HISTOPATHOLOGY OF BLACK PEPPER ROOTS INFECTED WITH *RADOPHOLUS SIMILIS**

FRANCISCO C.O. FREIRE[1] & JOHN BRIDGE[2]

[1] EMBRAPA, CPATU – Caixa Postal 48, 66.000 – Belem – Para - Brazil
[2] COMMONWEALTH INSTITUTE OF PARASITOLOGY, 395 A, Haftield Road, St. Albans, Herts AL4 OXU U.K.

(Accepted for publication in 22/02/85)

ABSTRACT

FREIRE, F.C.O. & BRIDGE, J. Histopathology of black pepper roots infected with *Radopholus similis*. Fitopatol. bras. 10:475-481.1985.

Histological sections of black pepper roots (*Piper nigrum* L.) infected with *Radopholus similis* were observed at 10, 20 and 30 days after inoculation. Penetration occurred preferentially through the root tips. At 10 days nematodes took up a feeding position intercellularly and intracellularly and cortex cells immediately around the nematodes were necrotic. Nuclei and nucleoli of cells upon which nematodes of *Radopholus similis* fed showed increased size. Twenty days following inoculation large necrotic lesions were detected throughout the cortex. Eggs were found laid in the necrotic cortical tissues. By 30 days nematodes reached vascular tissues and some xylem vessels were seen plugged with a "gum-like substance".

RESUMO

Histolopatologia de raízes de pimenta-do-reino parasitadas por *Radopholus similis*

Seções histológicas de raízes de pimenta-do-reino parasitadas por *Radopholus similis* foram observadas aos 10, 20 e 30 dias após a inoculação. A penetração ocorreu preferencialmente através das extremidades radiculares. Aos 10 dias os nematóides foram observados se movendo

* Portion of a Ph.D. thesis submitted by the senior author to London University.

intercelularmente e intracelularmente enquanto as células corticais próximas aos nematóides se mostravam necróticas. Núcleos e nucleólos das células sobre as quais os nematóides se alimentaram apresentavam-se hipertrofiados. Vinte dias seguindo-se à inoculação os tecidos corticais exibiam grandes áreas necróticas. Ovos foram vistos depositados nos tecidos corticais necrosados. Aos 30 dias os nematóides atingiram os tecidos vasculares e alguns vasos do xilema encontravam-se completamente obstruídos por uma substância gelatinosa.

INTRODUCTION

Radopholus similis is a nematode which has a world wide distribution. Is wide host range includes more than 250 plants species and its most devastating effects are confined to tropical and subtropical countries where national economies are essentially dependent on the export of agricultural products (O'Bannon, 1977).

Although *R. similis* has not been found parasitizing black pepper Brazil its presence infecting black pepper in Guyana, near the Brazilian border (Bisessar, 1969), is a constant menace to the farmers of Brazilian Amazonian region.

Pathological effects of *R. similis* in roots of important cash crops have been reported (DuCharme, 1959; Loos, 1962; Blake, 1966; O'Bannon *et al.*, 1967; Vilsoni *et al.*, 1976; Venkitesan & Setty, 1977). However, very little information is available on the histopathology of black pepper roots infected with *R. similis.* This paper describes the histological changes caused in black pepper roots by *R. similis* aiming to better understand the feeding habits of this nematode and its subsequent effect upon root cells.

MATERIALS AND METHODS

five month-old black pepper seedlings, cultivar Singapura, grown in 10 cm diameter plastic pots partially filled with an autoclaved mixture of loam and sand (3: 2), were inoculated with 50 specimens of *R. similis.* Nematodes of *R. similis,* rinsed in sterilized distilled water, were a mixed population of hand picked juveniles, mature females and males. The nematode population was originally collected from roots of black pepper plants showing typical yellows symptoms on the Bangka Island of Indonesia. Seedlings receiving only sterilized distilled water were kept as the control. Pots were kept on glasshouse benches where temperatures ranged from 23°C to 38°C.

For histopathological observations roots were collected 10, 20 and 30 days after inoculation. Roots of infected and noninfected plants were carefully washed free of soil in distilled water, cut into small pieces (0,5 to 1 cm long) and fixed in F.A.A. for 24 hours. Root pieces were serially dehydrated through an ethyl alcohol series, embedded in paraffin wax (melting point 56°C), sectioned (12 – 15 μm) longitudinally and transversally on a rotating microtome and attached to microscope slides using glycerin albumen as an adhesive. The sections were stained with safranin and fast green and mounted in Canada balsam (Johansen, 1940).

