Analysis of the feeding of *Xiphinema diversicaudatum*

David L. TRUDGILL*, Walter M. ROBERTSON* and Urs WYSS**

* *Zoology Department, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA, and*
** *Institut für Phytopathologie, Universität Kiel, 2300 Kiel, West Germany.*

SUMMARY

The feeding of *Xiphinema diversicaudatum* was studied on seedlings of rye grass and of other plants growing in agar. It fed at root-tips, where it induced galls, and in the piliferous area. Typically, *X. diversicaudatum* inserted its stylet through one or two cells and then commenced feeding on a column of progressively deeper cells. Salivation, which involved a rapid emptying of the ducts of the dorsal oesophageal gland cell, was observed between the frequent bouts of oesophageal bulb contractions. Usually, there were several such bouts during feeding on each cell. The rate of bulb contraction declined between salivations. *X. diversicaudatum* also had periods of apparent inactivity when feeding deep within either the root-tip or the piliferous region and these were also thought to be periods of a distinct type of salivation. This behaviour was associated with the formation of a spherical structure at the stylet tip and subsequent prolonged periods of ingestion without further salivation. Estimates were made from video recordings of the amounts ingested and these were found to be large, especially during periods of prolonged ingestion which occurred when nematodes were feeding deep within root-tip galls and the piliferous region.

RÉSUMÉ

Analyse de la prise de nourriture par Xiphinema diversicaudatum

La prise de nourriture par *Xiphinema diversicaudatum* a été étudiée sur des plantules de ray-grass et d'autres plantes croissant sur milieu gélosé. Le nématode se nourrit à l'extrémité des racines, où il induit la formation de galles, et dans la zone des poils absorbants. D'une manière typique, *X. diversicaudatum* fait pénétrer son stylet à travers une ou deux cellules, puis commence à se nourrir à partir d'une colonne de cellules de plus en plus profondément situées. La salivation, qui implique le vidage rapide des conduits de la glande œsophagienne dorsale, a été observée entre les nombreuses périodes de contractions du bulbe œsophagien. Plusieurs de ces périodes se succèdent généralement durant la prise de nourriture dans chaque cellule. La valeur des contractions du bulbe diminue entre les périodes de salivation. *X. diversicaudatum* présente également des périodes d'inactivité apparente lorsqu'il se nourrit en profondeur soit à l'extrémité des racines, soit dans la région des poils absorbants, ce qui est supposé correspondre à un type distinct de salivation. Ce comportement est associé à la formation d'une structure sphérique à l'extrémité du stylet, puis à des périodes prolongées d'ingestion sans salivation. Sur la base d'enregistrements vidéo, des estimations sont fournies qui concernent les quantités ingérées : celles-ci ont été trouvées importantes, particulièrement durant les périodes prolongées d'ingestion qui se produisent lorsque le nématode se nourrit en profondeur à l'extrémité des racines ou dans la zone des poils absorbants.

The *in vitro* feeding of *Xiphinema diversicaudatum* on seedlings of *Petunia hybrida*, of *X. index* on fig and of *X. vulgare* on tomato have been described in detail by Trudgill (1976), Wyss (1977), and Hunt and Towle (1979). More recently Wyss, Robertson and Trudgill (1988) used video enhanced contrast light microscopy to clearly show the rapid, solubilising effects of the dorsal gland cell secretions of *X. index* on host cell cytoplasm and nuclei.

X. diversicaudatum feeds on all parts of the root, but at the root-tip it induces galls containing modified cells which are enlarged and multinucleate with increased levels of DNA and RNA (Griffiths & Robertson, 1984, 1988).

This report describes the feeding behaviour, including salivation, of *X. diversicaduatum* on plants growing in agar, the volumes extracted are estimated and the relationship between salivation, ingestion and changes in the host root are assessed.

