Ectoparasitic feeding behaviour of the root lesion nematode, *Pratylenchus penetrans,* on root hairs of different host plants

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SUMMARY

The feeding behaviour of *Pratylenchus penetrans* on root hairs was observed using video-enhanced contrast microscopy. The behaviour could be separated into phases of probing, cell penetration by the stylet, salivation and food ingestion for brief and extended periods. After cell penetration, a small " salivation zone " was formed around the stylet tip. No feeding tubes were observed. The root hairs responses to nematode feeding included increased rate of cytoplasmic streaming and gradual hypertrophy of the root hair nucleus. The observations are discussed in relation to work on the ectoparasitic feeding behaviour of nematodes.

RÉSUMÉ

Comportement nutritionnel ectoparasitique de Pratylenchus penetrans *sur les poils absorbants de différentes plantes-hôtes*

Le comportement nutritionnel de *Pratylenchus penetrans* sur les poils absorbants a été observé en microscopie assistée par vidéo à haute résolution. Le comportement peut être divisé en plusieurs phases : reconnaissance, pénétration de la cellule par le stylet, salivation et ingestion de nourriture pendant des périodes de durée variable. Après sa pénétration dans la cellule, une « zone de salivation » restreinte se forme autour de l'extrémité du stylet. Il n'a pas été observé de tube nutritionnel. La réaction des poils absorbants à la nutrition du nématode se traduit par une augmentation des mouvements cytoplasmiques et une hypertrophie progressive du noyau. Ces observations sont discutées à la lumière des travaux relatifs au comportement nutritionnel ectoparasitique des nématodes.

Ectoparasitic species of phytoparasitic nematodes of the families Paratylenchidae, Tylenchidae, Belonolaimidae (Tylenchorhynchinae) 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). The effect of feeding of *Paratylenchus projectus* on root hairs varied with host plant; whereas the protoplasts of red clover root hairs contracted and disappeared after being fed on by juveniles for several days, the cytoplasm in tobacco root hairs continued to stream apparently normally during feeding (Rhoades & Linford, 1961).

The feeding behaviour of the migratory endoparasitic nematode, *Pratylenchus penetrans,* has only recently been studied in detail. Kurppa and Vrain (1985) observed penetration and feeding behaviour over a period of two weeks and found patterns of behaviour identical to those described for ectoparasitic nematodes (Doncaster, 1971; Doncaster & Seymour, 1973; Jones, 1978; Wyss, 1981). Although Kurppa and Vrain (1985) observed some individuals of *P. penetrans* exploring and occasionally penetrating root hairs, they did not observe nematodes feeding on them. However, Zunke and Inst. wiss. Film (1988), observed this species feeding on root hairs of various host plants. This paper presents the results of observations, using high resolution video-enhanced contrast microscopy, on the feeding behaviour of different developmental stages of *P. penetrans* on root hairs.

Materials and methods

P. penetrans was obtained from cultures supplied by R. M. Webb (Rothamsted) and J. Rössner (Giessen). Nematodes were subsequently maintained on monoxenic excised root cultures of maize *(Zea mays)* and rape (*Brassica napus* cv. Akela) grown on nutrient agar medium (Müller, 1978).

For observations of feeding behaviour, nematodes of all stages were inoculated onto seedlings of rape (*B. napus* cv. Akela), oil radish *(Raphanus sativus* var. *oleiformis),* tobacco (*Nicotiana tabacum* cv. Samsun) and potatoes (*Solanum tuberosum* cv. Hansa) which were grown in aseptic nutrient agar in special observation chambers as described by Wyss and Zunke (1985). The chambers were placed under a Reichert Polyvar light microscope with differential interference contrast optics.

Feeding behaviour was recorded on 2.5 cm video tapes using video-contrast enhancement (Wyss & Zunke, 1986*a;* Zunke & Wyss, 1986; Zunke, 1988) and were analysed where required by single frame evaluation (Wyss & Zunke, 1986*b;* Zunke, 1988; Perry, Zunke & Wyss, 1989).

