Occurrence of the Root-knot Nematode *Meloidogyne Incognita* on Guava in Malaysia

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**ABSTRACT**

Incidence of decline in growth of guava caused by the nematode, *Meloidogyne incognita*, in several states in Malaysia is reported for the first time. The nematode infested plant manifested a drastic reduction in plant growth, small leaves, absence of fine roots, a poorly developed root system, and a decline in yield quality and quantity. Severely infested roots are distorted by small and large multiple galls. Factors influencing the spread and survival of the nematode are also discussed.

**INTRODUCTION**

The profitability of the Kampuchean guava (*Psidium guajava* L.) cultivar, has made the crop popular with fruit growers in Malaysia. Area of guava cultivation has expanded considerably both in terms of farm size as well as geographical distribution. The cultivar introduced to Bidor, Perak in 1981, has spread to the states of Selangor, Negeri Sembilan, Malacca, Johore, Kedah and Pahang. Recently, the crop was introduced to Sarawak in East Malaysia. Likewise, the popularity of the crop among growers has upgraded the farm size from a smallholder farm of 0.5 – 2 hectares in 1981 to large orchard plantings of more than 20 hectares in 1986. Two more new cultivars have since been introduced, namely the apple-shaped, seedless “Donrom” and “Glom sali” — a round fruit cultivar from Thailand.

With the rapid expansion of the guava areas, the incidence of pests and diseases also increased (Lim & Razak, 1986; Lim et al., 1986). Hitherto, problems of poor plant growth and small fruit size were attributed to soil fertility, and attempts to arrest the problem by more frequent fertilizer applications were not successful. The growers destroyed infested stands by bulldozing and pushing the stubble to one end of the field but replanted on the same infested area with new seedlings.

Nematode disease on guava is unknown to the growers and has not been reported in Asia. The incidence of nematode disease in the field was first noted on 6-month old guava plants in Semenyih, Selangor in 1985 by the authors. Subsequently, the nematode decline problem on the same cultivar was found to be more severe on plants cultivated on light soils around Bidor,
Perak. Diseased plants were found to be associated with disfigured root systems caused by nematodes. Widespread decline was also found on the Kampuchean cultivar in Bentong and in Sungai Baging, Pahang (Chai, T.B., MARDI, Per. Comm.).

This paper reports on the incidence, symptomatology, causal agent, and possible factors influencing the spread of disease.

**MATERIAL AND METHODS**

Four guava farms in the Bidor-Sungkai area, 2 farms in the Nilai-Semenyih area, a farm in Sungai Buloh and a seedless guava farm in Sungai Ruang, Raub, were visited to assess the incidence of nematode infected trees. Both above-ground and below-ground symptoms of infested, bearing trees were recorded. One hundred and fifty marcotted and bud-grafted polybag seedlings were also taken from two farms in Bidor to assess the severity of galling on the roots. Five soil samples (auger probes) per tree were collected with an auger from a depth of 0.3m at a distance of 1 metre from the bases of trees showing symptoms of poor growth, and composited. A total of 100 trees each were sampled from two severely infested orchards in Bidor. Nematodes were extracted from the soil samples using a modified Whitehead Tray's technique (Whitehead and Hemming, 1965). A 200cc soil volume was used as one subsample from each soil sample. The remaining soil in the sample was bioassayed for nematode incidence using tomato seedlings.

Infected roots were washed clean of debris under running water and processed for both light and scanning electron microscopic examinations. For light microscopic studies, the young infected roots were stained in lactophenol cotton blue and cleared in clear lactophenol, or phloxine B for egg mass assessment. Perineal patterns from saccate, females and the second larvae stages were prepared for species identification.

Tissues for scanning electron microscopy viewing were prepared by selecting pieces of multiple-galled roots and fixed in buffered aldehyde fixatives according to Eisenback (1985). After washing out the aldehydes, the roots were teased carefully to expose the internal modified cells of the galled roots; post-fixed in osmium tetroxide, and dehydrated through a graded series of ethanol. Dehydrated specimens were critical point-dried in CO$_2$. Dried specimens were mounted on copper stubs, coated with gold and examined under the JEOL-JSM 55C scanning electron microscope at 25kV.

