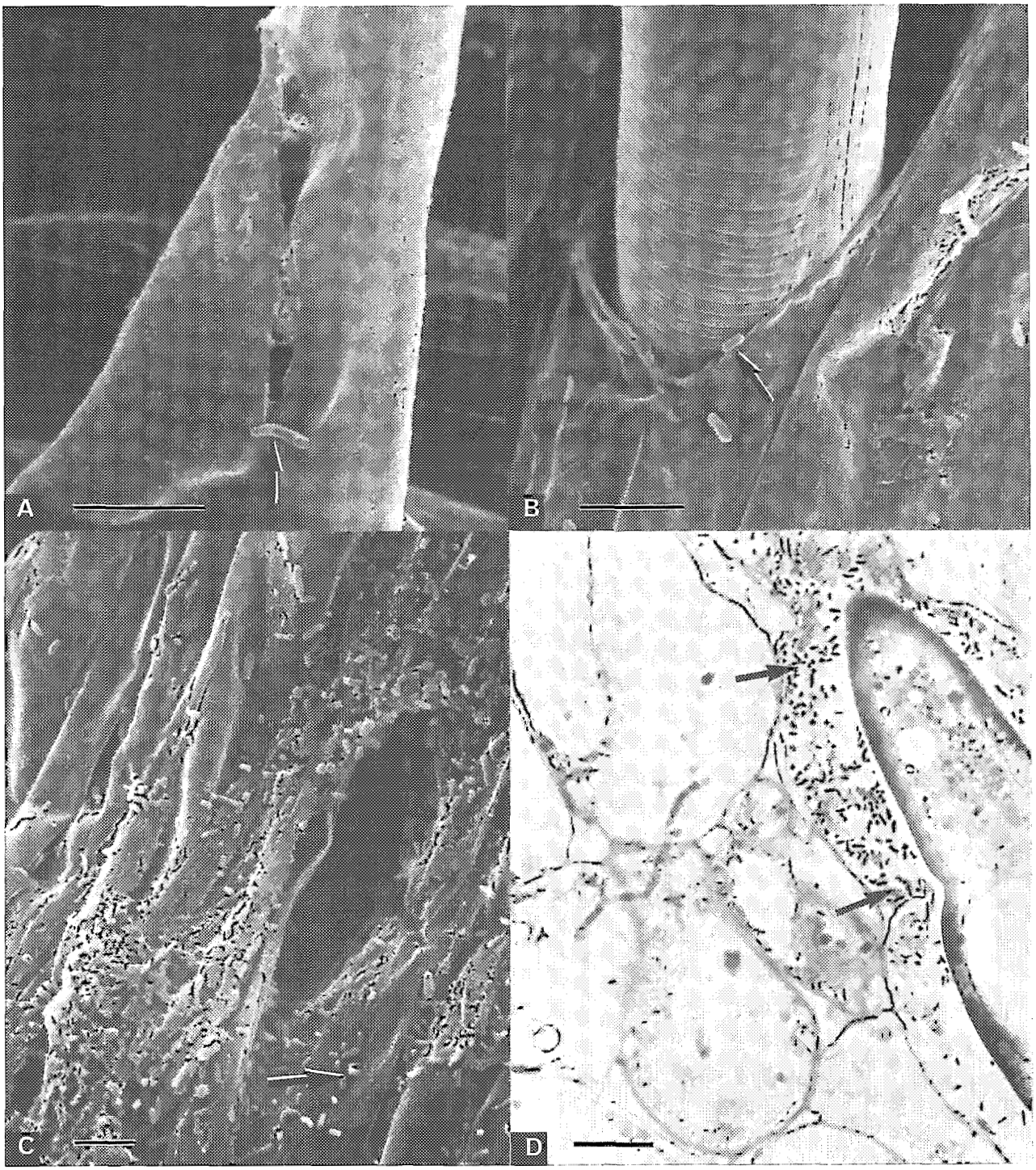

Fig. 1. A : penetration hole in root hair of strawberry after *Pratylenchus penetrans* entered the root and the root hair cell has collapsed. Arrow shows bacteria; B : nematode penetrating epidermal cell; C : penetration hole in epidermal cell after *Pratylenchus penetrans* entered the root; numerous bacteria are found near and in the opening; D : cavity formed by *Pratylenchus penetrans* in cortex of strawberry root; the cavity is rapidly colonized by bacteria. (A, B, C : SEM; bar = 5 µm).

ter, 1973; Wyss, 1981). The nematodes migrated almost exclusively to the root hair zone to feed and penetrate (widespread exploration). 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 (local exploration). The nematodes were somewhat immobile while injecting their saliva which appeared as opaque globules similar to those described in other nematodes (Wyss, 1981).