Results

The feeding behaviour of *P. penetrans* was the same on all host plants examined and could be separated into probing, salivation and food ingestion for brief or extended periods.

After inoculation, the majority of nematodes moved directly to the root hair region (Fig. 1 A) and, on contact with a root hair, started rubbing the root hair surface with the lip region and then probing it with the stylet (Fig. 1 B). The stylet was inserted (Fig. 1 B) slowly into the root hair with increasingly deeper thrusts. After the stylet had finally been inserted to a length of approximately 2 µm, the nematode commenced salivating for a few minutes and then the median bulb of the oesophagus started pumping, indicating food uptake.

If cytoplasmic streaming in root hairs was slow, the median bulb pulsated (Fig. 1 C) only for few seconds before the stylet was withdrawn leaving a drop of saliva within the cell, which probably aggregated with some of the thin cytoplasmic layer in this area (Fig. 1 D).

Although all stages were observed to probe and feed on root hairs, the adults soon moved into the root to feed endoparasitically. Juveniles, especially the second (J2) and third stages (J3), remained feeding on root hairs for longer periods, often over 10 min. Occasionally, juveniles were observed to enter epidermal cells through holes previously made by adults penetrating the root.

Brief feeding

Brief feeding on root hairs occurred for periods of a few seconds to about 2 min and could be contrasted with extended feeding which occurred over many minutes. During brief feeding, there was no noticeable change in the root hair cell of any host examined, except for a slight increase in the rate of cytoplasmic streaming. The initial period of salivation varied in duration.

A zone, termed " salivation zone ", developed around the stylet tip and probably included some accumulated cytoplasm in addition to saliva. This zone was always small (Fig. 1 E) and could not be easily differentiated, even at very high magnification (up to 3000 ×), from the cytoplasm. During cytoplasmic streaming, the zone was more easily visualized as it impeded the flow of cytoplasm, which aggregated around the salivation zone for a few seconds until sufficient had accumulated to pass over the stylet tip area.

After salivation, pulsations of the median bulb started as the nematode began to feed on the root hair and this caused pulsations of the salivation zone. Root hairs lived for about 10 h or more after brief feeding, whereas after extended feeding the cells survived for much shorter periods of up to about 1 h.

Extended feeding

The sequence of probing, salivation and food uptake noted for brief feeding periods occurred during extended feeding when the period of food uptake, and sometimes the salivation period, increased markedly. The longer the feeding period, the greater the rate of cytoplasmic streaming. Frequently, a cytoplasm-like connection developed between the salivation zone and the opposite side of the root hair (Fig. 1 F). These connections were not permanent and often only persisted for a few seconds.

During extended feeding it was possible to observe the oesophageal glands of *P. penetrans* (Fig. 2 A) and the changes in the root cell nucleus. The dorsal oesophageal gland cell nucleus and nucleolus appeared similar in size to those of the sub-ventral glands. Several secretory granules were visible in the dorsal gland, but the subventral glands appeared nearly empty (Fig. 2 B). Granules from the dorsal gland passed down the duct during salivation and accumulated at the ampulla behind the stylet (Fig. 2 C). Extended feeding had a noticeable effect on the root hair nucleus which hypertrophied (Fig. 2 D).

After completion of feeding, the stylet was withdrawn, leaving an aggregation of cytoplasm and saliva at the point where the stylet had been inserted (Fig. 2 D). The puncture in the cell wall closed and cell contents were never observed to leak out (Fig. 2 E-H) during subsequent cytoplasmic streaming. This streaming was more rapid in root hairs on which a nematode had fed. Several small vacuole-like structures were observed moving with the cytoplasm (Fig. 2 F, G).

Repeated feeding sequences on the same root hair

It was frequently observed that nematodes returned to the same root hair and started to feed again. During an observation sequence of nearly two hours, one nematode returned three times to the same root hair (Fig. 3 A-D), inserting the stylet into a different site on each occasion. The root hair nucleus hypertrophied gradually during this sequence and eventually, after the last feed, the nucleus was carried by the streaming cytoplasm to the end of the root hair (Fig. 3 E). Approximately 1 h later cytoplasmic streaming stopped as the root hair died (Fig. 3 F).