Materials and methods

X. diversicaudatum, maintained on ryegrass in a glasshouse at SCRI, were placed around the roots of seedlings of ryegrass *(Lolium perenne)*, strawberry (cv. Cambridge Favourite from meristem culture) and *Petunia hybrida* growing in 0.75 % water agar on large cover slips as described by Wyss (1977). Supporting observations were also made on rooted leaf cuttings of rose (*Rosa* spp.). No attempt was made to obtain sterile cultures. Feeding, mainly of adult nematodes, was observed with a light microscope with up to × 1250 magnification and recorded using a video tape recorder

and camera. When the tapes were replayed a light-sensitive diode placed against the screen of a television monitor and a chart recorder were used to analyse oesophageal bulb activity as described by Seymour, Minter and Doncaster (1978). During the periods of observation the cultures were maintained at temperatures between 20 and 22 °C.

Results

X. diversicaudatum fed on root-tips (terminal 400 µm), root-tip galls and the piliferous region. Usually, the stylet was inserted one or two cells deep before ingestion commenced. A progressively deeper column of cells was penetrated and fed upon until the stylet was almost fully protracted and five to six cells had been exploited.

Penetration of each cell took up to 1 min, with the cell wall being perforated by vigorous, repeated thrusts of the stylet. Once the wall of the cell was perforated the tip of the stylet was inserted only a little way before ingestion commenced. Typically, perforation of the cell wall was followed by a few slow contractions of the oesophageal bulb. When these ceased the anterior duct of the dorsal oesophageal gland cell was observed to gently empty (salivation). There was a subsequent, relatively long pause of several seconds followed by a bout of vigorous and rapid (two or more contractions per second) bulb contractions. This, and subsequent bouts of ingestion pumping were separated from one another by further brief pauses of only a few seconds for salivation, which was much more pronounced than the initial injection and during which the anterior and longitudinal duct of the dorsal gland cell were observed to empty. These injections of saliva were sometimes so vigorous that the flexed slender oesophagus was seen to jerk under the pressure of the flow. Also, after these vigorous injections of saliva, but not after the initial gentle injection the oesophageal bulb was observed to visibly shorten before ingestion pumping commenced. During ingestion the bulb gradually lengthened as the ducts of the dorsal gland cell were re-filled. No obvious changes were seen in the sub-ventral gland cells.

Typical patterns of feeding behaviour on rye grass are illustrated in Fig. 1. In cortical cells and in unmodified root-tips there were 6 to 16 separate bouts of ingestion separated from one another by injections of saliva as described above. During each bout of ingestion there were, on average, 14 to 17 bulb contractions (Table 1). The mean number of contractions ranged from 80 to 320 per cell. Fewer observations were made on *P. hybrida* but feeding behaviour was similar to that on rye grass (Table 2).

When feeding on the deeper cells within root-tip galls patterns of behaviour were similar to those at root tips but the bouts of ingestion were extended (Tables 1, 2, 3). However, the extended bouts of ingestion were still separated by the injection of saliva (Fig. 1). In cells five and six deep within rye grass galls there were an average of 110 to 142 bulb contractions between each salivation and 800-900 contractions associated with each cell. In strawberry, there were fewer bouts of ingestion which appeared to be more extended, even in cells closer to the gall surface, than in rye grass or *P. hybrida*, perhaps reflecting its status as a good host (Griffiths & Trudgill, 1983).

No relationship was observed between the time taken for salivation and the duration of the subsequent bout of ingestion. However, the rate of bulb contraction decreased during each bout of ingestion. Figure 2 shows that within a single cell of rye grass the rate (number of

