**Esophageal bulb function of Xiphinema index and associated root cell responses, assessed by video-enhanced contrast light microscopy**

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**SUMMARY**

Xiphinema index fed on a column of progressively deeper cells in root-tips of Ficus carica seedlings and in each the pattern of behaviour and responses was generally similar. Following penetration of a root cell wall, secretions (saliva) from the dorsal esophageal gland were injected by the nematode. Simultaneously globules of fluid, thought to arise from the two subventral glands, passed backwards through the esophago-intestinal valve. There then followed several periods of esophageal bulb pumping during which the contents of the perforated uninucleate cell were removed within about two minutes. Each period of pumping was separated by a short pause at the beginning of which further secretions from the dorsal gland were even more forcefully and rapidly injected. The pattern of feeding was similar on uninucleate and modified binucleate cells, except that it took longer on the latter. When saliva was injected, the cytoplasm of the root cell in the vicinity of the protruded stylet-tip lost its structure and viscosity within a few seconds. With each subsequent injection of saliva this change spread to the rest of the cytoplasm and the nucleus, facilitating the ingestion of their liquefied contents. As feeding continued the nucleolus progressively shrank until only a condensed residue remained. The amyloplast membranes were dissolved, releasing their enclosed starch grains but the plasmalemma and nuclear envelope apparently remained intact. Occasionally, feeding nematodes became inactive for c. 15 min immediately after their stylet-tip had penetrated the next deeper cell. A plug was then observed forming around the stylet-tip, but otherwise no cellular changes were recorded. During the extended periods of ingestion that followed an inactive phase, the plug interfered with the passage of saliva for some time so that in these cases the disintegration of the cell contents was retarded.

**RéSUMÉ**

Fonctionnement du bulbe asophagien chez Xiphinema index, et modifications des cellules radiculaires par le nématode, révélés par la microscopie en contraste de phase couplée à la vidéo à haute résolution

Xiphinema index se nourrit à partir d'une colonne de cellules s'accroissant progressivement en profondeur, et pour chacune de ces cellules le comportement du nématode et la réponse de l'hôte sont similaires. Après avoir percé la paroi cellulaire, le nématode injecte les sécrétions (salive) de la glande esophagienne dorsale. Simultanément, des globules de fluide, supposés parvenir des deux glandes subventrales, passent vers l'arrière, à travers la valve esophago-intestinale. Suivent plusieurs périodes de pompage du bulbe esophagien au cours desquelles le contenu de la cellule uninucléée perforée est vidé, en deux minutes environ. Les périodes de pompage sont séparées par une courte pause au début de laquelle de nouvelles sécrétions de la glande esophagienne dorsale sont injectées plus rapidement et avec plus de force. Ce schéma de prise de nourriture est semblable pour les cellules uninucléées et pour les cellules modifiées, binucléées, toutefois le processus est plus long pour ces dernières. Lorsque la salive a été injectée, le cytoplasme de la cellule radiculaire situé au voisinage de l'extrémité du stylet perd sa structure et sa viscosité en quelques secondes. Au cours des injections suivantes, ces modifications s'étendent au reste du cytoplasme et au noyau, facilitant ainsi l'ingestion du contenu cellulaire. La prise de nourriture continuant, le nucléole diminue progressivement et il n'en demeure qu'un résidu condensé. Les membranes des amyloplastes sont dissoutes, libérant les granules d'amidon, mais les plasmalemmes et l'enveloppe nucléaire restent apparemment intacts. Il arrive que le nématode reste inactif pendant environ quinze minutes immédiatement après que son stylet ait pénétré dans la cellule voisine, située plus en profondeur. On observe alors la formation d'un bouchon autour de l'extrémité du stylet, mais aucune autre modification de la cellule n'intervient. Pendant les périodes d'ingestion qui suivent une telle période de repos, ce bouchon gêne le passage de la salive pendant quelque temps, ce qui retarde la désintégration du contenu cellulaire.