Defaecation during feeding

During feeding by all stages, defaecation occured every 2-4 min. Thus, it was possible to observe defaecation only during extended feeding periods.

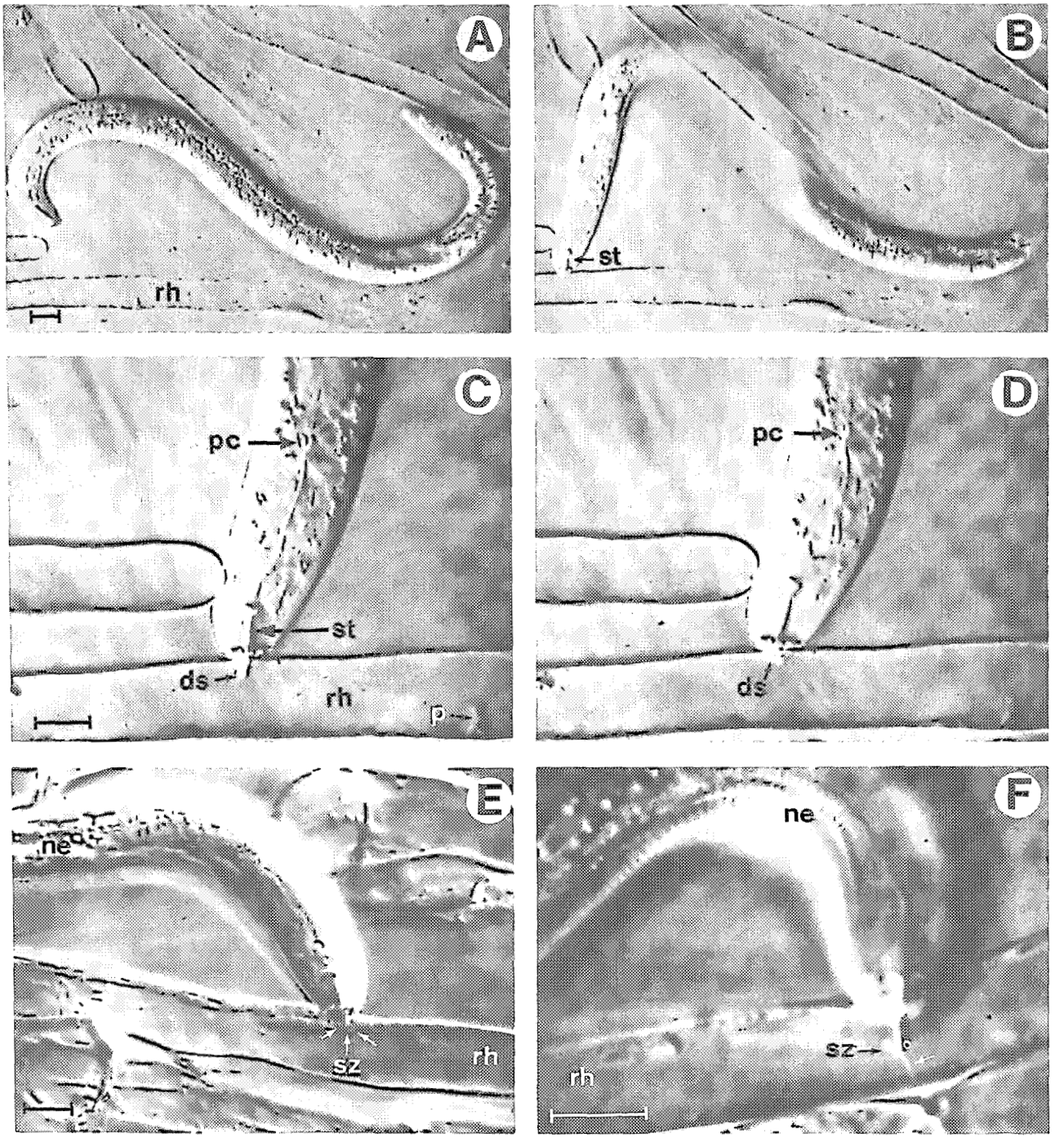

Fig. 1. A-E — Probing behaviour and brief feeding — A : *P. penetrans* J3 in an area of a root hair (rh) with a very thin layer of cytoplasm (oil radish); B : The nematode had just inserted its stylet (st) into the root hair; C : Attempted feeding from a root hair (rh), which had a very thin layer of cytoplasm (oil radish). [pc = open pump chamber (= pumping) of the median bulb; st = stylet; ds = droplet of saliva; p = previous puncture]; D : After few seconds, the nematode stopped pumping [pc = closed pump chamber of the median bulb; ds = droplet of saliva with some aggregated cytoplasm]; E : J4 (ne) feeding from a tobacco root hair (rh) that showed strong cytoplasmic streaming; when the cytoplasm passed the small zone of salivation (sz) its movement was impeded; F — Extended feeding — J3 fed for some minutes from a potato root hair (rh). The white arrow points to a small cytoplasm-connection between the salivation zone (sz) and the cell wall on the opposite side *(Bars = 10 µm).*

