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# Comparative host tissue reactions of *Musa acuminata* (AAA group) cvs Poyo and Gros Michel roots to three banana-parasitic nematodes

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## Summary

In a comparative histopathological investigation, Poyo and Gros Michel cultivars of *Musa acuminata* (AAA triploid) were inoculated with *Radopholus similis, Helicotylenchus multicinctus* or *Hoplolaimus pararobustus* and were grown in a greenhouse under tropical conditions. *R. similis* infected all the cortical parenchyma layers of the roots, reaching the vascular cylinder, but it stayed more superficial in Gros Michel roots. Red-brown cytoplasmic globules appeared in the cortical parenchyma cells of Gros Michel only. *H. multicinctus* infected much of the outer cortical parenchyma in roots of both cultivars with a few phenolic cells occurring around the superficial lesions. *H. pararobustus* penetrated only the immediate sub-epidermal tissues in both cultivars. The differences observed between nematodes and cultivars reflect specific host-nematode interactions on bananas.

**Key words**: Banana, histopathology, *Helicotylenchus multicinctus, Hoplolaimus pararobustus, Radopholus similis*

## Introduction

The histopathology of banana roots infected with *Radopholus similis* was studied by Blake (1961, 1966) on Dwarf Cavendish *Musa* AAA Cavendish sub-group. Nematodes migrate between root cortical cells, punch cell walls with their stylets, feed on the cytoplasm, and make cavities within roots. As the cells are destroyed and *R. similis* migrates, cavities coalesce to form red-brown lesions. These lesions do not expand into the vascular cylinder because the endodermis acts as a barrier towards the nematode.

*Helicotylenchus multicinctus* penetrates into banana roots and feeds on and destroys cortical cells, thereby causing small necrotic lesions (Luc & Vilardebo, 1961; Zuckerman & Strich-Harari, 1964; Blake, 1966). These lesions do not reach the vascular cylinder and can be distinguished from those of *R. similis* because of their very small size (Stover, 1972). Cytological disturbances are in the form of cytoplasmic contraction or distortion, nuclear enlargement, or even cell wall breakage (Blake, 1966).

Whitehead (1959) briefly described banana root diseases caused by *Hoplolaimus pararobustus*. Cortical cells become necrotic adjacent to the head of the nematode and form dark brown pustules which disintegrate into ulcerated lesions.

These three endoparasitic nematodes were chosen because of the high level of damage caused by the first two on banana and the widespread distribution of *H. pararobustus* in

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*Present address: ORSTOM, BP 1386, Dakar, Sénégal the banana growing areas in Côte d'Ivoire (Fargette & Quénéhervé, 1988) where this study was conducted. Poyo and Gros Michel cultivars were chosen because they have the same genetic origin (*Musa acuminata* wild species) and are pure triploid *acuminata*, but they have different responses to *R. similis* (Wehunt, Hutchison & Edwards, 1965, 1978).

In other studies, the population development of these three parasite nematodes was found to be different on Poyo and Gros Michel (Mateille, 1992). The objective of the present investigation was to study the pathology of banana roots infected by these three nematodes in order to determine if nematode damage is also related to specific banana cultivars.

## Materials and Methods

### *Nematode management*

After *in vitro* micropropagation (Mateille & Foncelle, 1988), Poyo and Gros Michel plantlets, belonging respectively to the Cavendish and Gros Michel sub-groups (AAA triploids) of *Musa acuminata*, were transplanted into pots in a greenhouse for acclimatisation, under natural tropical conditions in Côte d'Ivoire at 75% humidity with a 12-h photoperiod (226 $W/m^2$) at 32°C and 12 h at 25°C. After 3 wk, plantlets were individually transplanted in 21 containers filled with a substrate composed of two parts sandy soil and one part shredded coconut fibrous mesocarp. One week after the second transplant, plants of each cultivar were inoculated with a suspension of each of the three species *Radopholus similis* (Cobb) Thorne, *Helicotylenchus multicinctus* (Cobb) Golden or *Hoplolaimus pararobustus* (Schuurmans Stekhoven and Teunissen). Each nematode population had been reared on Poyo banana *vitro*-plants in pots and extracted from the roots in a mist chamber (Seinhorst, 1950) 2 days before inoculation. Each nematode inoculum had a variable percentage of juveniles, females and males, although the inoculation level was adjusted to 5000 individuals per plant. Each nematode-banana cultivar pair was replicated five times, and uninoculated plants were controls. Banana plants were harvested 2 months after inoculation.