Xiphinema index, the vector of grapevine fanleaf virus, is an economically important pest and hence one of the most intensively studied ectoparasitic nematodes. It feeds readily on the roots of host plants growing in agar culture, and several studies have been made of its relationship with its specific hosts. Feeding is largely
restricted to the root-tips which, when attacked, cease growing and develop into terminal galls (Wyss, 1981). Sections through these galls reveal areas of collapsed necrotic cells surrounded by metabolically active multinucleate cells (Wyss & Welscher, 1976; Wyss, 1978; Rumpenhorst & Welscher, 1978; Wyss, Lehmann & Jank-Ladwig, 1980; Bleve-Zacheo & Zacheo, 1983). However, little is known about how the necrotic cells have been killed or what induces the adjacent cells to become multinucleate.

Attempts to determine qualitative changes in free amino acids and proteins in aseptic *Ficus carica* fed upon by *X. index* (Poehling, Wyss & Neuhoff, 1980; Poehling & Wyss, 1980) gave only a few clues about the possible means of induction of the galls. However, it seems likely that any chemicals involved in gall formation are synthesized in the cesophageal bulb of the nematode. The cesophageal bulb is a complex organ used for ingestion and also contains three gland cells. There are two subventral glands which lie midway along the bulb. Each has a short duct which enters the pump chamber towards the rear of the bulb (Robertson & Wyss, 1979; 1983). The third gland cell is the dorsal, which is much larger than the subventrals and has a complex duct system. Two ducts extend dorsally along almost the entire length of the bulb and four ventral branches extend almost half-way along the bulb. These six ducts join together at the anterior end of the bulb and enter the food canal through a valve just anterior to the pump chamber. Fluids have been seen moving forward through the duct system of the dorsal gland a few seconds after the stylet-tip had perforated the wall of a root cell and also during the short pauses of ingestion pumping (Wyss, 1977; Wyss & Inst. Wiss, Film, 1977). However, although observations were made at high magnification with interference contrast light microscopy, it was not possible to confirm that these fluids were injected into the root cell. Also, the immediate response of the protoplast of the attacked cell, which was usually several cells deep, remained obscure. Video-enhanced contrast light microscopy (Wyss & Zunke, 1986a; 1986b; Wyss, 1987) enabled these limitations to be overcome and this paper reports more detailed studies on the functioning of the three gland cells during feeding and on the response of the attacked root cell.

**Materials and methods**

Aseptic fig (*Ficus carica*) seedlings and *X. index* were maintained in agar culture as previously described (Wyss, 1978). The observation chambers were the same as those described by Wyss and Zunke (1985) except that fine sand particles were omitted. Numerous periods of feeding by *X. index* (mainly adult females) were recorded using a high resolution video system (Wyss & Zunke, 1986a) and later analysed. In some feeds the functioning of the dorsal gland and two subventral gland cells were studied whereas in others the main objective was to study the associated root cell responses.

**Results**

**FUNCTIONING OF THE CESOPHAGEAL BULB**

Female *X. index* usually inserted their stylet two to three cells deep before commencing feeding. A few seconds after stylet projection had ceased, the bulb was observed to suddenly stretch and the duct system of the dorsal gland dilated, especially at the anterior end. Dilation of the ducts was accompanied by the rapid disappearance (they appeared to burst) of many of the secretory granules along the entire duct system. This was immediately followed by the ducts collapsing as their contents were passed forward. The whole process of duct dilation and collapse took, on average, 9 seconds (n = 30). After a further c. 8 seconds the bulb had shortened and ingestion pumping commenced (Fig. 1). Observations on the posterior end of the bulb showed that between the stretching and shortening of the bulb (i.e. before ingestion-pumping commenced) the pump plates were slightly opened and that there was a steady flow of fluids through the cesophageal-intestinal valve (Fig. 3 F). When ingestion-pumping started, fluids withdrawn from the root cell could be seen passing through the cesophageal-intestinal valve. Feeding on uninucleate cells within newly attacked root-tips typically comprised 2-4 periods of ingestion interspersed with short pauses (Fig. 1). During the pauses fluids from within the dorsal gland were again passed forward. These very rapid movements of dorsal gland cell fluids (hereafter termed saliva) were initiated by a sudden contraction of the dilator muscles (Fig. 3 D) that opened the valve where the main collecting duct enters the food canal in front of the pump chamber. During these movements of saliva no fluids were seen passing backwards through the cesophageal-intestinal valve. Depletion of the dorsal gland cell ducts usually took c. 1 second and 3-4 second later ingestion pumping was resumed (Fig. 1). During ingestion pumping the ducts in the dorsal gland cell progressively dilated (Figs 3 A, B). However, during salivations between periods of ingestion, a breakdown of secretory granules around the ducts of the dorsal gland cell was never observed, neither during duct dilation nor during depletion (Fig. 3 C).