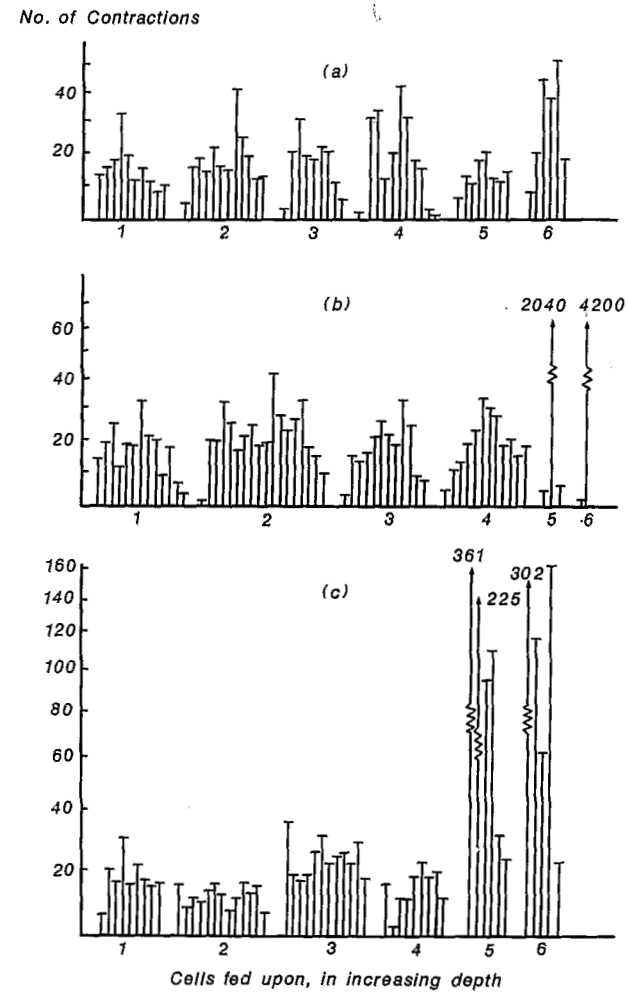

Fig. 1. Typical feeding on (a) an unmodified root-tip, (b) piliferous region of rye grass and (c) a root-tip gall. The vertical bars represent a period of ingestion, each separated by the injection of saliva.

oesophageal bulb contractions per second) was greatest in the first three bouts of ingestion and that it progressively declined within each bout. Following salivation, the initial rate of bulb contraction was largely restored to its previous level, but then progressively decreased. Towards the end of each period of feeding within a cell there were generally fewer contractions within each bout of ingestion and these were comparatively slow and laboured.

Long quiescent periods were observed in the middle of many feeds, confirming previous observations by Trudgill (1976). They generally occurred immediately after the penetration of one of the deeper cells in the piliferous region and in modified and unmodified root-tips. During the quiescent periods no obvious activity was observed in either the dorsal or subventral gland cells. Similarly, nothing was seen to pass through the oesophageal intestinal valve. Each quiescent period was usually, but not always, followed by an initial ingestion of saliva and then a long period of continuous ingestion within the same cell, as described by Trudgill (1976), when there were between 5100 and 8800 contractions without any further breaks for salivation. Substantial volumes of fluids were observed passing through the oesophageal intestinal valve with each contraction of the bulb. In one instance, such behaviour was observed just behind the root-tip of rose (*Rosa* spp.) when a nematode had its stylet tip inserted only two or three cells deep. During the quiescent period a ball-like structure (" stylet plug "; Wyss, Robertson & Trudgill, 1988) formed at the stylet tip. The volume of this cell was estimated to be *c.* 140 000 μm^3. During the subsequent period of ingestion which lasted 1 h 55 min this structure remained in place and was observed to pulsate at the same rate as the contractions of the oesophageal bulb. Also, the cytoplasm of the penetrated cell appeared not to be ingested; a vacuole was observed at the apical margin of the plasmalemma of the cell and this also pulsated at the same rate as the oesophageal bulb. Periodically, when this vacuole had reached a critical size it was suddenly drawn through the cytoplasm towards the stylet plug where it appeared to be ingested. Sometimes, stylet plugs were less clearly observed associated with the stylet-tips of other nematodes feeding continuously within deeper cells. During these long, uninterrupted periods of ingestion the rate of bulb contraction was relatively unchanged or slowly declined. Frequently, the long periods of continuous ingestion ended with several bouts of ingestion separated by normal salivation as described above.

On retraction of the odontostyle there were always a few, slow contractions of the oesophageal bulb during which small volumes of fluid were observed passing through the oesophageal intestinal valve.

Table 1

Feeding of *Xiphinema diversicaudatum* on rye grass.

