

Feeding studies on *Xiphinema vulgare* Tarjan, 1964 (Nematoda : Longidoridae)

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SUMMARY

In vitro studies of *Xiphinema vulgare* feeding on tomato roots are described. Nematodes did not attack the root tip but fed in the root-hair region. The time spent at any one site varied from 8 min to over 3 h 11 min with 2-5 penetrations of increasing depth being made at each site. Salivation was believed to have occurred in the short period between cell penetration and regular pulsation of the basal bulb. Two types of ingestion were observed : one, characterized by intermittent pumping of the basal bulb, lasted from 1-10 min with a mean of 3.1 min ; the other featured continuous pumping, occurred only at deep penetrations (86 and 104 μm), and lasted 76 min and over 157 min. Necrosis of cells at feeding sites developed within 2-3 days.

RÉSUMÉ

Etudes concernant la nutrition de Xiphinema vulgare Tarjan, 1964 (Nematoda : Longidoridae)

Les auteurs ont observé *Xiphinema vulgare* se nourrissant sur racines de tomates croissant dans un milieu gélosé à 2% pris entre deux lamelles de 80 mm de diamètre. Les nématodes commencent à se nourrir dans les 24 h suivant l'inoculation, mais, à l'inverse d'autres espèces de *Xiphinema*, restreignent leur action à la zone des poils absorbants. Le temps de nutrition sur chaque site varie de 8 mn à plus de 3 h 11 mn, la durée la plus fréquente étant de 15 à 20 mn. Deux à cinq pénétrations successives du stylet et à des profondeurs croissantes sont observées sur chaque site ; la profondeur maximale observée est de 108 μm . A la pénétration du stylet dans une cellule fait suite une période de 60-70 secondes durant laquelle on observe l'émission de salive et des contractions spasmodiques du bulbe basal de l'œsophage, puis la pulsation régulière du bulbe pendant la phase d'ingestion. Deux types d'ingestion ont été observés : l'un est caractérisé par un pompage intermittent du bulbe basal durant 1 à 10 mn (moy. : 3,1 mn) ; l'autre est un pompage continu observé seulement dans les cas de pénétration profonde du stylet (86 et 104 μm) et durant de 76 à plus de 157 mn. Après que le stylet se soit détaché de la racine, le bulbe basal a plusieurs pulsations, probablement pour nettoyer la lumière du stylet et de l'œsophage. Les cellules du site de nutrition commencent à se nécroser en deux à trois jours, mais ceci peut-être dû à une infestation secondaire par des micro-organismes car le milieu n'était pas aseptique.

In recent years increasing attention has been focused on the feeding of *Xiphinema* species because of their importance both as virus vectors and as plant parasites in their own right. Detailed descriptions of the feeding phases have been published only for *X. bakeri* (Sutherland, 1969) ; *X. diversicaudatum* (Fisher & Raski, 1967 ; Trudgill, 1976) and *X. index* (Wyss, 1978 a).

This paper reports on *in vitro* feeding of *X. vulgare* Tarjan, 1964, a common nematode in the Caribbean region found associated with a number of crops (Kermarrec & Scotto la Massese, 1972 ; Hunt, 1977) but perhaps most numerous under woody plants such as citrus. *X. vulgare* is not known to be a virus vector.

Materials and Methods

Tomato seeds, *Lycopersicon esculentum* Mill. cv. Roma, washed five times in sterile distilled water were placed on 2% water agar in Petri-dishes and kept in darkness at 25-27 °C for germination to take place. *X. vulgare*, extracted from soil under savanna grass, *Axonopus compressus* (Sw.), at Roseau, St. Lucia, were hand picked and 50 healthy-looking females inoculated around the developing roots when these were 3-4 cm long. Microscopical observations were made at $\times 375$ magnification.

For more detailed studies, a sterile 80 mm diameter cover-slip was placed on the bottom of a Petri-dish and the above procedure followed. When nematodes were feeding, the tomato shoots were excised and another 80 mm cover-slip placed on top of the agar. The sandwich was then removed from the Petri-dish and inverted for microscopical observations at magnifications up to $\times 1875$.

Results and Discussion

Nematodes usually commenced feeding within 24 hours of inoculation, the feeding process being similar in many respects to that observed for other *Xiphinema* spp. (Sutherland, 1969; Trudgill, 1976; Wyss, 1978 a). Unlike some species, adult *X. vulgare* did not feed on the root tips but restricted their attention to the root-hair zone, except in one case where the zone of elongation was attacked.

The total stay at any one site varied from 8 minutes to in excess of 3 hours 11 minutes, the usual period being 15-20 minutes with 2-5 penetrations of increasing depth at each site.

EXPLORATION

Nematodes tended to congregate along the roots, often remaining motionless, but occasionally moving and exploring the root surface by arching the head region and rubbing with the lips. During this phase the odontophore flanges were rotated, often vigorously, and the odon-

tostyle was moved back and forth but, as noted by several authors for *X. index*, (e.g. Wyss, 1978 a) the tip was never protruded beyond the base of the lip region.

