Observations on the cuticle surface of second stage juveniles of *Globodera rostochiensis* and *Meloidogyne incognita*

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SUMMARY

Longitudinal sections through the lips of sedentary second stage juveniles of *Globodera rostochiensis* within potato roots revealed the presence of numerous fibrils either free in the cell or part of a composite layer next to the cell wall. These fibrils originated in the third (median) layer of the cuticle and were extruded on to the surface of the nematode. Emigrant juveniles from potato roots also had an amorphous electron dense deposit in the annular grooves. No body pores were found, but longitudinal sections through the lip region of freshly hatched juveniles of *G. rostochiensis* and *Meloidogyne incognita* showed a previously unreported periodicity in the epicuticle. The fibrillar material may have been extruded through these striae. The function of the fibrils is unknown, but it is possible that they are involved in the anchorage of the nematodes during feeding.

RÉSUMÉ

Observations sur la surface de la cuticule des juvéniles de deuxième stade de Globodera rostochiensis *et de* Meloidogyne incognita

Des sections longitudinales au travers de la région labiale, de juvéniles de deuxième stade de *Globodera rostochiensis* fixés dans les racines de pommes de terre, ont révélé la présence de nombreuses fibrilles, soit libres dans la cellule, soit faisant partie d'une couche composite située près de la paroi cellulaire. Ces fibrilles proviennent de la troisième couche (= couche médiane) de la cuticule et sont expulsées à la surface du nématode. Les juvéniles migrant hors des racines de pomme de terre présentent de plus un dépôt amorphe, dense aux électrons, dans les sillons séparant les anneaux. Aucun pore n'a été observé sur le corps, mais des sections longitudinales au travers de la région labiale de juvéniles frais éclos de *G. rostochiensis* et de *Meloidogyne incognita* ont révélé une périodicité de structure dans l'épicuticule. La matière fibrillaire pourrait être expulsée à travers ces stries. La fonction de ces fibrilles est inconnue, mais il est possible qu'elles jouent un rôle dans l'ancrage du nématode pendant sa prise de nourriture.

The role of nematode saliva in compatible nematode/host plant interactions has long been a subject of study (Linford, 1937; Wyss & Zunke, 1986). When it was found that most if not all nematodes have saccharide residues on their surfaces (Spiegel, Cohn & Spiegel, 1982; Zuckerman & Jansson, 1984) the possibility that surface components might also be involved in compatible/incompatible interactions was considered (Rice, Leadbeater & Stone, 1985; Forrest & Robertson, 1986).

In contrast to animal parasitic nematodes, the juvenile surface of plant parasitic nematodes was generally considered to be inert and electron microscopical studies of the outermost layer of cuticle of *Globodera rostochiensis* (the epicuticle) indicated that it was amorphous (Günther & Kämpfe, 1967; Wisse & Daems, 1968). Shepherd, Clark and Dart (1972) also found that the cuticle structure in the adult male of potato cyst nematode was almost identical to that of the juvenile. The epicuticle was homogeneous, electron dense, without structure, and resistant to chemical breakdown (Shepherd, Clark & Dart, 1972). However, Endo (1987) demonstrated the presence of a uniform layer of fibrillar material on the cuticle in the head region of a second stage juvenile of *Heterodera glycines* sedentary within soybean roots which may have originated from the cuticle in advance of the moult.

In this paper the cuticles of second stage juveniles of *G. rostochiensis* which were freshly hatched, sedentary within roots, or which had invaded and subsequently left the root were examined to determine the structure and the production of fibrillar material. Freshly hatched second stage juveniles of *Meloidogyne incognita* were also examined for comparison.

Material and methods

Cysts of *G. rostochiensis* produced on susceptible potato cultivars for the previous two years were extracted from soil by flotation. One day-old, second stage juveniles were obtained by exposing soaked cysts to potato root diffusate (Forrest & Farrer, 1983).

Meloidogyne incognita second stage juveniles obtained from a population multiplied on tomato cv. Moneymaker were also examined for cuticle structure. Infected tomato plants growing in pots of gravel were watered to wash through the juveniles which were collected in trays (Fargette & Trudgill, unpubl.) decanted on to tissue to separate the juveniles and stored at 4°.

