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ULTRASTRUCTURE OF SYNCYTIAL CELL WALLS AND ASSOCIATED FEATURES AT THE HEAD REGION OF SECOND STAGE JUVENILES OF *GLOBODERA ROSTOCHIENSIS* IN SUSCEPTIBLE AND RESISTANT POTATO ROOTS

by

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Summary. In susceptible and resistant potato roots the area surrounding the initial syncytial cell wall at the head region of the second stage juvenile of *Globodera rostochiensis* was examined by transmission electron microscopy. In the small number of syncytia studied, partial or complete breakdown of the initial syncytial cell wall was found only in resistant roots, and is the probable cause of mass emigration or *in situ* death of nematodes.

Often fewer juveniles are found in the roots of resistant than susceptible plants (Forrest *et al.*, 1986; Mullin and Brodie, 1988; Herman *et al.*, 1991). When an inoculum composed of equal numbers of hatched juveniles is used to infest the roots, it can be demonstrated that roots of resistant plants are just as frequently invaded as susceptible ones, but that many of the juveniles leave the resistant roots instead of becoming sedentary (Forrest *et al.*, 1986; Mullin and Brodie, 1988; Herman *et al.*, 1991). It seems likely that this response occurs after initial attempts to settle and feed have failed. The crucial interaction between nematode and host plant may occur around the initial cell wall of the syncytium, and probably involves salivary products injected by the nematode or secretions from the surface of the head and body (Forrest and Robertson, 1986; Forrest *et al.*, 1988, 1989). Kim *et al.* (1987) previously reported that in the resistant soybean cv. Forrest necrosis was initiated adjacent to the lip region of *Heterodera glycines*, and Huang and Barker (1991) found an accumulation of glyceollin 1 in the same area of the incompatible cv. Centennial. The present paper reports a comparative study, made under sterile conditions, of the head region of *Globodera rostochiensis* (Wollen.) Behrens in resistant and susceptible potato roots, in an attempt to uncover key developments which could explain the differences in the behaviour of the nematode.

Materials and methods

Cysts of *G. rostochiensis* produced on susceptible potato cultivars for two years were extracted from soil by flota-

tion and freshly hatched J2 were obtained by exposing soaked cysts to potato root diffusate (Forrest and Farrer, 1983).