Site	*Cells of increasing depth*	*No. of observations*	*Mean no. bouts of ingestion*	*Mean no. of bulb contractions per bout*	*Mean no. of bulb contractions per cell*
Ummodified root-tip	1	6	9.2	16.7	157
	2	6	8.7	13.5	122
	3	6	11.0	14.5	152
	4	7	12.0	17.0	190
	5	7	9.4	15.7	144
	6	5	5.6	16.7	94
Root-tip gall	1	9	10.1	18.0	182
	2	9	14.1	16.1	227
	3	8	13.0	17.5	228
	4	7	10.0	52.3	523
	5	6	8.2	110.7	908
	6	4	5.8	141.7	822
Piliferous region	1	5	12.8	21.7	278
	2	7	15.9	20.9	332
	3	8	12.3	21.6	264
	4	8	11.3	17.7	200
	5	8	One	Continuous	8 760
	6	2	One	Continuous	5 100

Table 2

Xiphinema diversicaudatum feeding on *Petunia hybrida.*

Site	*Cells of increasing depth*	*No. of observations*	*Mean no. bouts of ingestion*	*Mean no. of bulb contractions per bout*	*Mean no. of bulb contractions per cell*
Ummodified root-tip	1	2	5.5	28.5	157
	2	2	7.3	22.8	166
	3	2	7.7	27.0	208
	4	2	6.0	28.0	168
	5	1	6.0	26.0	156
	6	1	5.0	35.0	175
Root-tip gall	1	1	16.0	17.0	272
	2	1	14.0	18.0	252
	3	1	24.0	26.0	624
	4	1	24.0	54.0	1 296
	5	1	21.0	172.0	3 612
	6	1	5.0	66.0	330
Piliferous region	1	1	9	22	198
	2	1	11	22	242
	3	1	11	25	275
	4	1	1	38	38
	5	1	1	Continuous	6 570

Table 3

Xiphinema diversicaudatum feeding on strawberry gall*.

Site	Cells of increasing depth	No. of observations	Mean no. bouts of ingestion	Mean no. of bulb contractions per phase	Mean no. of bulb contractions per cell
Root-tip gall	1**	1**	5.00	32	159
	2	2	2.50	68	170
	3	4	3.25	168	546
	4	7	4.14	127	526
	5	6	4.00	110	440
	6	4	1.75	382	669

* Results from a single gall – ** The depth of initial penetration was taken into account. Most nematodes penetrated 2-3 cells before commencing feeding – There were no quiescent periods.

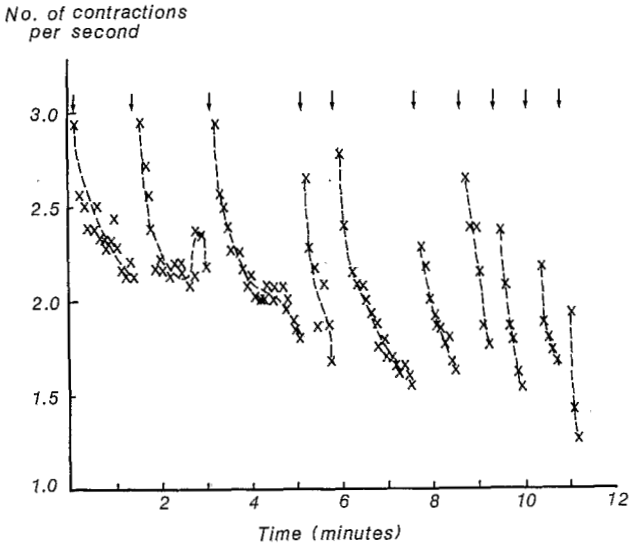

Fig. 2. Rate of oesophageal bulb contraction of a nematode feeding in a cell three to four deep in a 6-day old rye grass root-tip gall. The arrows indicate salivation, and the x are means over 5 seconds.