RESULTS

Histological sections of black pepper roots prepared 10 days after inoculation revealed adults and juveniles of *R. similis* in feeding positions within the cortex. Since invasion was not synchronous some nematodes were still observed penetrating the root tips, in the regions of meristematic activity. Small brown lesions were visible on the root surface at this time of nematode infection. Usually several nematodes were oserved associated with the same feeding site. Nematodes took up a feeding position intercellularly and intracellularly in the cor-

tex, and those cells immediately around the nematodes showed marked necrosis, contrasting with apparently healthy cells nearby (Figure 1). Nematodes of *R. similis* were found with the stylet inserted through the cell wall and sometimes with the head totally embedded inside collapsed cells. Nuclei and nucleoli of cells upon which *R. similis* fed exhibited increase in size compared with nuclei and nucleoli in cells still unaffected. Cytoplasm of those affected cells were dark, granular and contracted from the cell wall, which sometimes appeared intact but frequently appeared ruptured.

Twenty days following inoculation, necrotic lesions were found throughout cortical tissues. Cavities were commonly observed in the cortex, usually packed with nematodes (Figure 2). Eggs were also found laid in the necrotic cortex.

Fig.1 – L.S. Necrotic lesions caused by *R. similis* in the cortex of black pepper root. Nematode (N), Necrotic cells (NC).

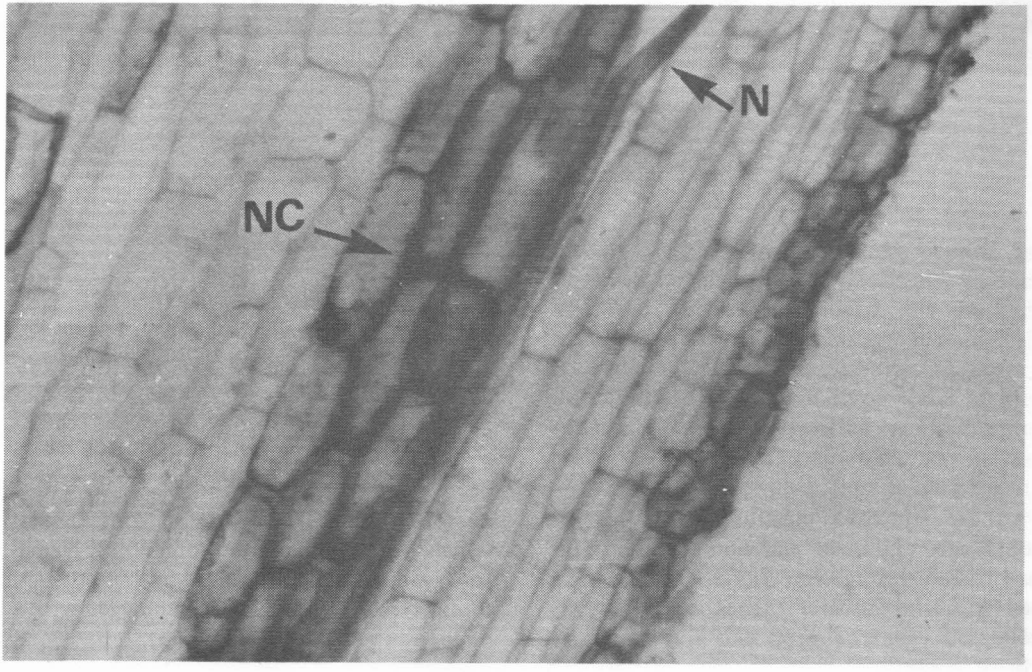
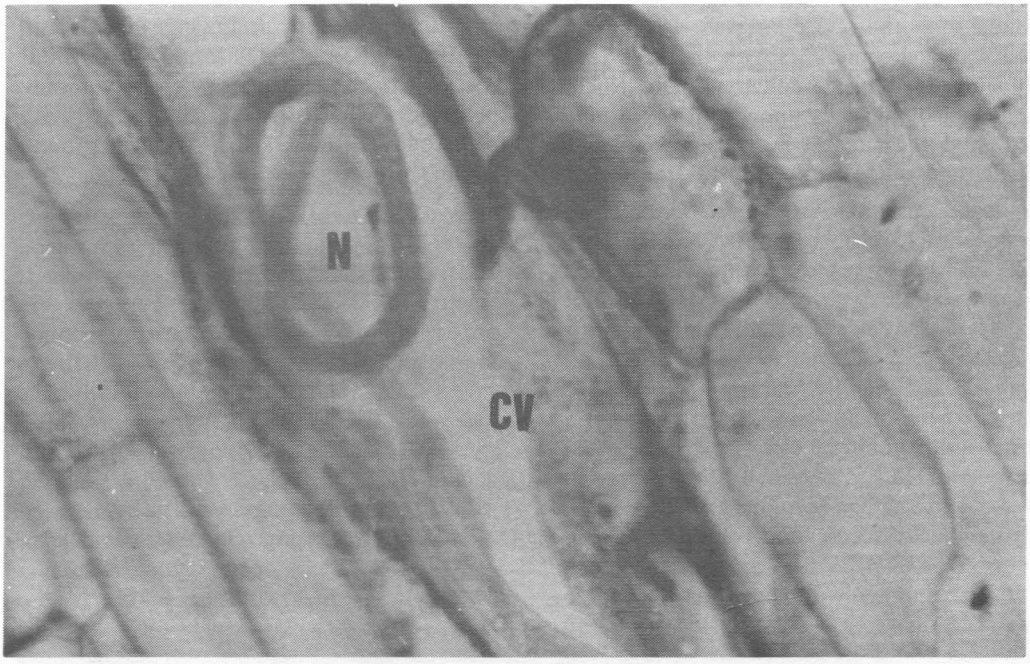
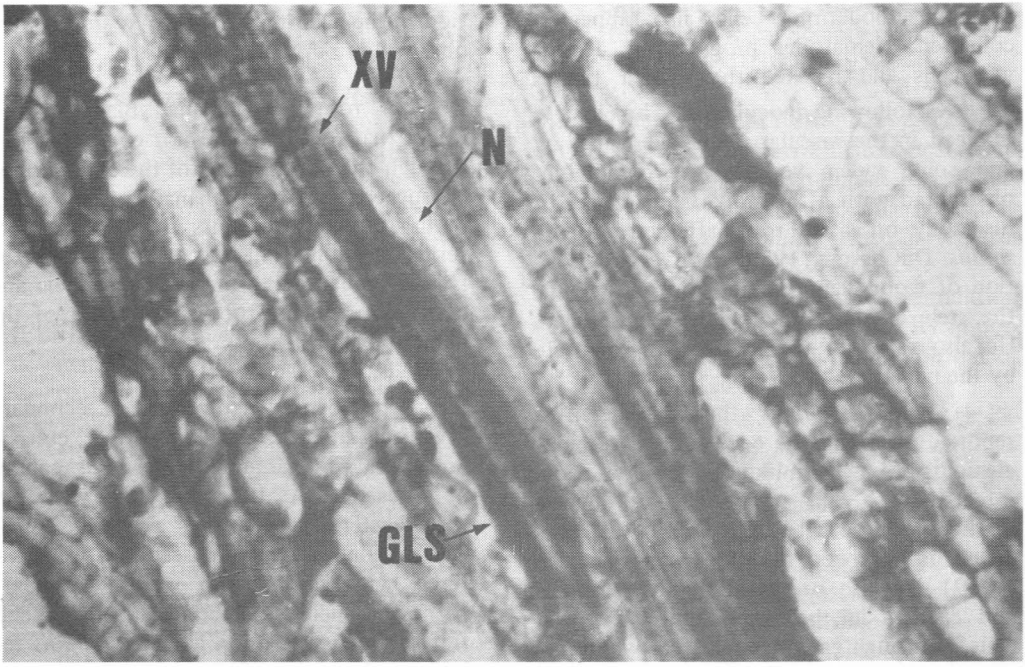