PENETRATION

Prior to root penetration a nematode positioned itself with the lips touching the root surface and the head region arched so that the odontostyle tip was approximately perpendicular to the root. Several short odontostyle thrusts of about one /second were then made followed by short bursts of 4-5 thrusts/second until penetration had been effected. Initial penetration was usually shallow (10-14 μm) and was followed by several short penetrations down to about 40 μm and then two or three deeper penetrations. A typical sequence was : 14, 30, 39, 64, 82 μm deep ; the mean distance between successive penetrations shallower or deeper than 40 μm being $12 \pm 1.2 \mu\text{m}$ and $22 \pm 1.8 \mu\text{m}$ respectively. Feeding occurred after each penetration.

One nematode departed from the usual pattern and penetrated directly to 68 μm and then to 91 μm . During the initial stages of penetration, down to a depth of about 35 μm , the odontophore flanges (and also, therefore, at least the basal region of the odontostyle) rotated whenever the odontostyle encountered a cell wall whereas below this depth the walls were penetrated by short, rapid thrusts with no rotation. Rotation of the odontophore flanges at cell walls has been recorded for other *Xiphinema* spp. (Sutherland, 1969; Trudgill, 1976; Wyss, 1978 a) and is probably more common when the walls to be penetrated are thick.

The maximum observed penetration was 104 μm , the basal 14 μm of the odontostyle not being protruded.

SALIVATION

During the course of each penetration secretions gathered in the oesophageal gland duct. As soon as odontostyle movement had ceased the basal bulb contracted 2-6 times at about 1-2/second and this was immediately followed by rapid dispersal of the secretions.

During the next 60-70 seconds the basal bulb contracted spasmodically in bursts of 1-4 pulsations, secretions which gathered in the quiescent interludes being dispersed. Secretions observed to gather during salivations were less copious than those produced during the ingestion phase proper. One nematode, which subsequently went on to feed for the longest recorded time, was quiescent for 18 minutes before initiating the above sequence. During this inactive period no secretions were seen to gather in the dorsal duct.

Trudgill (1976) suggested that the prolonged quiescent periods he observed in *X. diversicaudatum* could have been related to salivation. Wyss (1978 a), working with *X. index*, also observed extended quiescent periods but could detect no movement in the dorsal gland system. Instead, he suggested that salivation occurred during a period of prolonged bulb elongation between odontostyle penetration and regular bulb pulsation. In the present study, *X. vulgare* only showed a prolonged quiescent period in one case and, as recorded by Wyss (1978 a), no movement in the dorsal gland system could be discerned. Salivation appeared to take place during the short period of spasmodic bulb pulsation following odontostyle penetration. Although the fate of the secretions after dispersal could not be observed, it is probable that they moved forward as the bulb platelets appeared to be closed. Spasmodic bulb pulsations during the salivation phase were probably associated with 'tasting' the host cell contents.

INGESTION

Immediately after the salivation sequence the oesophageal bulb began to pump regularly at a rate of 4-5 contractions/second, the platelets opening from anterior to posterior. Two types of pumping activity were observed:

(a) *Intermittent pumping*: bursts of bulb pulsation lasting from 4-15 seconds were interspersed with quiescent periods of 3-5 seconds. Copious secretions gathered in the dorsal gland duct during pumping and were dispersed immediately after cessation. In some individuals secretions could be seen to gather as far as

50 μm posterior to the dorsal gland nucleus. This type of ingestion sequence was of short duration; individual feeds at any one depth lasting from 1-10 minutes with a mean of 3.1 ± 0.4 minutes ($n = 21$).

(b) *Continuous pumping*: observed on only two occasions at depths of 86 and 104 μm . The basal bulb pumped continuously at a rate of 3-4 contractions/second with only an occasional beat missed. Secretions from the dorsal gland were possibly dispelled almost continuously as only occasionally were concentrations seen to build up in the duct. One feed lasted for 76 minutes and the other was still continuing when observation ceased after 157 minutes.

It seems probable that the intermittent pumping was associated with feeding from a single cell whereas the continuous pumping occurred when phloem tissue had been penetrated. Trudgill (1976) came to a similar conclusion after observing the long continuous ingestion periods of *X. diversicaudatum* feeding on the older or piliferous regions of *Petunia hybrida* roots.

WITHDRAWAL

The odontostyle was usually not retracted until completely withdrawn from the root, but sometimes retraction was concurrent with nematode withdrawal from the feeding site. Cohn (1970) noted that *X. brevicolle* invariably withdrew the odontostyle from the root before it was retracted, although *X. index* retracted the odontostyle before withdrawing from the feeding site. After leaving the root the oesophageal bulb pulsated several times and the odontostyle was moved back and forth and the odontophore flanges rotated. Bulb pulsations after odontostyle retraction have also been recorded for *X. diversicaudatum* (Trudgill, 1976) and *X. index* (Wyss, 1978 a) and are probably associated with clearing the feeding apparatus of food.

HOST REACTION

No immediate host response was visible after feeding, but some necrosis of cells at the feeding sites developed within 2-3 days. As the roots were not growing under aseptic conditions the

necrosis could have been due to secondary infection although Wyss (1978 b) found that *X. index*, feeding under sterile conditions on tomato roots, produced a similar necrotic response.

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