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Meloidogyne (Nematoda: Meloidogynidae) induced root galls of the banana plant *Musa paradisiaca* — A study of histopathology

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Abstract. *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 was found to complete its life cycle within the roots of the banana plant *Musa paradisiaca* (cultivar Palayathodan). Egg sacs were confined entirely within the root. Second stage larvae penetrated the endodermis and on entering the stele succeeded in inducing multinucleate giant cells which were solely confined to the stele and never found in the cortex. Induction of giant cells and production of large number of females in banana roots showed that this is a susceptible host plant. Heavily infested roots showed histopathological changes which could lead to the blockage of conducting elements. This nematode is not very common in banana roots probably because second stage larvae emerging from the egg sacs reinfested the same roots and also because of the differential susceptibility of the various cultivars of the banana plant.

Keywords. *Meloidogyne incognita*; Nematoda; root histopathology; *Musa paradisiaca*; multinucleate giant cells; induction.

1. Introduction

The root-knot nematode *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 was reported from cultivars of *Musa paradisiaca*, an important crop plant in Kerala (Mohandas 1976). According to Blake (1972) this nematode is not a significant pest from the point of view of banana cultivation probably because the individual roots of the prolific adventitious root system of the banana plant is shortlived relative to the length of the life cycle of the nematode. Our preliminary studies were not in full agreement with the contention of the above author and hence the present work has been undertaken to provide information on aspects of the life cycle of *Meloidogyne incognita* within the root of *Musa* together with the histopathological changes brought about in the infected roots of the host.

2. Material and methods

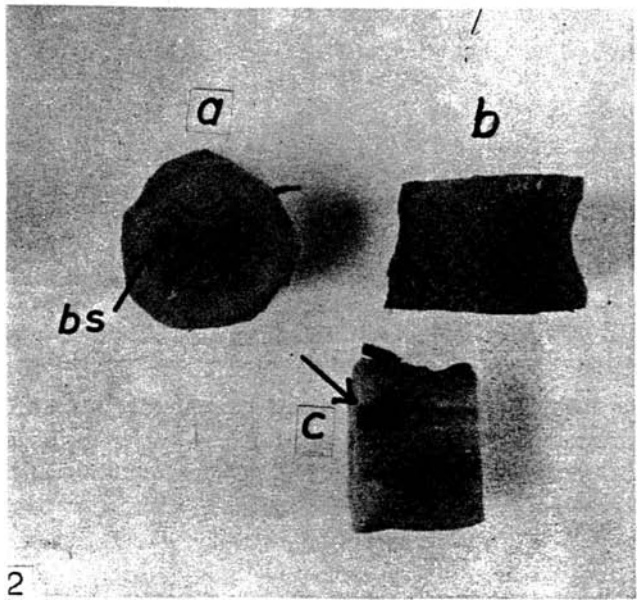
Thin hand sections of infected roots of *Musa paradisiaca* (cultivar Palayamthodan) fixed in FAA (formalin 6.5 ml; glacial acetic acid 2.5 ml; ethanol (50%) 100 ml) for 48 hr and preserved in 70% ethanol were used for histopathological studies. Nematodes *in situ* were stained using 0.1% acid fuchsin lactophenol (Goodey 1963). Dilute aqueous safranin gave good histological picture of the plant tissue. Nuclear and nucleolar conditions of giant cells were studied by staining sections using acetocarmine (saturated solution of carmine in 45% acetic acid).

3. Results and discussion

In sections of swollen roots (figure 1), thick, oval brown spots could be observed deep in the cortex and arranged radially around the stelar region just touching the vascular cylinder (figure 2 a,b). Each brown spot represented the position of an egg sac attached to the posterior end of the adult saccate female. Eggs at various stages of development and the second stage larvae hatched out from the eggs were seen in the egg sac. This means that all life stages of the nematode is found in the adventitious roots of the banana plant, a finding which is not in agreement with the contention of Blake (1972). Cortical parenchyma cells immediately surrounding the egg sacs became brown, thick-walled and opaque and formed a protective covering around the egg sac, except in the region where the latter was attached to the saccate female (figure 3). A similar condition was noted in the potato tubers (Finley 1981) except that in this case the protective covering completely surrounded the egg sac. It was suggested that the discolouration and the changes in the host cells might be due to the oxidation of phenolic substances and was induced by substances present in the gelatinous matrix of the egg sac. At the same time 5-7 layers of weakly stainable (by aqueous safranin), thin-walled and hypertrophied parenchyma cells in the cortex surrounded the brown area (figure 3), contributing to corresponding swellings (knots) formed in the root (figure 1). In *Musa* the egg sacs were never found protruding outside the root surface as has also been observed in the potato tubers (Finley 1981), contrary to the condition noted in most other host plants of *Meloidogyne* spp.