Sterile J2 in potato roots were obtained by growing surface sterilised sprouts of four genotypes on 1% Murashige and Skoog (MandS) plant salt and minimal organics mixture (Flow Laboratories, Irvine, Scotland) in 1.25% Davis Agar (Davis Gelatine Ltd., New Zealand) in sterile petri dishes. The dishes were sealed with Parafilm M and kept in the dark at 20°C for 5-7 days before inoculating the roots with hatched J2. These J2 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 sec with 0.01% mercuric chloride. The J2 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, the invaded J2 being located from entry tracks. Cultures contaminated during inoculation were discarded. After various time intervals petri dishes containing infested roots were cooled at 4°C for 30 min and chilled fixative was then added (Schuerger and McClure, 1983). Prior to fixation portions of root, containing nematodes, were cut and kept in fixative (2% tannic acid and 2% glutaraldehyde in 0.1 M phosphate buffer, PB, final pH 6.6) for 24 h at 4°C, rinsed in PB and embedded in 1% agar. Blocks containing processed specimens were placed through a graded ethanol series and finally propylene oxide. They were infiltrated with Spurr's resin for 24-30 h at 4°C, evacuated for *c.* 1 min and embedded in open blocks in Spurr's resin at 65°C overnight.
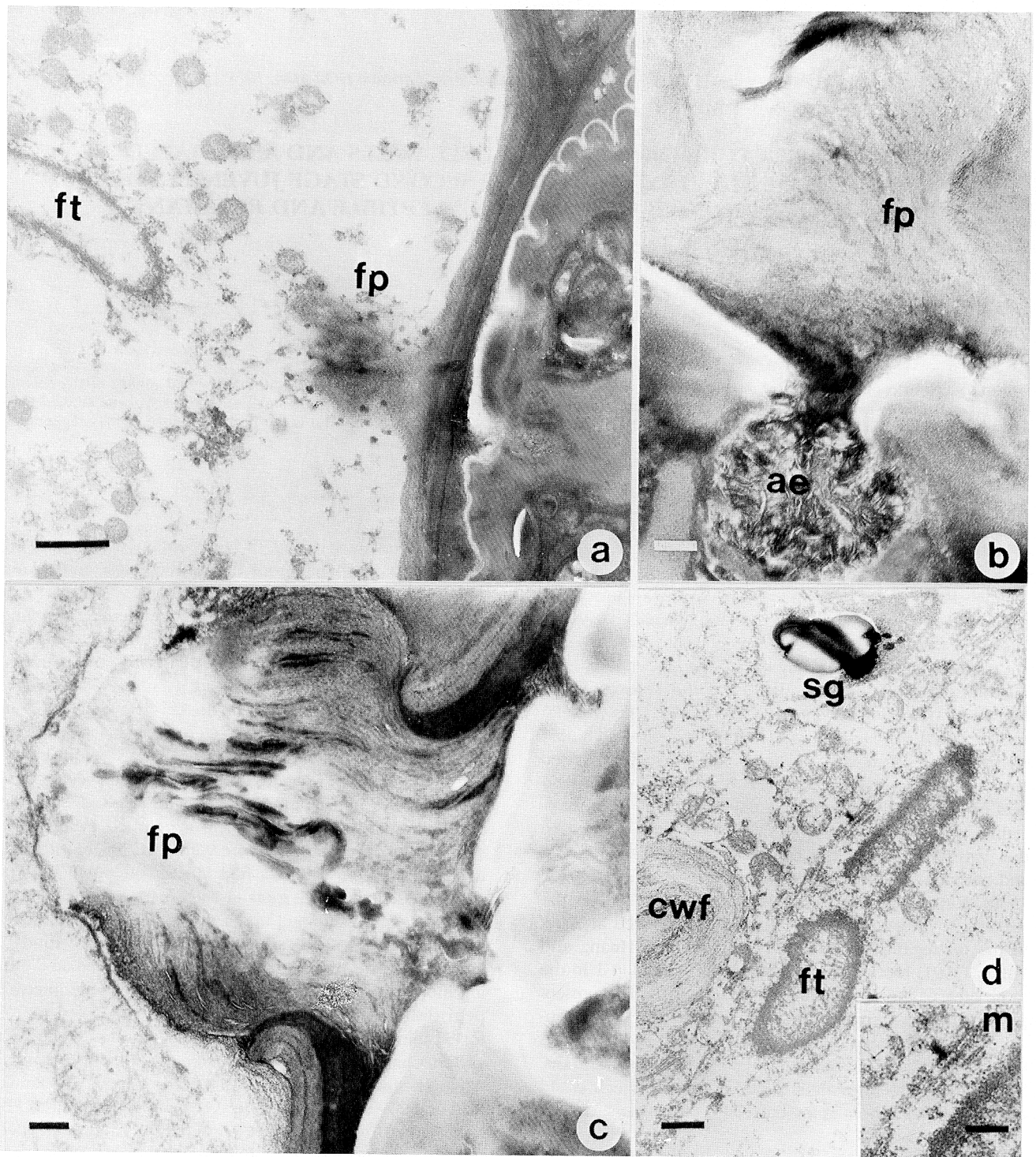
Fig. 1 - Susceptible potato roots, 5 days after inoculation: a, head of J2 against initial syncytial cell wall (ISCW), feeding plug (fp) and feeding tube (ft) are visible (cv. Arran Consul), scale bar = 1 μm; b, amphidial exudate (ae) and feeding plug (fp) are contiguous (cv. Arran Consul), scale bar = 250 nm; c, head of J2 against ISCW and feeding plug (fp) (cv. Home Guard), scale bar = 500 nm; d, sections of feeding tube (ft) with cell wall fragment (cwf) and starch grain (sg) close by, scale bar = 200 nm, inset: microtubules (m) run parallel to the feeding tube (cv. Arran Consul), scale bar = 250 nm.

Silver/grey sections of resin embedded root pieces containing 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 1200EX at 80 KV.

The host status of the potato cultivars and clones examined and the time of fixation after inoculation were as follows:

Genotype	Host status	Days (Post Inoculation)
Arran Consul	Susceptible	5
Home Guard	Susceptible	5
9559 ab(9)	Resistant	5,8
8917 b(3)	Partially resistant	5,9

Clones 9559 ab(9) and 8917 b(3) were derived from *Solanum vernei* Bitt et Wittm with subsequent intercrosses between *S. tuberosum* L. and *S. tuberosum* ssp. *andigena* (Juz et Buk) Hawkes. Clone 9559 ab(9) has major gene resistance (H_1) while 8917 b(3) routinely displays polygenic resistance of around 90% to *G. rostochiensis* (Forrest, unpublished).