### *Histology*

Sections of fresh or fixed tissues of roots were examined. Root tissues selected for microtome sectioning were immersed in a fixative of 9:0.5:0.5 70% ethanol:acetic acid:37% formaldehyde (Johansen, 1940). Root pieces were then dehydrated in a 50–100% ethanol solution series. After toluene substitution of ethanol, the root pieces were passed through 2:1 and 1:2 toluene:paraffin baths, and were then embedded in paraffin. Microtome root sections (15–20 $\mu$m) were placed on slides in a Haupt adhesive solution (10 mg/ml gelatine, 20 mg/ml phenol and 15 $\mu$l/ml glycerine) with one 4% formaldehyde drop.

Fresh or fixed sections were stained with safranin and fast-green (Berlyn & Miksche, 1976). With safranin, lignined cell walls appear red, and with fast-green celluloses are green. In some cases, roots were first cleared in 1.5% NaOCl, and nematodes were stained in the cleared roots with 3.5 mg/ml acid fuchsin in 25% acetic acid (Byrd, Kirkpatrick & Barker, 1983). Roots were preserved in 5 N HCl in glycerin, and they were pressed between two glass plates for microscope observations.

Phenols were detected according to Regaud's method (Langeron, 1949). Fresh root pieces were dipped in a 11:4 3% potassium dichromate:37% formaldehyde solution. After four days, they were dipped in 3% potassium dichromate for 7 days, and then rinsed for 24 h in water. Root sections were made manually and observed. Phenols appear brown.

## Results

Uninfested banana roots of both cultivars have the same histological structure as described by Riopel & Steeves (1964) on Gros Michel. They had a typical monocotyledon structure, but three layers of cortical parenchyma exist (Fig. 1A): an outer layer with large geometric cells and small intercellular spaces, a medial layer of lacunar parenchyma, and an inner layer of radical parenchyma with small round cells. These three cortical layers were affected differently by the nematode species.

The observation of healthy Poyo roots stained with potassium dichromate revealed many brown cells in the vascular parenchyma, with some of them appressed to the xylem vessels,