**RESULTS**

**Disease Incidence and Symptoms**

The visits showed that the slow decline of guava plants was rampant and widespread. On all the farms visited, trees exhibiting poor growth were found to be infested by nematodes. Symptom manifestation appeared distinct on 2½ year-old plants onwards, on both budgrafted and marcotted plants. The presence of the nematodes was indicated by the heavily galled exposed roots around the base of the plant. Severely stunted 2½ year-old plants could be easily pulled out of the ground, revealing the swollen, disfigured and discoloured root system (Plate 1). This symptom is most indicative of *Meloidogyne* spp. The primary and secondary lateral roots, distorted by the large multiple galls, were dark brown or black in colour with rough corky surfaces and cracks along the long axes of the roots. There were distinct reductions in the root systems which were almost devoid of fine feeder roots. Young, white roots emerging...
from the swollen infected tissues were also infested by the nematodes. Egg masses on the galled surfaces could clearly be seen as small red spots when stained in phloxine B (Plate 2).

Plate 2: Egg masses of Meloidogyne (arrowed) on the guava roots stained with Phloxine B stain

Roots of seedlings in the polythene bags also displayed a similar corky and distorted root system, with several young white roots sustaining the infected plant (Plate 3). These young roots were already showing signs of nematode infection. The roots still retained the white colour, although very heavily galled.

The effect of the nematodes on the roots results in stunted and unthrifty growth of the above-ground parts of the plants. The trees bore small, pale green to yellowish chlorotic foliage (Plate 4). On severely nematode infested trees, the leaves matured early and were observed to defoliate during dry periods or when under water stress, leaving a few clusters of pale yellow leaves at the apical end of the branches (Plate 5). The branches became exposed to intense insolation and were rendered susceptible to secondary infection by Botryodiplodia theobromae.

The quality and quantity of fruits produced by nematode infested trees were also affected. On younger trees (2 years old), the plants tended to produce a lot of young fruits which aborted readily. The fruits lacked the smooth, glossy, green appearance. Fruits which developed to maturity, matured early, were smaller in size and became more susceptible to fungal infection. Similar quality fruits were also found on older bearing trees which survived through the heavy infestation of the nematodes. Fruits collected from the nematode-infected plants weighed between 200 to 250gms per fruit (Grade C) instead of the usual average weight of between 450 - 650gms (Grades A & B). This was observed in all the farms visited.

The Causal Organism
The soil extracts showed that at least two genera of plant parasitic nematodes, Meloidogyne sp and Pratylenchus sp were present. The population of Meloidogyne sp (mean 9300 per 200cc of soil) was predominant. Pratylenchus sp. was present in comparatively smaller numbers (mean 33 per 200cc of soil). Roots stained in cotton blue

Plate 3: Roots of an infected guava seedling obtained from the nursery displaying a similar corky, galled and distorted root system. Note the few young white roots supporting the infected plant and which are already infested by nematodes

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Meloidogyne infested tree characterized by small, pale, greenish-yellow, chlorotic foliage and stunted growth.

Plate 5: Severely nematode infested trees showing heavy defoliation, with bare branches bearing clusters of chlorotic leaves at the apices.

A larva of Pratylenchus was not identified. Based on the morphometric measurements of larvae and perineal pattern of saccate females, the Meloidogyne species was identified as *Meloidogyne incognita* (Kofoid and White) Chitwood. The species of *Pratylenchus* was not identified.

All developmental stages of *Meloidogyne incognita* were present in the tissues of the infested roots, often with different stages present within the same locality in the large galls. The electron micrograph of Plate 6 shows a developing third stage larva surrounded by several infective second stage larvae. Likewise, it was not uncommon to see several nematodes of the same developmental stage feeding on adjacent tissues (Plate 6). Cells surrounding the nematodes in the galled tissues appeared to be similar in shape and size, and assumed a honey-comb pattern.

Plate 6: SEM micrograph showing several nematodes at the various developmental stages, feeding on adjacent tissues.

The tunnels formed by dislodged saccate females were characterized by the conical-shaped impres-
pressions of the anterior part of the body (Plate 7). A pea-sized gall could easily sustain 50 to 100 nematodes of different developmental stages. The saccate females laid their eggs within the modified root tissues in a gelatinous matrix (Plate 8), numbering about 200 eggs per egg mass.

