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

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).

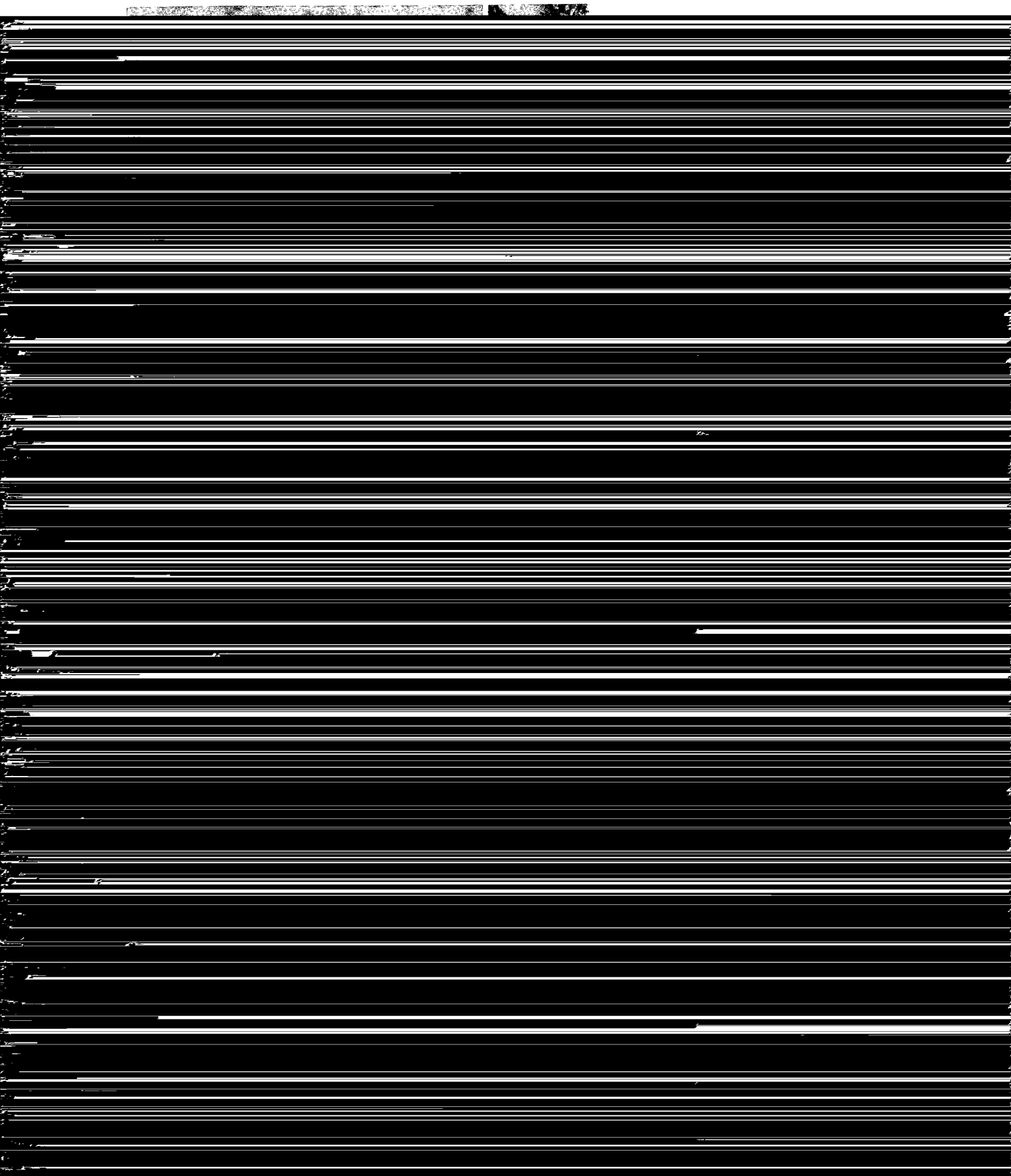
Second stage juveniles of *G. rostochiensis* developing in sterile potato roots were obtained by growing surface sterilised sprouts of potato cvs Home Guard (susceptible) or 8917b (3) (ex *vernei*, 90 % resistant) on 1 % Murashige and Skoog (M & S) plant salt and minimal organics mixture (Flow Laboratories, 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

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



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* *chiensis*, and so did not determine if the fibrils also occurred nearer the tail (Zunke, 1986).

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