Figure 1 represents typical events observed in the anterior feeding apparatus as a female *X. index* fed progressively deeper on four uninucleate meristematic cells within a newly attacked root-tip. On slightly swollen root-tips containing binucleate cells, feeding was identical to that on newly attacked root-tips, with the exception that the time spent on one cell was now longer (up to 8 min) with an average of 12 ingestion periods.
In addition to this general pattern of feeding, most nematodes showed occasional periods of cesophageal bulb inactivity when feeding within the root-tip. The inactive phases, which lasted on average 15 minutes \((n = 18)\), apparently occurred at random but always immediately after penetration of the wall of a living cell. The ducts of the dorsal gland cell stayed slightly dilated throughout the periods of inactivity and, apart from a very occasional twitching of muscles near the cell nucleus and a slight movement of secretory granules, the bulb appeared to be completely inactive. The ducts and the nuclei (Fig. 3 E) of the subventral glands were then, as always, surrounded by many secretory granules and most were seen to move. Occasionally, some of the...
Fig. 3. Females of *X. index* feeding from cells within root-tips of *Ficus carica* seedlings. All bars = 5 μm. A-C: anterior end of oesophageal bulb. A: bulb pulsating during food ingestion, showing nucleus (nd) of dorsal gland cell and one of four dilated ventral branches (vd) of its duct system; main duct (md) of dorsal gland cell slightly dilated. g, some of secretory granules surrounding duct system; B: 13 sec. after A, main duct (md) and ventral branch now more dilated, just before duct depletion; C: 0.8 sec. later, ducts depleted; D: anterior end of oesophageal bulb during depletion of dorsal gland duct system. Arrow points to region of bulb pulled in by contraction of dilator muscles that open orifice of food canal (fc) into which main duct (md) enters. nd, nucleus of dorsal gland; pc, pump chamber; E: one of two subventral gland nuclei (nv), densely surrounded by secretory granules (g); F: posterior end of oesophageal bulb, showing passage of secretory fluids (arrows) from two subventral glands into intestine (i); pc, pump chamber slightly opened; G-H: feeding from root-tip cell (cell C in Fig. 2), seven layers beneath root surface. G: stylet-tip (St) just inserted into this cell, marked by four arrows. Nucleus (nu) intact like that (nu 3) of an unaffected cell. nu 1; nu 2, degraded nuclei of cells A and B (Fig. 2), previously fed on; H: 58 sec. after G, contents of cell partially ingested, nucleolus (n) now more refractive, here just before second injection of saliva; I: 1 sec. after H, showing amount of saliva injected (compare location of arrows with H); J: 32 sec. after I, after a total of three injections of saliva. Stylet-tip now just being pushed deeper.
granules around the ducts were observed breaking down, but breakdown was rather less rapid than that previously described for the dorsal gland cell. Following periods of inactivity the duration of ingestion prior to penetrating the next deeper cell was considerably prolonged (8-39 min). However, pumping was still typically intermittent, i.e. periods of ingestion alternated with short pauses during which the ducts of the dorsal gland cell were depleted.