Second stage juveniles of *G. rostochiensis* which had invaded and subsequently left the roots of susceptible and resistant potatoes were collected as described by Forrest, Trudgill and Cotes (1986). One week-old rooted potato sprouts in canisters of sand were inoculated with 2 500 juveniles at 20° and after three days the roots were thoroughly washed before transfer into grit in plastic containers. Plants were watered daily and emigrant juveniles which had left the roots were washed into trays, collected, and stored for up to seven days at 4°.

Some emigrants were labelled with 100 µg/ml of concanavalin A-tetramethylrhodamine isothiocyanate (TRITC) in phosphate buffered saline as described by Forrest and Robertson (1986) for visualisation in transmission electron microscopy after tannic acid fixation (Roholl *et al.*, 1981).

Second stage juveniles of *G. rostochiensis* developing in sterile potato roots were obtained by growing surface sterilised sprouts of potato cvs Home Guard (susceptible) or 8917b (3) (ex *vernei*, 90 % resistant) on 1 % Murashige and Skoog (M & S) plant salt and minimal organics mixture (Flow Laboratories, Irvine, Scotland) in 1.25 % Davis agar (Davis Gelatine Ltd, New Zealand) in a Petri dish. The dishes were sealed with Parafilm M and kept at 20° for 5-7 days, before inoculating the roots with hatched juveniles. The juveniles had been stored for 1-14 days in 50 ppm streptomycin, washed in sterile distilled water and treated in a gauze-tipped 1 ml plastic syringe (Forrest, 1986) for 2 s with 0.01 % mercuric chloride. The juveniles were then washed in distilled water and 50-100 placed close to the roots in each Petri dish.

Roots were examined under a low power stereo binocular microscope 24 h after inoculation to determine if juveniles had penetrated. After various time intervals Petri dishes containing infested roots were cooled at 4° for 30 min and chilled fixative added (Schuerger & McClure, 1983). Portions of root containing nematodes were then excised and kept in fixative (2 % tannic acid and 2 % glutaraldehyde in 0.1 M phosphate buffer (PB, final pH 6.6) for 24 h, rinsed in PB, enclosed in blocks of 1 % agar which were passed through a graded ethanol series and finally liquid propylene oxide. They were infiltrated with Spurr's resin for 24-30 h at 4°, evacuated for *c.* 1 min and embedded in open blocks in Spurr's resin at 65° overnight.

Freshly hatched juveniles and emigrants of *G. rostochiensis* and juveniles of *M. incognita* were processed as described by Forrest and Robertson (1986) with minor modifications. They were washed as above and decapitated in the fixative. The heads were rinsed three times in phosphate buffer, osmicated in 1 % OsO_4 then embedded in 1 % agar prior to dehydration in an ethanol in phosphate buffer, osmicated in 1 % OsO_4 then pylene oxide then infiltrated with Emix (medium) resin (Emscope Laboratories Ltd., Ashford, Kent) at 37° for 2 h. Specimens were placed in fresh resin and polymerised at 65° ovenight.

Silver/grey sections of resin embedded nematodes and root pieces containing sedentary nematodes were cut on a Reichert ultra-microtome and stained with saturated uranyl acetate in 50 % ethanol and 2.4 % lead citrate in citrate buffer. They were then examined by transmission electron microscopy on a JEOL JEM 1 200 EX at 80 KV.

Results

When sections through sedentary second stage juveniles of *G. rostochiensis* within the roots of resistant and susceptible potatoes were examined by TEM, abundant fibrils were often seen surrounding the head region (Fig. 1*a*). These fibrils were associated with the annules which appeared to have inverted. In other cases they were present in the uninverted grooves. Lacunae had developed in the third layer of the cuticle below each annule and the components for the fibrils appeared to have been extruded on to the surface (Fig. 1*b*). These extrusions were found on the lip region and on at least the front third of the body, sometimes as a uniform fibrillar layer. Many of the fibrils were also found loose in the cell in which the juvenile settled, or forming a deposit composed of plant and nematode material next to the cell wall (Fig. 1*c*).