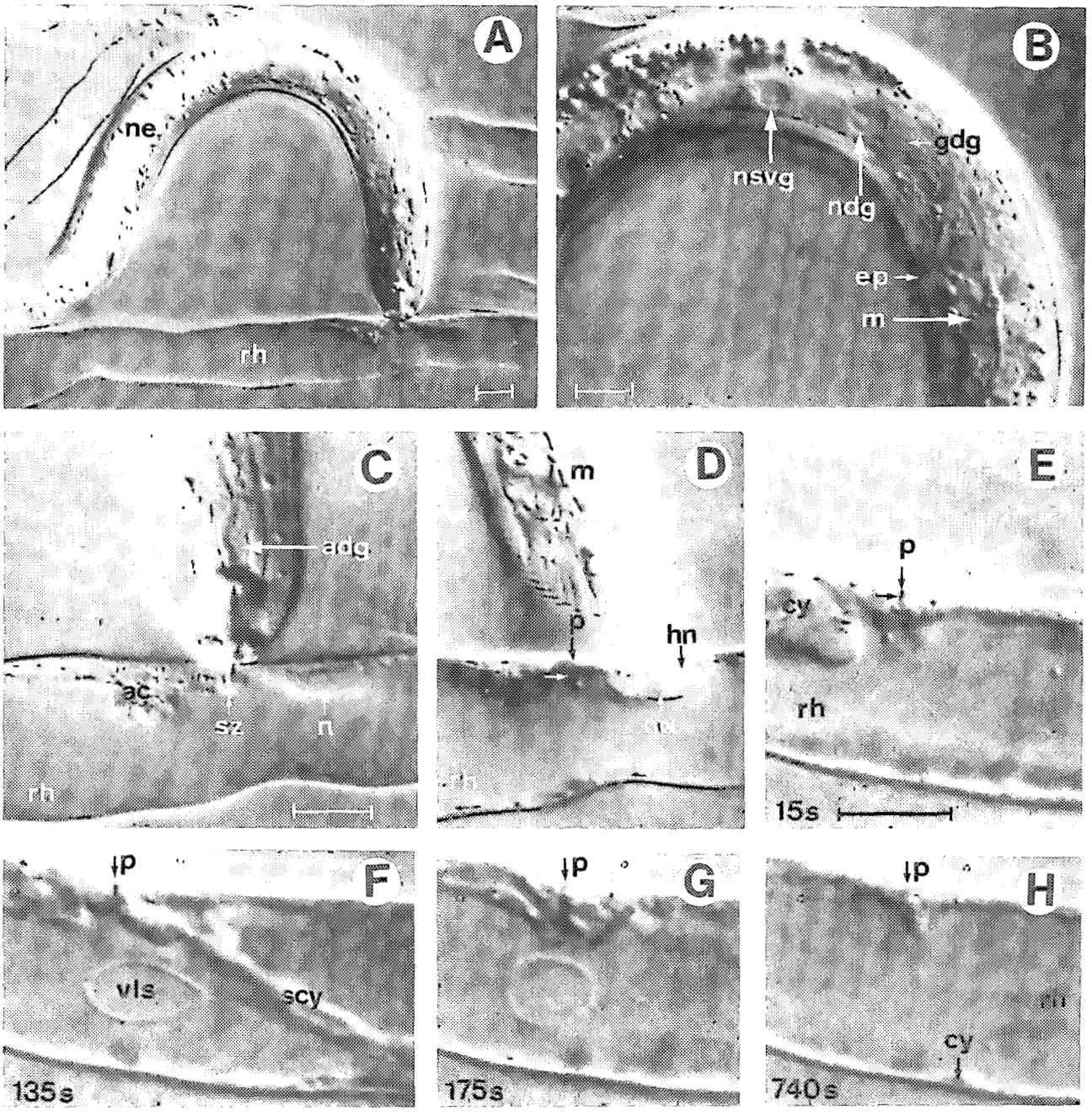

Fig. 2 — Extended feeding — A : An adult male (ne) during extended feeding : stylet inserted into a root hair (rh) of rape seed; B : Median bulb area of the oesophagus of an adult male : the nucleus of the dorsal gland (ndg) with its nucleolus was only slightly bigger than the nuclei of the sub-ventral glands (nsvg). Only a few secretory granules of the dorsal gland (gdg) were visible during food uptake. The pump chamber of the median bulb (m) of this male was open (= pumping) [ep= excretory pore]; C : Head of a *P. penetrans* male with stylet inserted into the root hair. In this period of extended feeding, the root hair nucleus (n) was slightly hypertrophied [rh = root hair; ac = aggregated cytoplasm; sz = salivation zone; adg = ampulla of the dorsal oesophageal gland]; D : Just after the nematode had left the root hair (rh) [nu = nucleolus of the hypertrophied nucleus (hn) of the root hair; m = median bulb; p = puncture (see text)]; E-H : Sequences over a period of 12 min after a J4 had left its feeding site (potato root hair). There was no change in the rate of cytoplasmic streaming within the root hair (rh) during the observation [cy = cytoplasm; vls = vacuole like structure; scy = strands of cytoplasm; p = puncture] *(Bars = 10 µm).*