Results

Susceptible cultivars, 5 days after inoculation

Typically, the head of the J2 was observed close to the initial syncytial cell wall, which was always intact. A characteristic association is the presence of a feeding plug inserted through the cell wall at the head of the J2. In Fig. 1a (from cv. Arran Consul) one amphid has been sectioned tangentially and the amphidial exudate is shown to be contiguous with part of the feeding plug, which is at least partially of nematode origin. The position of the amphid indicates that the nematode has been sectioned with its amphids in the vertical plane. On the cell wall in contact with the syncytium, the edge of the feeding plug is visible, and beyond it a section through a feeding tube. Numerous atypical mitochondria are present in the cytoplasm, giving it a characteristic appearance (Fig. 1a).

Another section from the same sample shows at the lower level the breach in the syncytial cell wall, which is filled with the feeding plug. The amphidial exudate is clearly contiguous with the feeding plug (Fig. 1b).

In another susceptible cultivar (Home Guard), the head of a J2 is close to the initial syncytial cell wall, at the point where it has been penetrated by the stylet. The hole
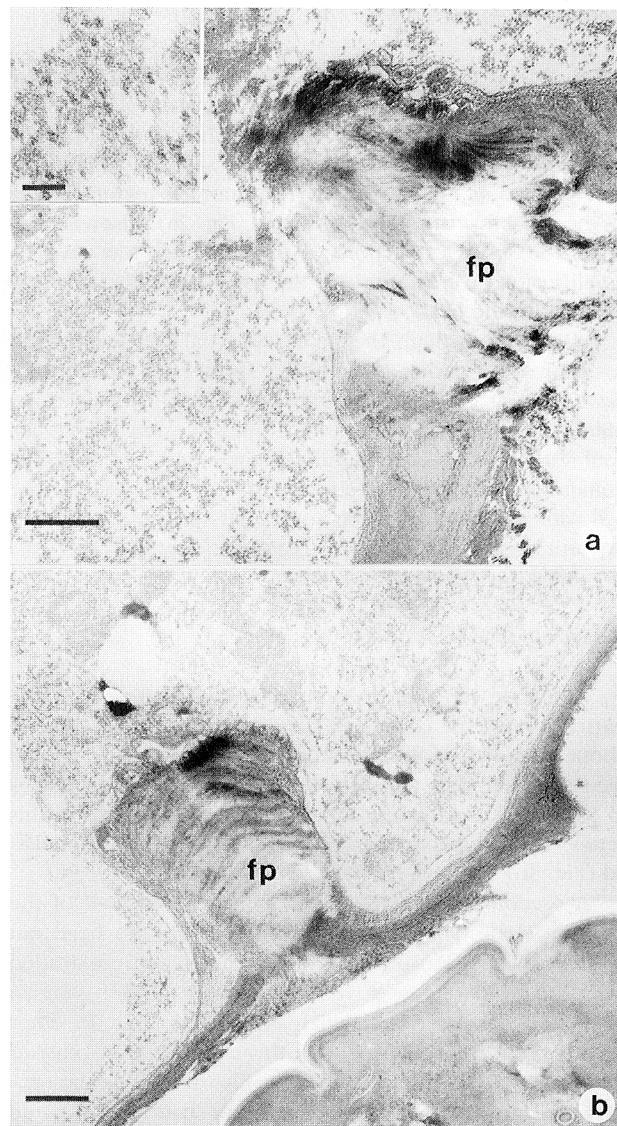

Fig. 2 - Resistant potato roots, 5 days after inoculation: a, feeding plug (fp) in ISCW, cytoplasm with characteristic granular appearance (clone 8917 b3), scale bar = 500 nm; inset, organelles in cytoplasm may be ribosomes, scale bar = 100 nm; b, feeding plug (fp) in ISCW which is disintegrating on the exterior (clone 9559 ab9), scale bar = 500 nm.

is completely filled with feeding plug material which has a striated structure (Fig. 1c). Feeding tubes are present in syncytia of both susceptible cultivars. Fig. 1d shows numerous microtubules arranged parallel to the tube (inset) with mitochondria, a circular cell wall fragment, and a starch grain.

Resistant cultivars, 5 days after inoculation

A section through the initial syncytial cell wall of the partially resistant clone 8917 b(3) is shown in Fig. 2a. The head of the nematode is not in view. The cytoplasm in the syncytium differs from that of the two susceptible cultivars with few or no mitochondria. The granular texture is due to the presence of organelles (inset, Fig. 2a). Elsewhere in the same series of sections, a feeding plug was present. Small lesions were found on the initial syncytial cell wall adjacent to the head of the J2.