The cuticle of a juvenile of *G. rostochiensis* in longitudinal sections was seen to consist of four layers : the outermost epicuticle, a fibrillar second layer, an electron transparent third layer, possibly composed of fluid, and a striated fourth layer with alternating dark lines and transparent spaces. An electron-dense ball (Wisse & Daems, 1968) was visible in the third layer. Lectin-labelling with concanavalin A-TRITC followed by treatment with tannic acid revealed an electron dense deposit in the annular grooves of emigrant juveniles (Fig. 1*d*) which was not found in similarly treated freshly hatched juveniles.

Longitudinal sections through the head region of freshly hatched second stage juveniles of *G. rostochiensis* revealed a definite structure in the epicuticle (Fig. 1*e*). The periodicity which ran perpendicular to the surface was also visible in the vestibule extension around the stylet (Fig. 1*f*). The same periodicity was evident in the epicuticle of emigrants. The epicuticle of *M. incognita* also had a similar structure (Fig. 1*g*).

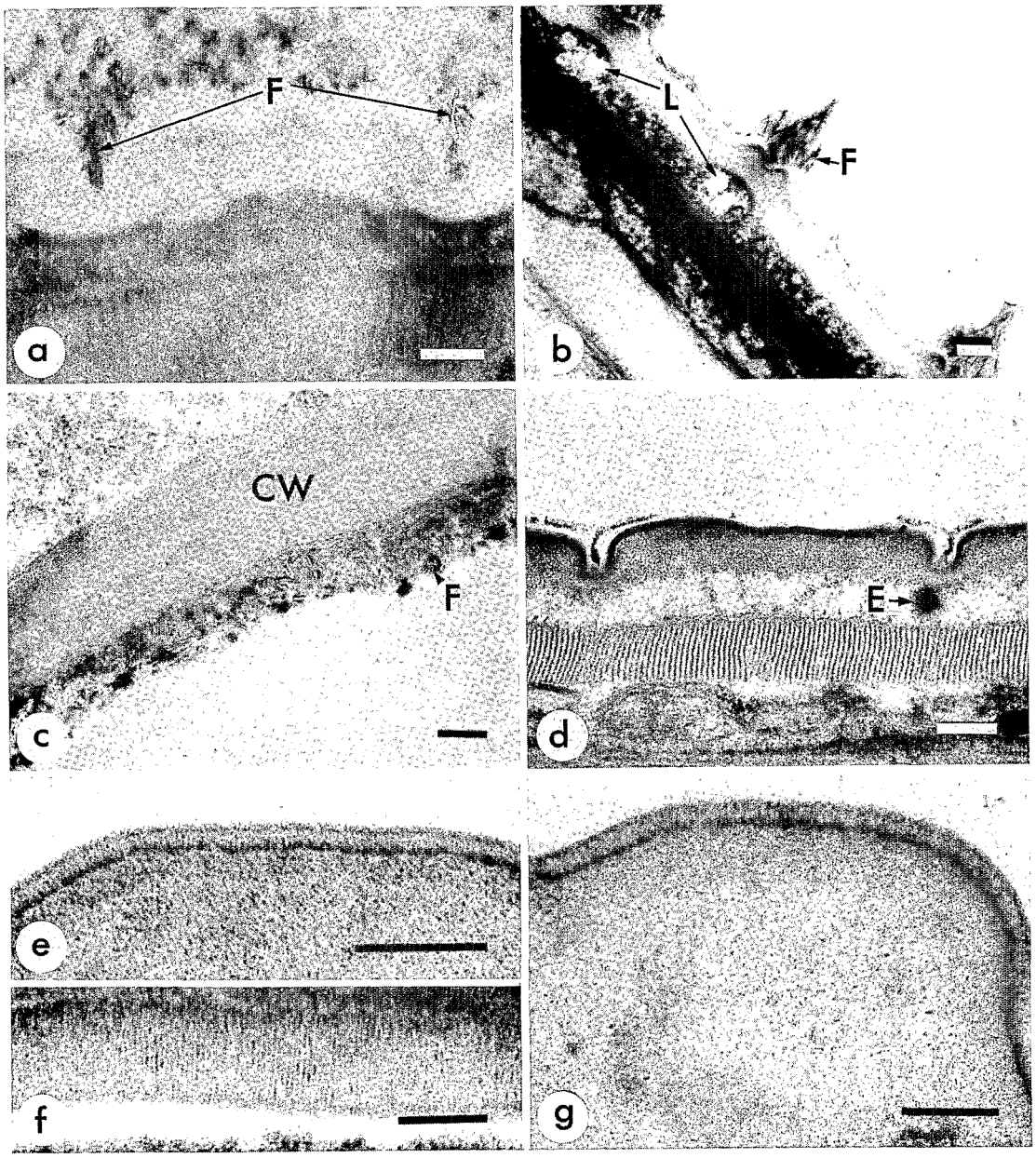

Fig. 1. *a* : Fibrils (F) of nematode origin in the head region of sedentary second stage juveniles within potato root, Scale bar ≡ 250 nm; *b* : Fibrillar material extruded on to the cuticle surface of a second stage juvenile of *G. rostochiensis* sedentary in potato root. Fibrils (F) may originate from lacunae (L) in the third layer, Scale bar ≡ 250 nm; *c* : Composite layer of plant and nematode fibrillar (F) material on the initial syncytial cell wall (CW) close to the head of the juvenile, Scale bar ≡ 250 nm; *d* : Électron dense deposits in the annular grooves of emigrant juveniles treated with concanavalin A-TRITC and then tannic acid. An electron dense ball (E) is visible in the third layer, Scale bar ≡ 250 nm; *e* : Longitudinal section through head region of a freshly hatched juvenile of *G. rostochiensis* showing the periodic structure of the epicuticle, Scale bar ≡ 100 nm; *f* : Longitudinal section through head region of freshly hatched juvenile of *G. rostochiensis* showing periodicity on the vestibule extension, Scale bar ≡ 100 nm; *g* : Longitudinal section through head region of freshly hatched juvenile of *Meloidogyne incognita* showing the structure of the epicuticle, Scale bar ≡ 100 nm.

Discussion

Endo and Wergin (1973) and Endo (1987) illustrated electron dense deposits respectively on the cuticle of second stage juveniles of *Meloidogyne incognita* and *H. glycines* endoparasitic in roots but were uncertain as to whether they originated from nematode, plant, or a combination of both. The deposit surrounding *H. glycines*, like that surrounding sedentary *G. rostochiensis* was fibrillar in structure. There were no body pores on second stage juveniles of *G. rostochiensis* or *H. glycines* apparent in TEM sections but there may, however, be areas of weakness in the epicuticle delineated by the periodicity from which material could be extruded.