Normally the endodermis of *Musa* root was strongly developed with deposits in the cell wall which was thickened at the inner tangential side, except in the case of the passage cells which were distributed sparsely. Infective second stage larvae of the nematode penetrated this layer easily and entered the stele fully, where they started enlarging into saccate females. Endodermal cells adjacent to the region of infection, became highly enlarged and thin-walled (figure 4), and sometimes they showed a tendency to divide radially into two or more layers (figure 4). The larvae entered the stele presumably attracted by the phloem contents (Finley 1981) and induced the formation of large giant cells in the region of the pericycle, phloem and xylem parenchyma (figure 5). The number of giant cells observed at each site of infection varied, but not more than five radial cells were found in one group (figure 5). Taylor and Sasser (1978) and Arya (1980) suggested that giant cells appeared to be dependent on continual chemical stimulus from the feeding nematode. Giant cells were noticed only inside the stele and never in the cortex in *Musa* roots, as has been found by Krusberg and Nielsen (1958), Rao and Kumar (1973), Trivedi (1978) etc. But Balasubramonian and Rangaswami (1964) noted the presence of giant cells in the cortex also in sugarcane, while Chaturvedi and Khera (1979) found that giant cells occurred more frequently in the cortex of the roots of jute.

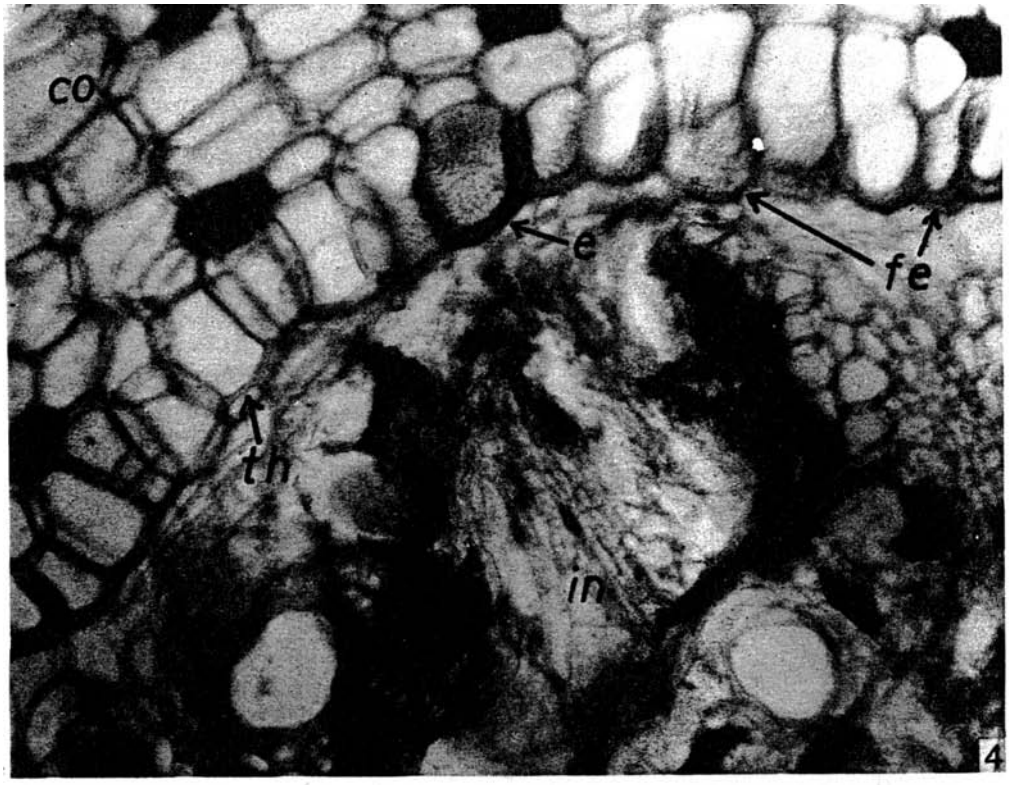
Giant cells had thick walls, dense granular cytoplasm and several nuclei (figure 6). Nuclei and nucleoli of giant cells showed hypertrophy. Some nuclei were irregularly spindle shaped or dumb-bell shaped (figure 6) about 24 μm long and 8.6 μm wide. The enlarged nuclei, nucleoli and dense granular cytoplasm in the giant cells indicated high metabolic activity as reported by Endo (1971). Bird (1974) suggested that the giant cells function as selective transporters over short distances