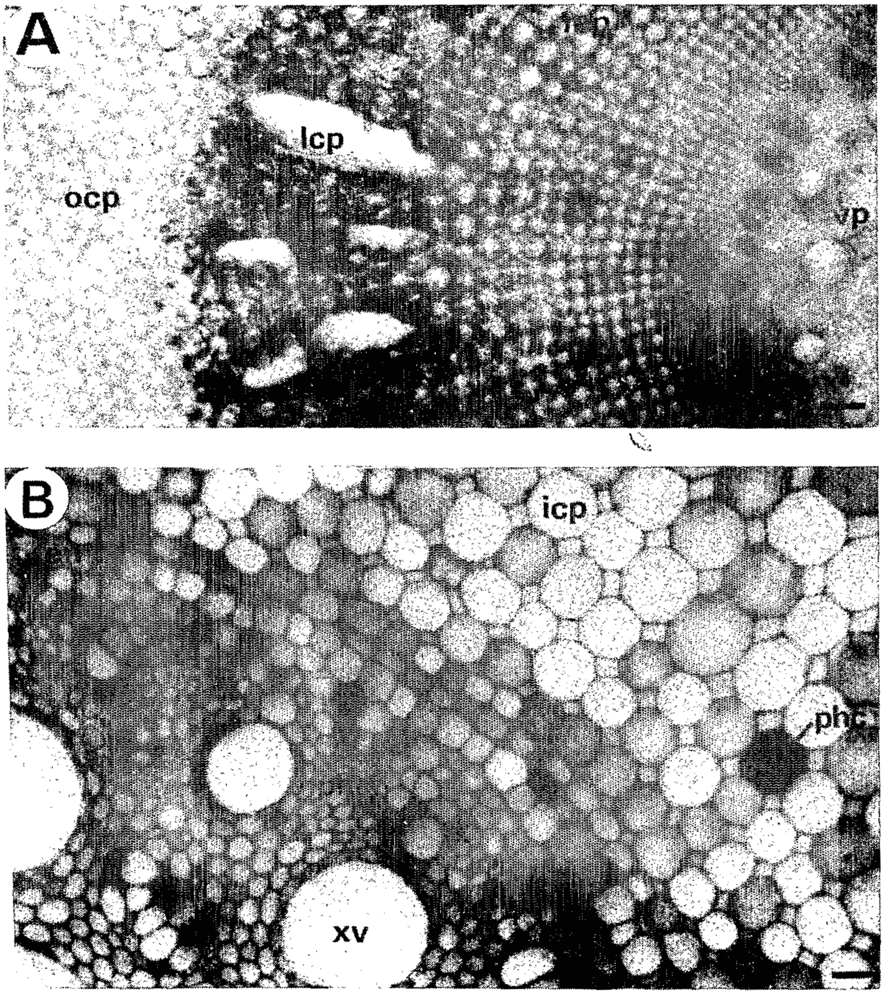

Fig. 1. A–B) Cross sections of healthy banana cv. Poyo roots. A) Three layer structure of the cortical parenchyma (en = endodermis; icp = inner cortical parenchyma; lcp = lacunar cortical parenchyma; ocp = outer cortical parenchyma; vp = vascular parenchyma; bar = 84.5 μm). B) Stained phenol cells in both vascular and cortex parenchymas (en = endodermis; icp = inner cortical parenchyma; phc = phenol cell; xv = xylem vessels; bar = 34.5 μm). C–D) Poyo roots infected by *Radopholus similis*. C) Cross section: deep lesion reaching the vascular cylinder (icp = inner cortical parenchyma; 1 = lesion; n = nematode; vp = vascular parenchyma; bar = 34.5 μm). D) Root crushed between two glass plates: forward migration of a female and egg-laying (e = eggs; n = nematode; bar = 115 μm).