A section through the initial syncytial cell wall of the resistant (H_1), clone 9559 ab(9) shows the nematode's head nearby (Fig. 2b). A large feeding plug is present. Mitochondria are visible in the cytoplasm. Areas of the cell wall are thin and disintegrating on the exterior close to the nematode. In another section from the same series, the cell wall adjacent to the feeding plug is clearly disintegrating and in one part is absent (Fig. 3a).

Resistant cultivars, 8-9 days after inoculation

A section through an initial syncytial cell wall in the partially resistant clone 8917 b(3) shows a large breach about the width of the nematode's head containing several pieces of cell wall which have fragmented at right angles to its length. No feeding tubes or plugs are visible in this series. The cytoplasm is exceptionally dense and vacuolated (Fig. 3b).

A section through the initial syncytial cell wall of the resistant clone, 9559 ab(9), with the nematode's head out of view (Fig. 4) illustrates fibrils similar to those extruded from the head region of J2 (Forrest et al., 1989) lying in pockets in the cell wall which is disintegrating. In other sections from the same series these fibrils have passed through breaches in the cell wall to the interior of the syncytium. The breaches in the wall occur in the same plane as in Fig. 3b, and could eventually lead to fragmentation. No feeding tubes are visible.

Discussion

Because of the difficulty of locating and sectioning J2 within the roots it was only possible to examine the interaction between *G. rostochiensis* Ro$_1$ and two clones, representing respectively major gene resistance derived from *S. tuberosum* ssp. *andigena* and polygenic resistance from *S. vernei*. The two susceptible cultivars are not widely grown, but were chosen for their ability to produce a succession of fresh sprouts; Home Guard breaks dormancy early in the season, and Arran Consul very late. As potato sprouts age, it becomes more difficult to achieve sterile
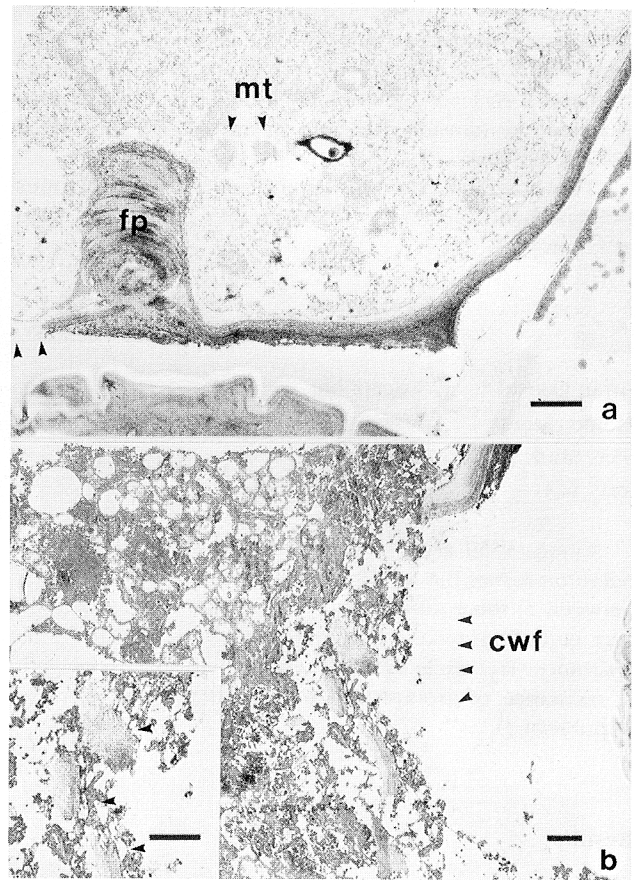

Fig. 3 - a, Resistant potato root, 5 days after inoculation, a section from the same series as 2b. ISCW is absent to left of feeding plug, mitochondria (mt), scale bar = 500 nm; b, resistant potato root, 9 days after inoculation, large breach in ISCW with three cell wall fragments (cwf) (clone 8917 b3), scale bar = 1 μm, inset, cell wall fragments, scale bar = 200 nm.

root cultures, which are essential if changes are to be attributed to nematodes and not other pathogens.