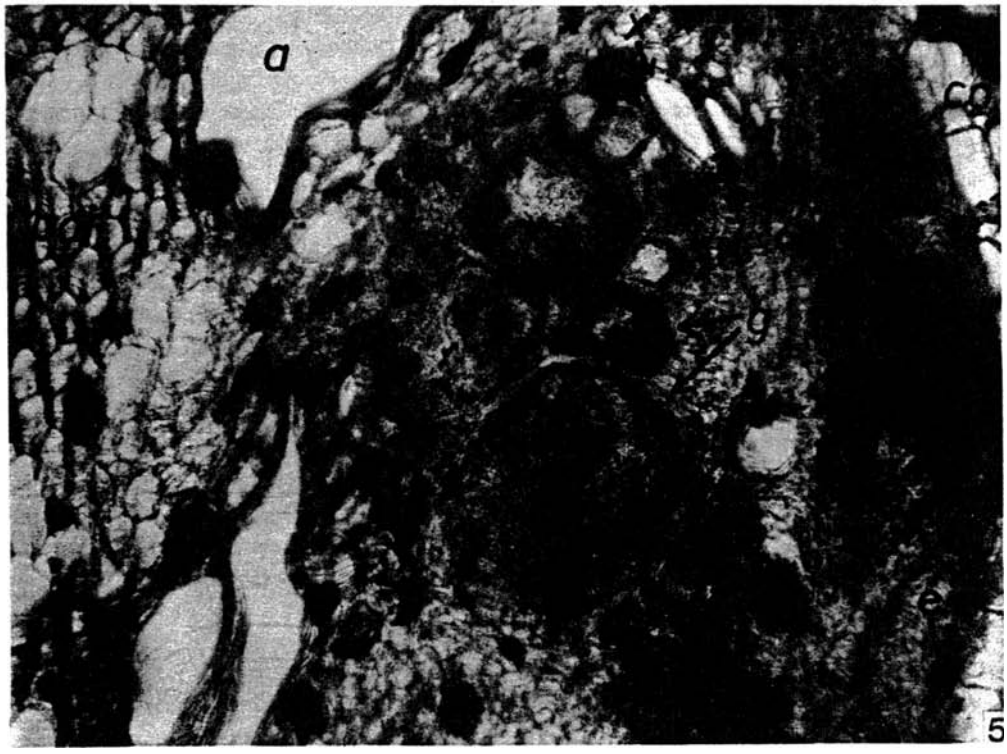


Figures 1-2. *Meloidogyne* infested roots of *Musa paradisiaca*. Arrows show regions of swelling. **2.** Sections of banana roots, **a.** TS through region of prominent swelling showing the position of mature females with egg mass, as brown spots (bs); **b.** Surface of the swollen root without any lesions; **c.** Longitudinal section through region of swelling, arrow indicating position of egg mass and mature female.

Figure 3. Transverse section of root stained in aqueous safranin (nc — cortical parenchyma cells which became brown, necrotic and thick walled; ba — brown host cells together forming a protective basket for the eggs sac; hc — hypertrophied cortical cells around the brown area; ed — exodermis; c — crack in the epidermis).

and that the developing larvae were entirely dependent on these cells for their nutritional requirements. The feeding nematode always had its head embedded in the stele adjacent to the giant cells. Endo (1971) suggested that those larvae which could induce large giant cells and derive sufficient nutrients became females, which later turned saccate with their body mostly pushed out from the stele in *Musa* roots. The formation of giant cells, developing of large number of females and production of several eggs by each female were considered as signs of high susceptibility of the host (Endo 1971; Sasser and Taylor 1978). In spite of the high susceptibility of the host roots the infestation of *Musa paradisiaca* by *Meloidogyne incognita* was not very common. The probable reason for this was apparently provided by a kind of re-infestation of the host roots noted in *Musa* in the course of the present study in which the second stage larvae emerging from the egg sacs in