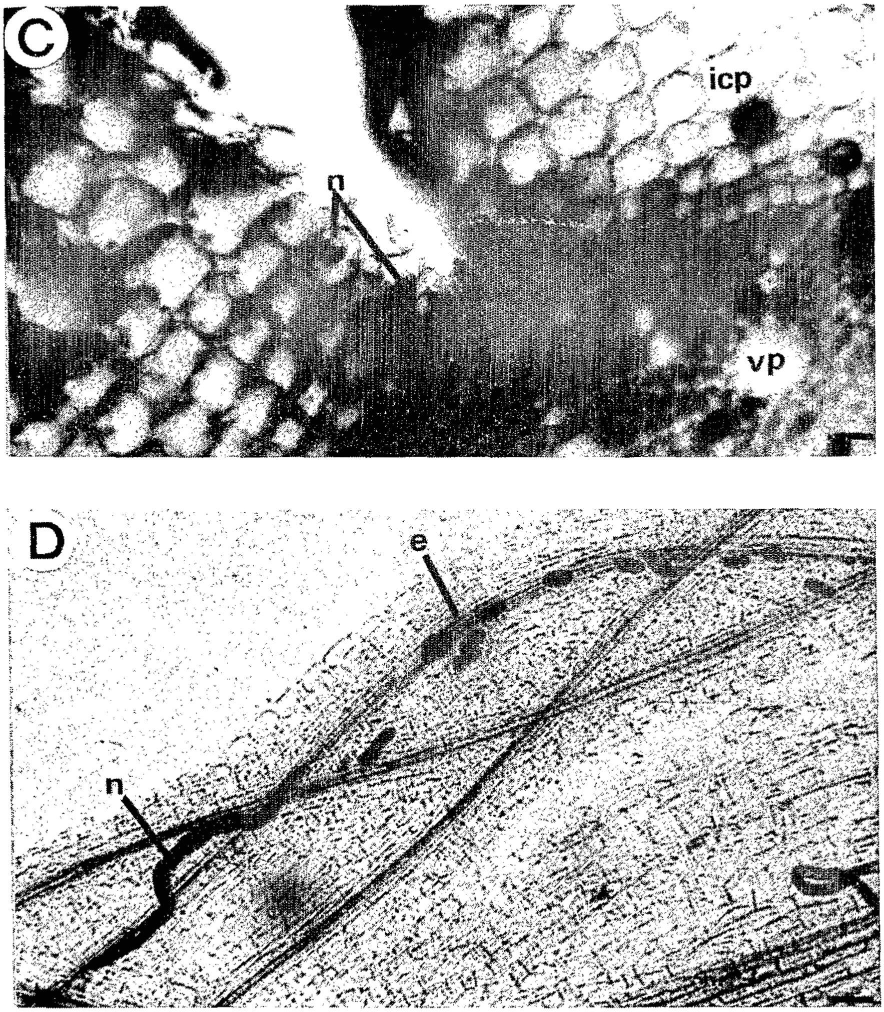

Fig. 1 cont.

and a few in the cortical parenchyma (Fig. 1B). According to Mace (1963), these cells are phenolic cells: around the vacuole, the phenols are partitioned in globular structures which can aggregate. The same phenolic cells were observed in the healthy Gros Michel roots, but they were significantly more numerous than in Poyo roots, in both cortical and vascular parenchyma.

### Radopholus similis *infection*

On both Poyo and Gros Michel, necrotic tissues occurred near the root surface. Histology indicated that necrosis was restricted to sub-epidermal cortical cells, but on Gros Michel, these necrosis were less expanded than on Poyo. Nematodes were not found in the damaged subepidermal areas, but they migrated through the outer cortical parenchyma. In Poyo roots, nematodes induced large cavities and the lacunas of the medial parenchyma coalesced in lesions which extended through the internal cortical parenchyma and reached the vascular