**ROOT CELL RESPONSES TO FEEDING**

On young, healthy root-tips (with uninucleate meristematic cells) *X. index* fed progressively deeper on a column of cells (Fig. 2). Food was usually not withdrawn from root cap and epidermal cells. However, in the adjacent deeper cells penetration was followed by ingestion.

The stylet-tip of a female *X. index* which had just been inserted into a meristematic cell, seven cell layers below the root surface, is shown in Fig. 3 G. This cell was the fifth in the series that were fed upon (cell C, Fig. 2). No changes were observed within perforated cells in the first 12-19 seconds (n = 25). However, coincident with the breakdown of secretory granules and depletion of the ducts in the dorsal gland cell, fluids were seen entering the cell through the stylet-tip (1.S in Fig. 2) and, from these observations, it is certain that saliva from the dorsal gland cell is injected into the root. The injection of this saliva caused immediate changes in the cytoplasm around the stylet-tip with a loss of structure and an apparent decrease in viscosity. The injection of saliva was followed after about 10 seconds by withdrawal of cytoplasm (Fig. 2). During ingestion, the wall of the root cell vibrated and the nucleus oscillated at the same rate as the pulsations of the esophageal bulb. After about 15-40 seconds saliva was again injected (2.S in Fig. 2), but now with much greater volume and force than in the first injection (Fig. 3 H-I). As illustrated in Fig. 2 the contents of uninucleate meristematic cells were usually consumed within 2 minutes by 2-4 periods of ingestion and a total of 3-5 injections of saliva from the dorsal gland cell (n = 25).

A full sequence of cell penetration, and responses following salivation and ingestion is shown in Fig. 4 A-L. Perforation of the wall of this cell (Fig. 4 A) was achieved by a few stylet thrusts and the stylet-tip came to rest 2-4 μm within the cytoplasm (Fig. 4 B). Following the injection of saliva, liquefaction of the cytoplasm spread rapidly (Fig. 4 C), even while the bulb was pumping (Fig. 4 D). The viscosity of the nucleoplasm was also reduced. The nucleolus was drawn towards the stylet-tip and started to oscillate at the same rate as the pulsations of the esophageal bulb. The second injection of saliva was so large that the nucleolus was visibly repelled (compare Figs 4 D and 4 E). At the end of the third and final period of food withdrawal the remaining cytoplasm was totally liquefied, and the size of the nucleolus still retained within the nuclear envelope, had been decreased considerably (Fig. 4 F). The last (fourth) injection of saliva (Fig. 4 G) was followed by a further disintegration of the nucleolus which was reduced to a dense core (Fig. 4 H). When the stylet-tip was pushed deeper through the dead cell by a series of short jabs at a rate of 2-3 second, it appeared that the nuclear envelope had not been dissolved (Fig. 4 I). The cellular responses described above were typical of those observed in all uninucleate root-tip cells even when, occasionally, epidermal cells were attacked. Feeding on an epidermal cell is illustrated in Fig. 5 A-D. The cell was killed within 72 seconds after stylet-tip insertion (Fig. 5 A). The cytoplasm disintegrated rapidly after the first injection of saliva (Fig. 5 B). The plasmalemma appeared not to be destroyed during feeding, as it was pulled away intact from the cell wall by the suction created during food withdrawal (Fig. 5 C). Also, occasionally the plasmalemma was observed cloaking the tip of the protruding stylet during penetration to the next deeper cell.

A few feeds by juveniles were recorded and, except that their stylets were shorter and could not penetrate so deeply, their effects (Fig. 5 E and F) and behaviour were similar in all respects to those of adult females. Not yet swollen root-tips of fig were sufficiently small for the fully protracted stylet of a female to reach the outside of the root on the opposite side. Females usually fed from these cells, which contained amyloplasts (e.g. cell D in Fig. 2), illustrated by Figs 5 L-M). The first injection of saliva produced a rapid disintegration of the amyloplast membrane surrounding groups of starch grains. The released grains (Fig. 5 M) were not ingested. Root-tips attacked by two to three females soon formed galls which contained columns of collapsed, necrotic cells surrounded by expanding meristematic cells (Fig. 5 G). When the stylet-tip of a feeding nematode encountered a necrotic cell it had more difficulty in penetrating the wall because of the lack of turgor pressure. Feeding nematodes appeared to recognise when they had reached a dead cell and immediately penetrated more deeply until a living cell was contacted.