Figures 4-5. (*see captions on page 473.*)

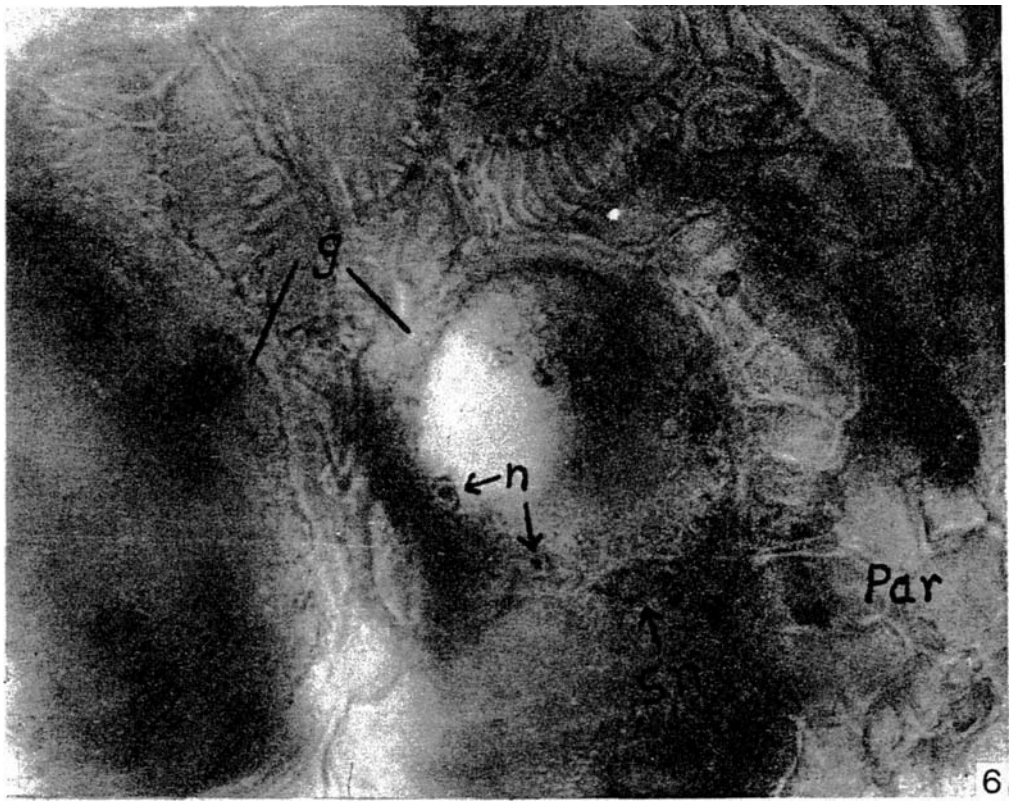


Figure 6. Transverse section of infected root stained in acetocarmine. (g — giant cells; n — nuclei; sn — spindle shaped nucleus; par — undifferentiated parenchyma cells within stele).

the cortex entered the stele of the same root. Further it is also probable that only certain cultivars of *Musa paradisiaca* are susceptible to the attack of *M. incognita* and 'Palayamthodan' is one such cultivar.

As a result of the enlargement of several females and also of many giant cells associated with the former, inside the stele, the phloem and xylem elements became displaced, malformed (figure 5) and at least partially blocked. Similar observations were made on other host plants by Trivedi (1978), Arya (1980), Rao and Kumar (1973) etc. Heavily infected banana plants showed such symptoms as poor growth, discolouration and yellowing of leaves etc., which have been reported on other host plants also (Singh 1973; Prasad 1972; Milne 1972; Shah and Raju 1977). From this it could be reasonably concluded that though the infestation of the banana plant by *Meloidogyne incognita* is not widespread in Kerala, the former is a susceptible host and that heavily infested plants showed severe phytopathological conditions of roots and external symptoms in the shoot system similar to those produced in other hosts heavily infested by this nematode.

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Figures 4 and 5. 4. TS through root stained in aqueous safranin showing modifications of endodermis. (co — cortex; e — normal endodermis cell; th — thin walled endodermis cell; fe — endodermis cells showing a tendency to divide radially; in — infected region within the stele). **5.** Transverse section of the infected root stained in acetocarmine. (g — giant cells; co — cortex; e — endodermis; par — undifferentiated parenchyma cells in stele; x — dislocated xylem vessels; a — compressed 'trachea').