The time scale chosen for examining changes at syncytial feeding sites in resistant clones corresponded to the period of emigration, which reaches a peak after 8 or 9 days (Forrest et al., 1986). Only J2 are shown because they are still capable of leaving the roots. Roots were not sectioned at 8-9 days after inoculation because the majority of juveniles will have reached the third stage. In total only six syncytia were examined, two from susceptible and four from resistant or partially resistant clones. Five of these initial syncytial cell walls contained feeding plugs, which are of potential interest both with regards to their components and their special position at the interface of nematode, cell wall, and plasmalemma. The amphidial exudate is clearly contiguous with the feeding plug, and so may contribute to its composition, as previously suggested for

other cyst nematodes (Endo, 1978; Bleve-Zacheo et al., 1990a). The ends of the cell wall in contact with the feeding plug (Fig. 1c) are neatly invaginated as described by Endo (1991). Breakdown of the initial syncytial cell wall occurred in syncytia of three of the resistant roots, while a fourth showed lesions on the wall adjacent to the head of the nematode. Neither of the syncytia in susceptible roots was affected in this way. The finding that the initial syncytial cell wall breaks down close to the feeding plug was unexpected, and possibly of major significance. Although the syncytium itself enlarges by controlled cell wall dissolution internally, and at its extremities (Jones, 1981; Rice et al., 1985), breakdown of the initial syncytial cell wall at the feeding site will eventually lead to the complete destruction of the syncytium. Under these circumstances there are a number of possible outcomes. If cell wall breakdown occurs early and the juvenile is mobile, it may respond by emigrating. Later, as mobility is gradually lost it is more likely to die in situ. Many individuals, however, must be able to avoid causing cell wall breakdown, and develop into males.

Cell wall breakdown may be caused by a number of factors, including plant or nematode enzymes or possibly aberrant behaviour such as stylet-thrusting to inflict mechanical damage to the initial syncytial cell wall. Degradation of the initial syncytial cell wall adjacent to the nematode is suggestive of enzyme activity, while perpendicular breaches in the cell could perhaps be attributed to mechanical damage inflicted by a 'frustrated' nematode.

Another potentially important difference is the apparent lack of feeding tubes associated with nematodes on resistant plants. Where sections have been cut in susceptible roots through feeding plugs, feeding tubes were found in the vicinity. None was observed close to the feeding site in the resistant combination sectioned after 5 days, and after 8 or 9 days conditions prevailing in the syncytia probably mean that any feeding tubes present have been destroyed or obscured.

Pictorial information on the production of feeding tubes in syncytia of resistant potato roots is scarce. Rice et al. (1985) found none in sections of cultivars with H1. Bleve-Zacheo et al., (1990a) showed none in their published micrographs, but they were present in other samples examined (Bleve-Zacheo, personal communication). Also, Rumpenhorst (1984) did not show any feeding tube either, but specifically remarked on the unusual distribution in a section of resistant potato root of a single feeding tube which ran along the cell wall, and did not extend into the cytoplasm.

While this scarcity may be entirely coincidental, we cannot rule out the possibility that there are abnormalities in the production, longevity or distribution of feeding tubes in syncytia developing in resistant potato roots. There is evidence, however, that they are produced by
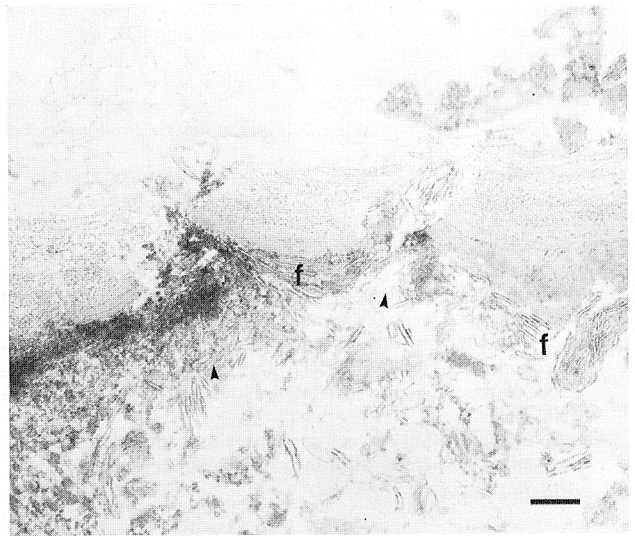

Fig. 4 - Resistant potato root, 8 days after inoculation; fibrils (f) of nematode origin are lying within pockets in the ISCW which is disintegrating (clone 8917 b3), scale bar = 200 nm.

other Heterodera spp. in resistant hosts (Bleve-Zacheo et al., 1990b; Endo, 1991).

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