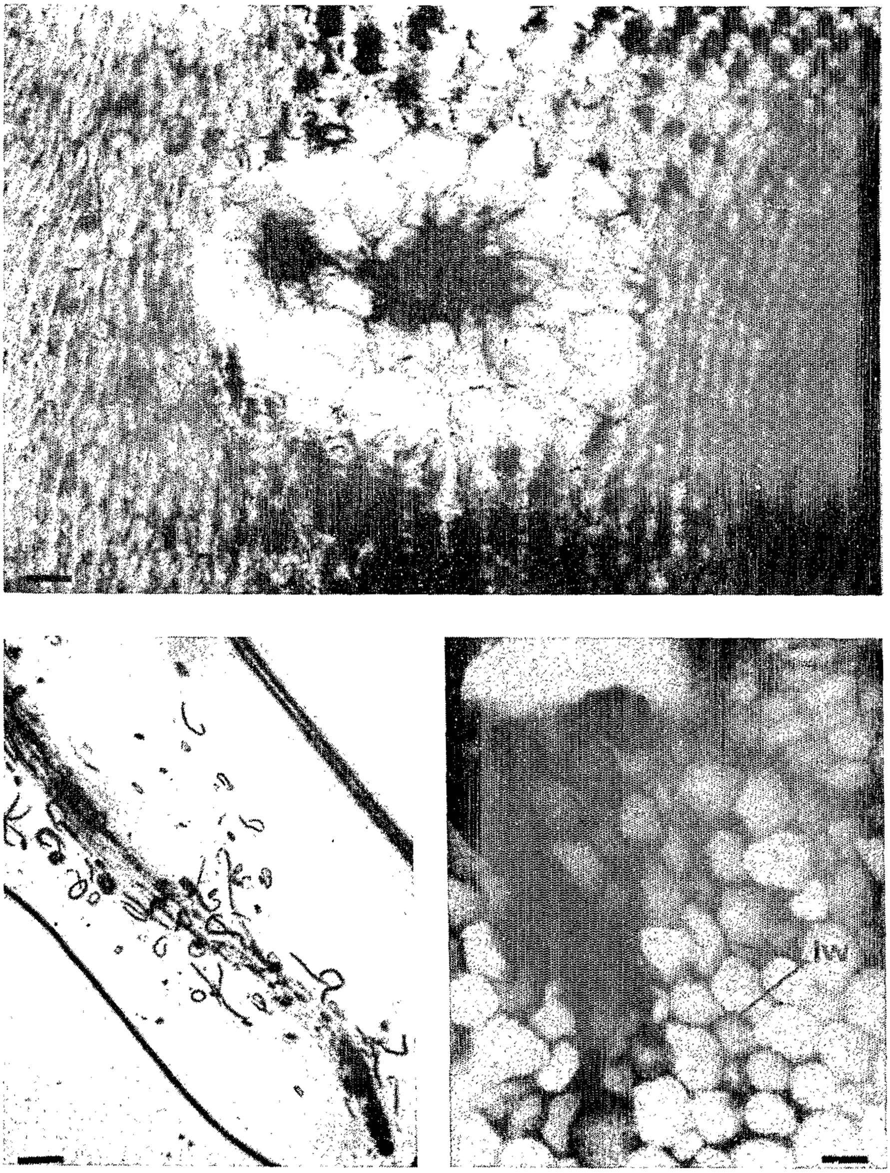

Fig. 2. A–C) Gros Michel roots infected by *Radopholus similis*. A) Cross section: necrotic cells surrounded by hypertrophied cells in the lacunar cortex parenchyma (bar = 76.8 $\mu$m). B) Root crushed between two glass plates: bent and coiled nematodes aggregated in the root (bar = 460.5 $\mu$m). C) Cross section: lignined cell walls around necrosis (lw = lignined walls; bar = 34.5 $\mu$m).

cylinder (Fig. 1C). *R. similis* females laid their eggs in the roots as they migrated forward (Fig. 1D). This was represented by a string of eggs. In Gros Michel roots, lesions did not occur in the cortical parenchyma; instead, small areas of necrotic cells were surrounded by hypertrophied cells (Fig. 2A). *R. similis* remained in the medial layer of the cortical parenchyma and did not migrate more deeply. Nematodes often appeared bent or coiled and were aggregated in the roots (Fig. 2B).