Root-tips which had been fed upon for one day were visibly swollen and contained binucleate cells (Fig. 5 H) which could be recognised up to five cell layers below the root surface with the video system used. Feeding from binucleate cells (Fig. 5 I) was similar to that on uninucleate meristematic cells, except that it took longer (average 5 mn) and there were more periods of ingestion (average twelve per cell, n = 12). As described previously, degraded nucleoli were left behind (Fig. 5 J). Two days after the initial nematode attack the modified cells contained four nuclei (Fig. 5 K). Feeding from the larger and denser multinucleate cells was, however, more difficult to observe but took even longer than in binucleate cells. During the periods when nematodes became inactive...
Fig. 4. Female of *X. index*, feeding from a root-tip cell of *Ficus carica* seedling. Bar = 5 μm. A: stylet-tip (st) just before cell wall perforation. nu, nucleus; n, nucleolus; B: stylet-tip inserted and at rest, 12 sec. before first injection of saliva from dorsal esophageal gland cell; C: 11 sec. after first injection of saliva, cytoplasm around stylet-tip liquefied, marked by arrows; D: nucleus and nucleolus drawn towards stylet-tip during food withdrawal, here 1 sec. before second injection of saliva; E: saliva just injected, nucleolus pressed back (compare length of arrow with that of D); F: just before fourth and last injection of saliva. Nucleus degraded, cytoplasm of whole cell liquefied; G: saliva just injected; H: cell partially emptied, nucleolus shrunk to a dense core, stylet-tip now being pushed deeper; I: stylet-tip approaching opposite wall of fed-on cell, nuclear envelope (ne) of degraded nucleus (arrows) apparently still intact.
Immediately after cell penetration, secretions emanating from the styllet-tip were observed forming a plug-like structure at the end of the styllet-tip (Fig. 5 N-O). A clear zone developed in the cytoplasm surrounding the growing plug, but otherwise no cell changes were observed. Once ingestion had commenced, the usual forceful injections of saliva were attenuated by the presence of the plug, which was never seen to become dislodged from the styllet-tip. Degradation of the cytoplasm and nucleus within the cell containing the plug was slow, in this particular case, taking about five times as long as during normal feeding. In other cases it was even more prolonged. When the nematode injected saliva, fluids were seen to escape through the slit in the styllet into the previous adjacent cell where, if it had not been previously fed upon, they caused a gradual degradation of the cell's protoplast (Fig. 5 O).

Discussion

Our observations show that when X. index feeds on root-tip cells the activities within the esophageal bulb generally follow a fixed pattern which is similar for uninucleate and bi- to multinucleate cells except that more salivation and ingestion phases occur for the latter, uninucleate and bi- to multinucleate cells except that feeding then lasts longer.

The dorsal gland cell appears to have at least two and possibly three ways of functioning viz. 1) Following penetration of a root-cell, the dorsal gland ducts fill with fluid coming, at least in part, from the breakdown of secretory granules lining the gland ducts. 2) In subsequent salivations into the cell between periods of ingestion duct contents are again injected into the root cell. However, duct dilation and depletion is then not accompanied by granule breakdown, indicating that the dorsal gland cell is capable of producing at least two types of secretion. There are many nerves running through the esophageal bulb which probably control its complex activities. 3) During the inactive periods slight movements of the muscles in the region of the dorsal gland nucleus were observed indicating that the dorsal gland cell may be slowly secreting the material which forms the plug at the styllet-tip in the root cell. These inactive periods were not confined to particular cells or to certain depths within the root and did not involve the dilation and depletion of the dorsal gland duct system. Otherwise they resemble the salivation phase reported for Longidorus species (Towne & Doncaster, 1978; Robertson, Trudgill & Griffiths, 1984). Similar inactive periods have been reported for X. diversicaudatum (Trudgill, 1976).