Plate 8: Egg mass of the saccate female laid within the proliferated cells of the gall instead of on the surface of the enlarged roots

DISCUSSION

Hitherto, nematodes have not been reported as important pests of guava in Malaysia or other Asian countries although Meloidogyne incognita has been recorded on guava in Hawaii (Martinez, 1973) and Meloidogyne arenaria, in the Federation of Rhodesia and Nyasaland (Martin, 1959).

This study clearly demonstrates that Meloidogyne incognita is an important pest of guava. The presence of the nematode was distinctly manifested by the grotesque root system of the plant. The discoloured infected roots were grotesquely enlarged into galls, almost completely devoid of feeding roots and provided poor anchorage for the plant. Cracks along the longitudinal axis of the root as noted on pepper (Razak, 1978b) were observed on the surface of the diseased main roots. Consequently, the above ground parts of the plant developed unthrifty growth, bearing small fruits which matured early. It was unlikely that Pratylenchus sp contributed to the poor growth, although it was present in the soil extract, and has been reported to cause damage to other fruit trees (McElroy, 1972). The failure to detect Pratylenchus nematode in the stained young roots further supports the statement. Alternatively, the presence of a high density of M. incognita in the roots as revealed by the electron scanning studies possibly deprived the Pratylenchus of suitable feeding sites. Galls or enlargement of the roots are generally associated with symptoms of Meloidogyne attack although other nematode genera can also cause root galling (Taylor and Sasser, 1978). Likewise root galling can be induced without the nematode actually entering the roots (Lowenberg et al., 1960).

Meloidogyne incognita is the dominant species accounting for approximately 64% of the population occurring in the tropical countries (Sasser, 1979) and has a wide host range (Taylor et al., 1982). In Malaysia, the species is ubiquitous and associated with several economically important agricultural crops as well as weeds (Razak, 1978a, 1981; Abdul-Karim, 1985; Loh and Ting, 1970). However, this is the first time the species is reported to be a significant pest of guava. Although no direct assessment was made, yield loss in terms of quality and quantity of fruits produced could be estimated to be around 50%. The eradication of 7-years old infested stands by some growers further reflected the magnitude of the problem on guava which has a viable life span of more than 20 years. The loss estimated far exceeds the 30% reported in Central America and Caribbean by Sasser (1979).

The extremely high incidence of rootknot on guava in the two farms in Bidor could be attributed to various factors among which are host susceptibility, soil condition and indigenous population of the nematode in the area. Guava appears to be a good host of M. incognita. Despite heavy nematode infestation the plant was still able to survive. Soil structure has a great influence on the host-parasite relationship involving M. incognita, and in light textured soils, the nematode could cause considerable damage to many crops (Lamberti, 1979). The light, sandy and sandy-loam soil on which most of the guava plants are grown in Bidor, Selangor and Pahang thus favour the development of the population of M. incognita. It was rather difficult to distinguish between the introduced and the indigenous population of Meloidogyne, especially among the established plants in the field where the root systems of the trees inter-
twined with those of neighbouring plants. Nevertheless, the concentration of high numbers of nematode population on the roots of young plants (2½ years old) and the absence or very little galling on roots of nematode susceptible weeds in between the planting rows suggest that the population was mainly introduced with the planting materials. The observation was further supported by the heavily infested roots of the seedlings in the polythene bags in the nurseries of the two farms in Bidor or those put up for sale at the roadside. Furthermore, most of the plantations visited obtained their planting materials from Bidor. Therefore, it was most likely that the nematodes in most of the plantings visited came with the planting materials from Bidor through the soils used to fill the polythene bags when the marcotted plants or the stock were transplanted to the bags. The spread of the nematodes can further be accelerated throughout the country by the farmers who are not aware of the existence of nematodes as pests. It is therefore necessary for the growers to use sterilized soil for the rooted marcotted soil bags. Alternatively, the movement of the planting materials from the infected field should be restricted to prevent further spread of the nematodes. In the meantime, efficacy and residual trials on several nematicides coupled with selected cultural measures to save the existing stands in some infested orchards are being investigated.

REFERENCES


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