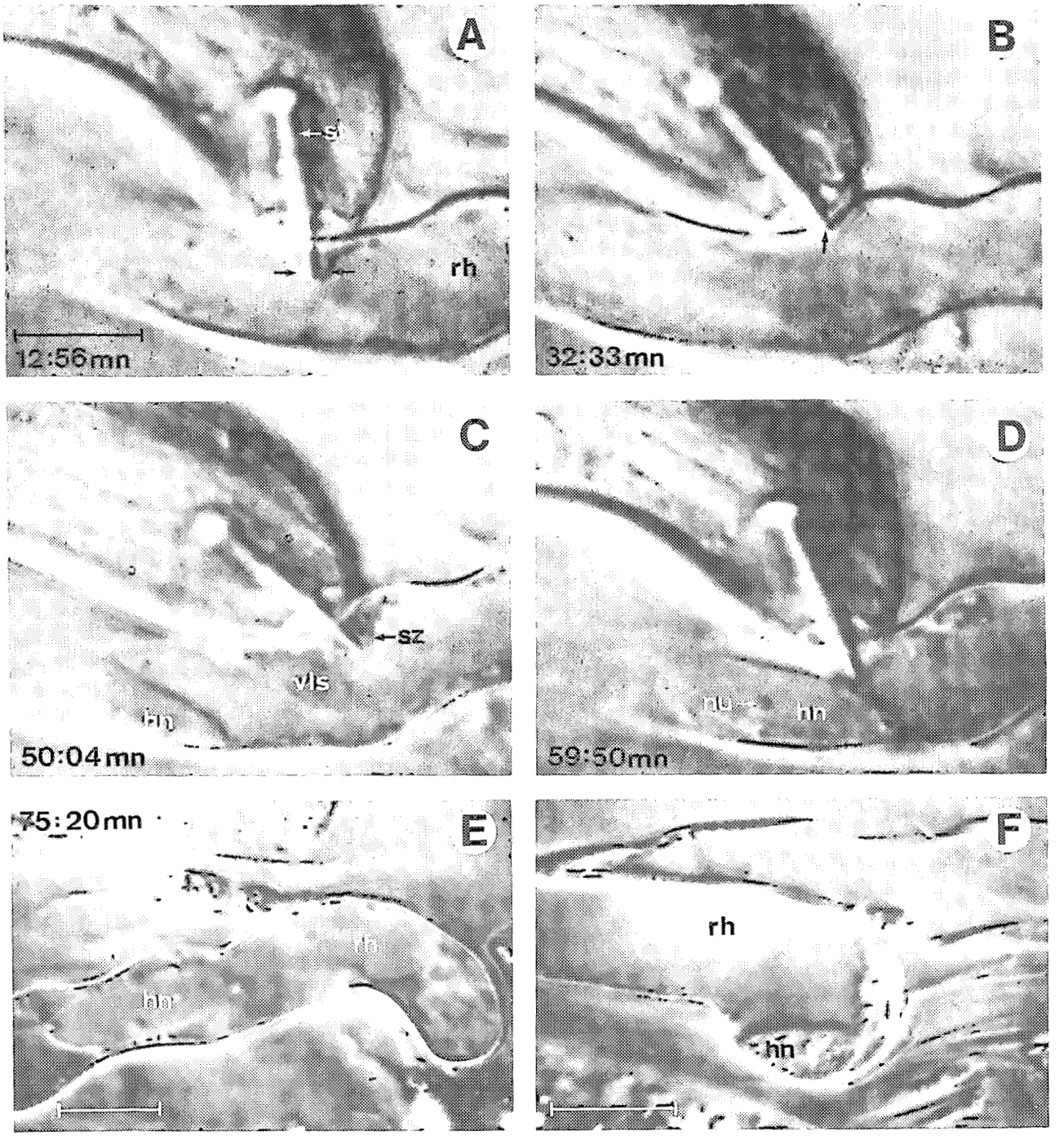

Fig. 3 — Repeated feeding sequences on the same root hair (potato) by a third stage juvenile (J3) — A : First feeding period (1 min 50 sec-13 min 5 sec). The salivation zone around the stylet tip (arrows) pulsated in synchrony with the median bulb [st = stylet, rh = potato root hair]; B : Just before the second feeding period : the nematode gently inserted the stylet (arrow) into the root hair at another area; C : Second feeding period (34 min 42 sec-54 min 29 sec). Near the salivation zone (sz) a vacuole-like structure (vls) and the hypertrophied nucleus (hn) were present; D : Third feeding period (59 min 0 sec-64 min 11 sec). After the nematode had stopped feeding for a few seconds, it returned to the same area of the root, but inserted the stylet at a different place [hn = hypertrophied nucleus; nu = nucleolus]; E : After the nematode had left the root hair, the hypertrophied nucleus was transported 15 min later close to the root hair tip [rh = root hair]; F : A different potato root hair with a coagulated nucleus. This root hair died some minutes before *(Bars = 10 µm).*

Discussion

The small size of *P. penetrans* makes observations on feeding behaviour very difficult to undertake, even with video-enhanced microscopy. For this work, over 500 nematodes were observed in order to obtain a film record of the life cycle biology of *P. penetrans* outside and inside the root (Zunke & Inst. wiss. Film, 1988). The film contains detailed observations of various stages of the life cycle feeding on root hairs and this paper presents an analysis of these data. Clearly, root hairs provide a source of nutrients for all stages, especially the early juvenile stages. It may be more difficult for the younger stages to perforate the relatively thick epidermal cell walls and so the root hairs are more attractive. Several features of the feeding process are similar to those described for other nematodes. The sequence of events includes probing, cell perforation by the stylet, salivation and ingestion of food. However, *P. penetrans* shows no regular rhythm of salivation and feeding or defaecation of set durations as has been observed in *Heterodera schachtii* (Wyss & Zunke, 1986*b*).