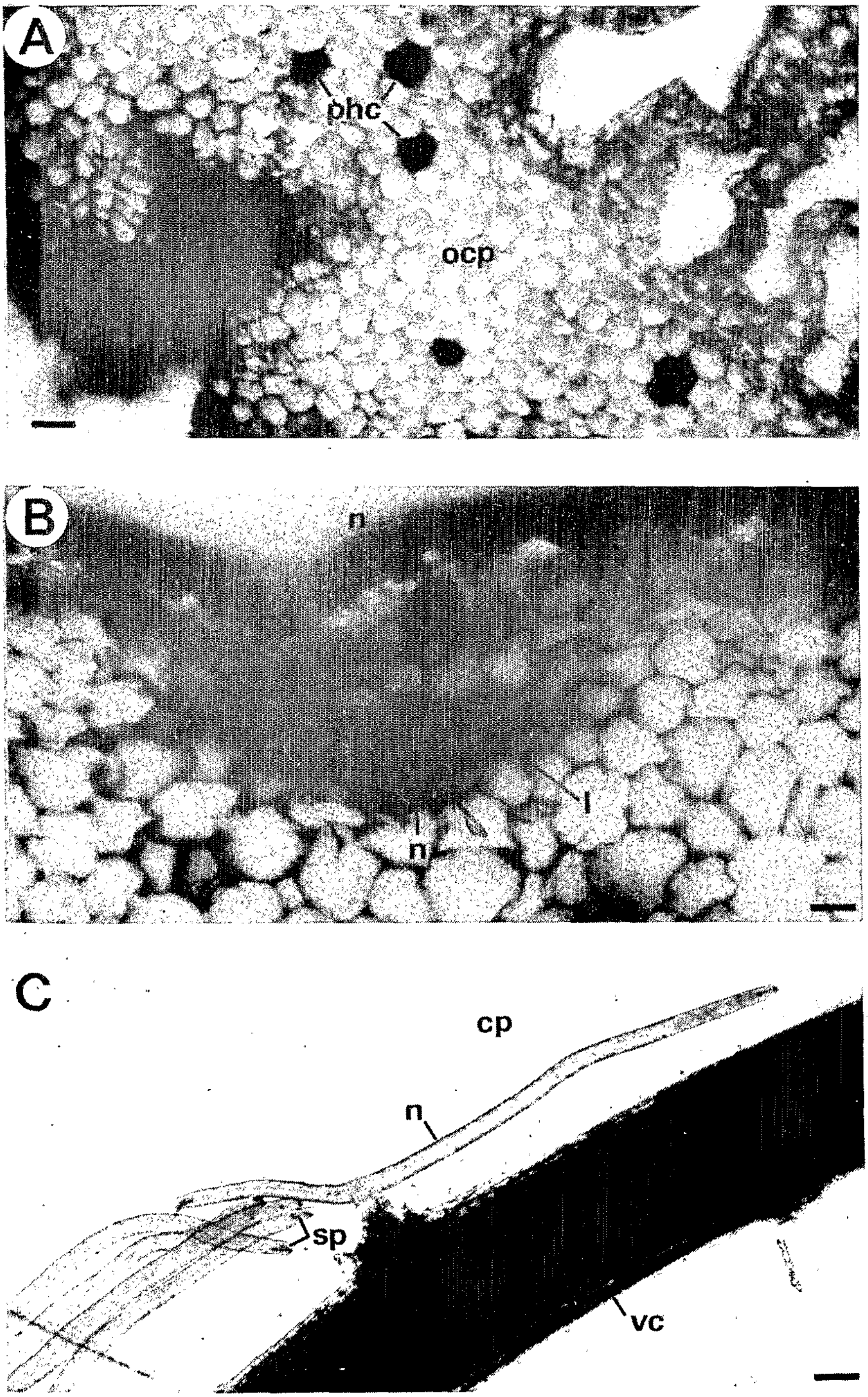

Fig. 3. A) Cross section of a Poyo root infected by *Helicotylenchus multicinctus*: superficial necrosis and stained phenol cells around it (ocp = outer cortical parenchyma; phc = phenol cell; bar = 84.5 μm.B–C) *Hoplolaimus pararobustus* infected banana roots. B) Cross section of a Poyo root: sub-epidermal necrosis and migration of the nematode (l = lesion; n = nematode; bar = 34.5 μm). C) Gros Michel root crushed between two glass plates: forward migration of males (cp = cortical parenchyma; n = nematode; sp = spicules; vc = vascular cylinder; bar = 84.5 μm).

In Gros Michel roots stained for phenols, brown globular structures were observed in cells around necrosed tissues. Such structures were not observed in Poyo roots.

The root staining with safranin and fast-green revealed no discoloration of the cell walls in infected Poyo roots, but a pink discoloration appeared around cells near the necrosed tissues in Gros Michel roots (Fig. 2C).

### Helicotylenchus multicinctus *infection*

In both banana cultivars, necrosis extended from just below the sites of penetration and spread into a few cells of the external cortical parenchyma, which often contained nematodes (Fig. 3A). *H. multicinctus* did not reach the lacunar and internal parts of the cortical parenchyma. Within the root tissues, nematodes usually adopted a coiled position. Females laid their eggs in a cluster in the lumen of empty cells. In both Poyo and Gros Michel roots stained for phenols, large brown cells appeared in the cortical parenchyma near and around the necrosis caused by the nematodes.