The striate structure of the epicuticle of freshly hatched juveniles of *G. rostochiensis* and *M. incognita* may only have been revealed because of the use of tannic acid which is a particularly good preservative of the structure of collagen-like proteins (Chaplin, 1985). Roholl *et al.* (1981) used tannic acid fixation for EM visualisation of fluorochrome-labelled antibodies and lectins attached to cell surface antigens. In our studies it revealed small electron dense deposits in the annular grooves of emigrants but not of freshly hatched juveniles of *G. rostochiensis*. It seems possible that these deposits are the precursors of the fibrillar material found on sedentary second stage juveniles. It is perhaps this material on the surface of emigrants which labels strongly with a number of TRITC-conjugated lectins (Forrest, Robertson & Milne, in press).

In most second stage juveniles sedentary within the root the material surrounding the head of the nematode was distinctly fibrillar in structure. We consider that these fibrils originated from the third or median layer of the cuticle (Wright, 1987) where lacunae may have developed due to the extrusion of material into the annular grooves. The fibrils could be the result of an interaction between different nematode products or between nematode and plant. Various macromolecules can act as matrices in lignin polymer formation (Siegel, 1957) and lignification is a common defence mechanism against pathogens (Hammerschmidt & Kuc, 1982). Regardless of their origin, a function of these interlocking bundles of fibrils could be to anchor the second stage juveniles to their feeding site. Bundles of fibrils provide tensile strength and are frequently used in nature for adhesion, for example, by nematode-trapping fungi (Veenhuis, Nordbring-Hertz & Harder, 1985).

Zunke (1986) reported the development of a sub-crystalline layer consisting of fibrils on the surface of second stage juveniles of *Heterodera schachtii* under sterile conditions a few hours after feeding on the syncytium began. These fibrils were similar to those described on *G. rostochiensis* and illustrated in the fibrillar layer of *H. glycines* (Endo, 1987). We examined only the front third of second stage juveniles of *G. rostochiensis*, and so did not determine if the fibrils also occurred nearer the tail (Zunke, 1986).

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