Discussion

The feeding behaviour of *X. diversicaudatum*, which closely resembled that described by Wyss (1977) for *X. index*, and the changes it induces indicate a complex relationship between this nematode and its host. *Longidorus* spp. also have a relationship with their hosts which appears to be more complex than that of nematodes in most other ectoparasitic genera (Robertson, Trudgill & Griffiths, 1984). Root-tip galls induced by *X. diversicaudatum* contain multinucleate, enlarged cells with elevated levels of DNA, RNA and cytoplasm compared with normal cells (Griffiths, Robertson & Trudgill, 1982). Some plant species are more responsive to the induction of cellular changes and support greater rates of nematode reproduction than less responsive species (Griffiths & Trudgill, 1983), probably because of the quality and quantity of food that can be extracted, especially from the cells within the galls.

It is suggested here that there must be distinct inductive and exploitive processes in the relationship between *X. diversicaudatum* and the root-tip galls it induces. However, the inductive process could not be identified with certainty. Most of the observed feeding by *X. diversicaudatum* was clearly exploitive. Sections through root-tip galls reveal, in addition to enlarged, multinucleate cells, columns of empty cells containing only condensed residues of nucleoli (Wyss, 1978; Griffiths & Robertson, 1988). Wyss, Robertson and Trudgill (1988) have shown very clearly that the injection of saliva from the dorsal gland cell by *X. index* rapidly liquifies the contents of the cell being fed upon and that during the subsequent bouts of salivation and ingestion most of the cell contents are progressively withdrawn. If the plant is virus infected, this will also include virus particles, with some of those for which *X. diversicaudatum* is the specific vector being retained in association with the food canal wall in the odontophore and narrow oesophagus.

The induction of galls and associated cellular changes may be associated with the periods of inactivity occasionally observed in *X. diversicaudatum* feeding at root-tips. Similar behaviour has been reported for *X. bakeri* (Sutherland, 1969), *X. vulgare* (Hunt & Towle, 1979) and *X. index* (Wyss, 1977; Wyss, Robertson & Trudgill, 1988). This behaviour appears to closely resemble that shown by *L. elongatus* and *L. leptocephalus* immediately after stylet insertion (Robertson, Trudgill & Griffiths, 1984). Saliva is probably injected by *Longidorus* spp. during the quiescent period, as this phase alone is sufficient to initiate gall formation (Cohn, 1970). Also, as *Longidorus* spp. lack the extensive duct system found in *Xiphinema* spp. (Robertson, Trudgill & Griffiths, 1984), it seems likely that salivation could not be accomplished in the few seconds observed for *X. diversicaudatum* during its exploitive behaviour.

The removal of large volumes during the prolonged periods of ingestion that typically follow the quiescent periods may also be involved in gall formation. The stylet plug formed at the stylet tip during the quiescent phase may act as a sieve, allowing the ingestion of large amounts of cytosol but not of cytoplasm. In this respect it may resemble the feeding tubes induced by some sedentary endoparasite species (Rumpenhorst, 1984). One of us (U.W.) suspects that the stylet plug impedes ingestion in *X. index*, but observations on *X. diversicaudatum* did not support this view. Another possible difference is that in *X. index* the prolonged periods of

ingestion following the quiescent phase were punctuated by brief pauses for salivation (Wyss, Robertson & Trudgill, 1988) but in *X. diversicaudatum* they were continuous for long periods and salivation only occurred towards the end. Whether *Longidorus* spp. also produce stylet plugs during their inductive phase is unknown.

Wyss, Robertson and Trudgill (1988) had strong observational evidence that the stylet plug was of nematode origin. If so, there may be at least three different roles for, and hence possibly up to three different types of saliva injected into plants by *X. diversicaudatum.* These are the secretions involved in liquifying the cell contents during the exploitive phase, the secretions which form the stylet plug during the quiescent phase, and those involved in the induction of multinucleate cells and gall formation.