If we assume that the role of the saliva is to lower the viscosity (predigestion) 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 *Pratylenchus crenatus* (Klinkenberg, 1963), and *Helicotylenchus dihystera* (Jones, 1978).

Temperature influences the speed of feeding and penetration behavior (Townshend, 1978, Boag, 1980). In our experiment the speed of stylet thrusting and metacorpus pulsations 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 endoparasites. 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 two weeks of our experiments, although others (Acedo & Rohde, 1971; Mamiya, 1971; Corbett, 1972; Pinochet, 1978) have shown that *Pratylenchus* spp. will eventually migrate to and feed on this tissue.

References

Acedo, J. R., & Rohde, R. A., (1971). Histochemical root pathology of *Brassica oleracea capitata* L. infected by *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven (Nematoda : Tylenchidae). *J. Nematol.*, 3 : 62-68.

Boag, B. (1980). Effects of temperature on rate of feeding of the plant parasitic nematodes *Rotylenchus robustus*, *Xiphinema diversicaudatum* and *Hemicycliophora conida*. *J. Nematol.*, 12 : 193-195.

Corbett, D. C. M. (1972). The effect of *Pratylenchus fallax* on wheat, barley and sugarbeet roots. *Nematologica*, 18 : 303-308.

Doncaster, C. C. (1971). Feeding in plant parasitic nematodes. Mechanisms and behavior. In : Zuckerman, B. M. & Rohde, R. A. (Eds) : *Plant Parasitic Nematodes*, Vol. II, New York, Academic Press : 137-157.

Doncaster, C. C. & Seymour, M. K., (1973). Exploration and selection of penetration site by Tylenchida. *Nematologica*, 19 : 137-145.

Jones, R. K. (1978). The feeding behavior of *Helicotylenchus* spp. on wheat roots. *Nematologica*, 24 : 88-94.

Klinkenberg, C. M. 1963. Observations on the feeding habits of *Rotylenchus uniformis*, *Pratylenchus crenatus*, *P. penetrans*, *Tylenchorhynchus dubius* and *Hemicycliophora similis*. *Nematologica*, 9 : 502-506.

Mamiya, Y. (1970). [Parasitism and damage of *Pratylenchus penetrans to Cryptomeria* seedlings.] *J. Jap. For. Soc.*, 52 : 41-50.

Pinochet, J. (1978). Histopathology of the root lesion nematode *Pratylenchus coffeae* on plantains, Musa AAB. *Nematologica*, 24 : 337-340.

Townshend, J. L. (1962). The root lesion nematode *Pratylenchus penetrans* (Cobb, 1917) Filip. & Stek. 1941, in celery. *Can. J. Pl. Sci.*, 42 : 314-322.

Townshend, J. L. (1963a). The pathogenicity of *Pratylenchus penetrans* to celery. *Can. J. Pl. Sci.*, 43 : 70-74.

Townshend, J. L. (1963b). The pathogenicity of *Pratylenchus penetrans* to strawberry. *Can. J. Pl. Sci.*, 43 : 75-78.

Townshend, J. L. (1978). Infectivity of *Pratylenchus penetrans* on alfalfa. *J. Nematol.*, 10 : 318-323.

Troll, J. & Rohde, R. A. (1966). Pathogenicity of *Pratylenchus penetrans* and *Tylenchorhynchus claytoni* on turfgrasses. *Phytopathology*, 56 : 995-998.

Van Reempts, J., & Borgers, M. (1975). A simple polychrome stain for conventionally fixed epon embedded tissue. *Stain Technol.*, 50 : 19-24.

Wyss, V. (1981). Ectoparasitic root nematodes : Feeding behavior and plant cell responses. In : Zuckerman, B. M. & Rohde, R. A. (Eds) : *Plant Parasitic Nematodes*, Vol. III. New York, Academic Press : 325-321.

Accepté pour publication le 22 mai 1985.