### Hoplolaimus pararobustus *infection*

Stained root sections revealed that in both banana cultivars, *H. pararobustus* parasitism was very superficial. Nematodes caused brown open necrosis around the penetration sites (Fig. 3B). Observations of stained roots crushed between two glass plates indicated that *H. pararobustus* migrated either parallel to the root axis (Fig. 3C) or crosswise around it into the cortical parenchyma, where the external part was colonised. Necrotic tissues appeared crushed and peripheral cells increased. Females scattered their eggs in the parenchyma as they migrated randomly in all parts of the roots. Second generation males were observed more in Gros Michel roots than in Poyo roots. In both cultivars, no change has been detected in *H. pararobustus* infected roots stained for phenols.

In roots stained with safranine and fast green, a very clear discoloration was noticed in Poyo and Gros Michel roots infected by *H. multicinctus* and *H. pararobustus*.

## Discussion

Although the three species investigated are endoparasites of bananas, they feed in different areas of the root cortex, regardless of the cultivar. *Hoplolaimus pararobustus* maintains a sub-epidermal position, whereas *Helicotylenchus multicinctus* remains in the outer cortex, and *Radopholus similis* feeds in all parts of the cortex in Poyo roots. Their migratory behaviours are different too; the habitus of the nematodes in the roots and the mode of egg-laying indicate that *H. multicinctus* and *H. pararobustus* are less migratory than *R. similis*.

The most important varietal character concerns host tissue reactions, especially towards *R. similis*. First, the same tissue lesions observed in Poyo roots were not seen in Gros Michel roots. Second, necrosis expands differently in the two cultivars. In Poyo tissues, necroses expand all along nematode migration ways, from the epidermal layer to the vascular cylinder. In Gros Michel roots, the migration of the nematodes seems to be slown down in the outer cortex by a tissue reaction around nematodes with accumulation of lignins in the cell walls. This could be compared with hypersensitive reactions. That is confirmed both by the coiled habitus and the aggregative distribution of the nematodes in Gros Michel roots. Third, cytoplasmic globules in cells adjacent to necrosis appeared only in Gros Michel roots. Pinochet (1978) had observed heavily stained globules in the cytoplasm of cortical cells in plantain (*Musa* AAB) infected by *Pratylenchus coffeae*. He suggested that these globules are an indirect response to nematode infection, rather than a direct consequence of nematode feeding. This study reveals that this reaction depends on the banana cultivar. Reaction of the globules to potassium dichromate staining indicates the probable phenol composition of their content. That confirms the increase of the root phenol concentration in *R. similis* infected roots of Gros Michel only (Mateille, 1993).

Even though *H. multicinctus* and *H. pararobustus* caused tissue damage, no differences were detected between cultivars, except for the abundance of *H. pararobustus* males seen in Gros Michel roots, which confirmed the different development of its sex-ratio on these two cultivars (Mateille, 1992).

This histopathological study shows that responses of *Musa acuminata* (AAA group) depend on the nematode species and are cultivar-specific. The same conclusion was made in relation to population development (Mateille, 1992). The different host reactions between the two cultivars can be related to the physiological events associated with the secondary metabolism involved in incompatibility. Biochemical studies (Mateille, 1993) had revealed that the lower sensitivity of the cultivar Gros Michel to *R. similis* is due to an increase of the secondary metabolism which does not occur in Poyo. It is not known if the tissue reactions are directly caused by *R. similis* or due to subsequent nematode invasion, but the phenol increase in the roots can be related to the occurrence of the cytoplasmic brown globules. The appearance of brown cells around root necrosis caused by *H. multicinctus* can also be related to an activation of the secondary metabolism. But as no significant phenol increase was detected in the roots by biochemical analysis, this histological phenomenon could be short-lived. Similarly *H. multicinctus* and *H. pararobustus* did not change the secondary metabolism in either cultivar roots, nor did the damage to the roots differ. So, banana tissue reactions depend on the parasitic nematode species and on the cultivar.

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