The subventral gland cells are presumed to act in unison and appear to have one or possibly two or three functions viz. 1) They supply secretions to the intestine immediately prior to the first period of ingestion pumping. 2) Breakdown of granules during the inactive period indicates that subventral gland secretions may pass forwards during plug formation and, if they are produced, may act in combination with dorsal gland cell secretions. 3) Secretions may be added to the food taken from the plant during ingestion pumping. Our observations therefore demonstrate the complex way in which the esophageal bulb of X. index functions and complement earlier ultrastructural studies (Robertson & Wyss, 1979; Robertson, in press).

Changes in the root cells during feeding also have a regular pattern. The first injection of saliva (involving breakdown of the dorsal gland granules) which follows penetration of the cell wall causes liquefaction of the cytoplasm and nucleoplasm, thereby enabling them to be readily ingested. Subsequent injections of saliva accelerate the liquefaction process. This could be caused by pH, ionic or enzymic effects. The release of secretory proteins is strongly suggested by the observed bursting (exocytosis) of secretory granules derived from the rough endoplasmic reticulum via the Golgi complex (cf. Robertson & Wyss, 1979). Neither the plasmalemma nor the nuclear membranes are destroyed, but the nucleoplasm is extracted leaving only a residue of the nucleus condensed within the membrane. In contrast, amyloplast membranes are readily broken down to release starch grains which are not ingested. Feeding on bi-to multinucleate cells follows a similar pattern but takes longer because the cells are larger and metabolically highly active with an increased cytoplasmic density.

The most obvious response to feeding is the formation of a gall containing multinucleate cells. It is not certain which of the individual effects of feeding is responsible for these changes and it may be that several are involved. Production of the plug material during the inactive periods had little immediate effect either in the cell penetrated or in surrounding cells. However, the inactive periods, and the more prolonged periods of ingestion which follow may play a crucial role in gall formation; little damage takes place during the inactive periods and there is ample time before ingestion begins for a gall-initiating substance to be transported to neighbouring cells. Similarly, the withdrawal of large volumes of solutes may initiate some of the changes invoked in the surrounding cells.

Our observations on the functioning of the esophageal bulb during feeding also pose a number of questions regarding the role of its secretions during transmission of grapevine fanleaf virus by X. index. The force with which saliva is expelled during ingestion pauses is considerable and may be sufficient to dislodge virus particles from their site of retention on the food canal wall without any specific release mechanism being required. However, potentially three different types of salivation behaviour have been identified, any one of which could involve a specific release mechanism. Release of virus during the inactive phase is an attractive proposition as the cell is then not rapidly destroyed as it is during normal feeding. The possibility that the subventral gland secretions may be involved in plug formation also raises the possibility that particles adsorb-
Fig. 5. *X. index* feeding from root-tip cells of *Ficus carica* seedlings. All bars = 5 μm. A-D: female feeding from an epidermal cell. A: stylet-tip (st) just inserted. n, nucleolus; B: during food withdrawal, 23 sec. after first injection of saliva from dorsal oesophageal gland cell. Most cytoplasm liquefied; C: during food withdrawal, 19 sec. after second injection of saliva. Plasmalemma (arrows) pulled away from cell wall by suction created; D: 36 sec. later, stylet-tip now inserted in next deeper cell. Nucleolus (n) of fed on cell shrunk to a dense core. E-F: L 3-juvenile feeding from subepidermal cell in region of root elongation; E: stylet-tip (st) just inserted. nu, nucleus; F: 2 min. later, arrow points to remnants of nucleus; G: collapsed necrotic cell (nc) between...
ed onto the walls of the pump chamber (Taylor & Robertson, 1970) could be passed forward into the root and are not lost for the purpose of transmission as had been previously supposed. However, it could equally be argued that the formation of the plug material would prevent the passage of the virus particles into the plant cell. Detailed experiments are now required to resolve these questions and to identify the mechanisms of gall formation.

REFERENCES


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