Our observations indicate that during feeding by *X. diversicaudatum* substantial volumes of plant material are withdrawn. Assuming that the oesophageal bulb opens only to a triangle (Towle & Doncaster, 1978; Robertson, Topham & Smith, 1987) rather than a hexagon (Robertson, Topham & Smith, 1987) and that on each contraction it is only 30 % efficient it is calculated that adult *X. diversicaudatum* ingest upto 460 μm^3 with each bulb contraction. Other estimates, made from rough measurements of the volume of expansion of the oesophageal bulb during ingestion pumping, indicate this is an under rather than an overestimate. In contrast, measurements of the rectum which, during continuous ingestion, was emptied at *c.* hourly intervals showed that it had a maximum volume of *c.* 200 000 μm^3, implying a much smaller volume was ingested (*c.* 27 μm^3) during each contraction of the oesophageal bulb or that most of the fluid ingested was excreted in other ways. However, based on the assumption that 460 μm^3 is extracted, the mean volumes extracted from cortical cells of ryegrass by *X. diversicaudatum* range from about 56 000 μm^3 at the root-tip to about 150 000 μm^3 in the piliferous region (Table 4). This is equivalent to cells 38 µm to 53 μm^3. In the centre of the gall, the amount ingested increases to 419 000 μm^3 per cell. However, the greatest volumes are extracted during continuous ingestion when *X. diversicaudatum* feeds deep within the piliferous region. Here, an average of *c.* 4 000 000 μm^3 was estimated to be extracted from one cell; such a volume is equivalent to a cell of 160 μm^3. Long periods of continuous ingestion in the piliferous region usually followed periods of quiescence indistinguishable from those observed at the root-tip. However, it is unclear if the same processes are involved.

Table 4

Estimated average volumes $(\mu m)^3$ extracted from different cells of rye grass assuming each bulb contraction withdraws 462 μm^3

Site	*Cell No.**	*1*	*2*	*3*	*4*	*5*	*6*
				*X. diversicaudatum***			
Ummodified root-tip		72 534	56 364	70 224	87 780	66 528	38 808
Root-tip gall		84 084	104 874	105 336	241 626	419 496	379 764
Piliferous region		128 436	153 384	121 968	92 400	4 047 120	–

* Cells of increasing depth from root surface – ** Based on data in Table 1.

The exceptionally long bodies of many *Xiphinema* spp., compared with most other plant parasitic nematodes, may be of benefit in absorbing nutrients from the large volumes ingested. Whatever the reason for the great length of *X. diversicaudatum,* the complexity of this nematode is becoming increasingly evident. Its beha viour is complex and interactive with its host. Although typical patterns of behaviour have been described here, many variations were also observed e.g. quiescent periods being followed by a series of short bouts of ingestion and salivation instead of a long period of continuous ingestion. In addition, Robertson (1975) and Robertson and Wyss (1979) have shown that the related species, *X. index* has a complex feeding apparatus with putative sense organs in the odontophore. Nerves from these sense organs, which are probably gustatory, connect via the narrow oesophagus to the oesophageal bulb. Other branches of these nerves, which connect to the nerve ring via the hypodermal cords, have part of their length free in the pseudocoel. This enables these nerves to move freely backwards and forwards during retraction and protraction of the stylet (Robertson, unpubl). The role of *X. diversicaudatum* as a virus vector adds to its interest and, although Robertson and Henry (1986) have demonstrated the occurrence of carbohydrates associated with virus retained in *X. diversicaudatum,* the mechanisms of retention and release are unknown. It is interesting to note that the observed behaviour during the initial injection of saliva into each newly penetrated cell seems to differ from that of subsequent injections which are so forceful that virus particles could be physically dislodged from their site of retention. However, Wyss, Robertson and Trudgill (1988) have shown that the host cell is rapidly killed and emptied during this type of exploitive feeding by *X. index.* Alternatively, virus release and transmission may be associated with the quiescent periods. If so, this is an additional reason for suggesting that any saliva produced during this behaviour may have different properties to that produced during exploitive feeding. These, and several other questions relating to the functions of the saliva remained unsolved.

ACKNOWLEDGEMENTS

The collaborative part of this work was conducted with the aid of a NATO grant 0102/88.