Contact of lips of *P. penetrans* with the root hairs is likely to involve sensory perception prior to selection of a penetration site and has been described in many nematodes including species of Tylenchida (Doncaster & Seymour, 1973) and *T. dubius* (Wyss, 1973). Often the head of the nematode moves from side to side, effectively rubbing the lips along root hairs, presumably to select a penetration site within a restricted region of the root hair. Touching of root hairs by *P. penetrans* individuals was observed by Kurppa and Vrain (1985) but they did not report rubbing of the lips or eventual penetration and feeding. However, lip rubbing by *P. penetrans* was slow and over a small area compared to the extensive, and very active rubbing observed with *Aphelenchoides hamatus* (Zunke, Rössner & Wyss, 1986).

Some ectoparasitic mycophagous nematodes, such as *Aphelenchus avenae* (Fisher & Evans, 1967), *Aphelenchoides bicaudatus* (Siddiqui & Taylor, 1969) and *A. hamatus* (Zunke, Rössner & Wyss, 1986), show no evidence of salivation after stylet insertion and before feeding. By contrast, after the stylet of *P. penetrans* had been inserted in the root hair a salivation phase commenced. Compared with *T. dubius* (Wyss, 1987) the salivation zone formed around the stylet tip of *P. penetrans* is very small and probably reflects the small body size and reduced dorsal gland cell volume which can contribute only limited amounts of saliva. Few granules and no large organelles, such as mitochondria, were observed near the stylet tip and, as suggested for *T. dubius* (Wyss, 1987), the zone may act as a barrier for organelles which may otherwise have blocked the narrow stylet orifice.

Food uptake by *P. penetrans* is clearly limited, firstly by the small size of the nematode and the associated salivation zone and, secondly, by the narrow opening of the stylet tip. This is reflected in the cycles of slight reduction and expansion in size of the salivation zone which synchronised with the pumping of the medium bulb of the oesophagus (Zunke & Inst. wiss. Film, 1988). Much larger volumes of fluid have been observed to be ingested by *T. dubius* and by *Trichodorus* spp. (Wyss, 1987), where a feeding tube is formed, and by the Aphelenchida (Siddiqui & Taylor, 1969; Zunke, Rössner & Wyss, 1986) where the stylet lumen is considerably larger than that of *P. penetrans.*

The reaction of root hairs of rape, oil radish, tobacco and potatoes to parasitism by *P. penetrans* dit not differ. The rate of streaming of the root hair cytoplasm increased markedly in cells being fed on by nematodes, and this response is a common feature of many plant cells attacked by different pathogens (Schlösser, 1983).

Vacuole-like structures became apparent in the cytoplasm of cells being fed on by *P. penetrans,* but their origin is unknown although they appear not to be related to removal of cytoplasm by the nematode. Cytoplasm-like connections between the stylet and the opposite side of the living root hair were often observed but may not be the same structures as the strands of cytoplasm described in dying plant cells (von Sengbusch, 1989).

When the nematode moved away from the root hair the puncture hole left by the nematode stylet closed or becomes blocked. It is possible that the salivation zone containing saliva and partly predigested cytoplasm becomes hardened and seals the hole when the nematode departs. However, a feeding plug, produced by the amphids, the openings of the inner labial receptors and the stylet, occurs in several Tylenchida, for example *Heterodera glycines* and *H. schachtii* (Endo, 1987). Further work, using electron microscopy, is required to determine the exact mechanism involved. With *T. primitivus,* for example, the saliva remaining in the feeding tube after the nematode had moved away hardened and prevented leaking of cell contents (Robertson & Wyss, 1983).

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