References

Cohn, E. (1970). Observations on the feeding and symptomatology of *Xiphinema* and *Longidorus* species on selected roots. *J. Nematol.*, 2 : 167-173.

Griffiths, B. S. & Robertson, W. M. (1984). Nuclear changes induced by the nematode *Xiphinema diversicaudatum* in root-tips of strawberry. *Histochem. J.*, 16 : 265-273.

Griffiths, B. S. & Robertson, W. M. (1988). A quantitative study of changes induced by *Xiphinema diversicaudatum* in root-tip galls of strawberry and ryegrass. *Nematologica*, 34 : 198-207.

Griffiths, B. S., Robertson, W. M. & Trudgill, D. L. (1982). Nuclear changes induced by the nematode *Xiphinema diversicaudatum* and *Longidorus elongatus* in root-tips of perennial ryegrass, *Lolium perenne*. *Histochem. J.*, 14 : 719-730.

Griffiths, B. S. & Trudgill, D. L. (1983). A comparison of the generation times of, and gall formation by *Xiphinema diversicaudatum* and *Longidorus elongatus* on a good and a poor host. *Nematologica*, 29 : 78-87.

Hunt, D. J. & Towle, A. (1979). Feeding studies on *Xiphinema vulgare* Tarjan, 1964 (Nematoda : Longidoridae). *Revue Nématol.* 2 : 37-40.

Robertson, W. M. (1975). A possible gustatory organ associated with the odontophore in *Longidorus leptocephalus* and *Xiphinema diversicaudatum*. *Nematologica*, 21 : 443-448.

Robertson, W. M. & Henry, C. (1986). An association of carbohydrates with particles of arabis mosaic virus retained within *Xiphinema diversicaudatum*. *Ann. appl. Biol.*, 109 : 299-305.

Robertson, W. M., Topham, P. B. & Smith, P. (1987). Observations on the action of the oesophageal pump in *Longidorus* (Nematoda). *Nematologica*, 33 : 43-54.

Robertson, W. M., Trudgill, D. L. & Griffiths, B. S. (1984). Feeding of *Longidorus elongatus* and *L. leptocephalus* on root-tip galls of perennial rye grass *(Lolium perenne)*. *Nematologica*, 30 : 222-229.

Robertson, W. M. & Wyss, U. (1979). Observations on the ultrastructure and function of the dorsal oesophageal gland cell in *Xiphinema index*. *Nematologica*, 25 : 391-396.

Rumpenhorst, H. J. (1984). Intercellular feeding tubes associated with sedentary plant parasitic nematodes. *Nematologica*, 30 : 77-85.

Sutherland, J. R. (1969). Feeding of *Xiphinema bakeri*. *Phytopathology* 59, 1963-1965.

Towle, A. & Doncaster, C. C. (1978). Feeding of *Longidorus caespiticola* on ryegrass *(Lolium perenne)*. *Nematologica*, 24 : 277-285.

Trudgill, D. L. (1976). Observations on the feeding of *Xiphinema diversicaudatum*. *Nematologica*, 22 : 417-423.

Weischer, B. & Wyss, U. (1976). Feeding behaviour and pathogenicity of *Xiphinema index* on grapevine roots, *Nematologica*, 22 : 463-470.

Wyss, U. (1977). Feeding phases of *Xiphinema index* and associated processes in the feeding apparatus. *Nematologica*, 23 : 463-470.

Wyss, U. (1978). Root and cell response to feeding by *Xiphinema index*. *Nematologica*, 24 : 159-166.

Wyss, U., Lehmann, H. & Jank-Ludwig, R. (1980). Ultrastructure of modified root-tip cells in *Ficus carica*, induced by the ectoparasitic nematode *Xiphinema index*. *J. Cell Sci.*, 41 : 193-208.

Wyss, U., Robertson, W. M. & Trudgill, D. L. (1988). Oesophageal bulb function of *Xiphinema index* and associated root cell responses, assessed by video-enhanced contrast light microscopy. *Revue Nématol.*, 11 : 253-261.

Accepté pour publication le 8 février 1990.