Fig. 2 – L.S. Large cavities created by the burrowing of *R. similis* in black pepper root. Nematodes (N), Cavities (Cv).



By 30 days, destruction of feeder roots was extensive and some nematodes had passed through the endodermis and moved into the vascular tissues. Cavities and collapsed cells coalesced and formed large necrotic lesions, involving all root tissues, which were completely disorganized. Nematodes were frequently found in these tissues. Some xylem vessels were seen plugged with a "gum-like substance" (Figure 3).

Fig. 3 – L.S. Complete necrosis in the vascular tissues of black pepper root caused by *R. similis*. Note obstruction of xylem vessels with a "gum-like substance". Nematode (N), "gum-like substance" (gls), Xylem vessels (XV).



DISCUSSION

The extensive necrosis and formation of cavities in roots of black pepper, cultivar Singapura, caused during parasitism of *R. similis,* are very much like cellular damage reported by various workers in roots of other host plants (DuCharme, 1959; Blake, 1966).

DuCharme (1959) reported that, in roots of grapefruit, *R. similis* penetrated the epidermis and migrated through the root cortex, apparently causing more damage by dissolving the cells rather than just by mechanical disruption. It seems reasonable to assume that in roots of black pepper, as observed in this work, *R. similis* also caused cell wall dissolution. Indeed, the marked necrosis detected in many histological sections indicates this possibility. However, formation of tumour or hyperplasia in the vascular cylinder, as reported by DuCharme (1959) in roots of grapefruit, was never observed in black pepper roots in the present study. According to DuCharme (1959), the formation of tumours occurred only when nematodes passed through the endodermis, indicating that endodermic cells act as a barrier that either prevents passage of nemic metabolites into the stele or changes them

so that they become innocuous. This was not the case of *R. similis* in roots of black pepper. Migration of nematodes through the endodermis did not produce any visible reaction, and during further stages of infection endodermic cells also appeared collapsed. Nematodes progressively destroyed cells and reached the vacular tissues 30 days following inoculation. The changes induced in the vascular tissues were striking and some xylem vessels were completely obstructed with a "gum-like substance". In his study on citrus roots affected with *R. similis,* DuCharme (1959) observed a deposition of wound gum in the courtex, next to the stele at the site of nematode activity. He also reported the invasion of the stele by the nematodes.

As for *R. similis,* there has been no report concerning the occlusion of vascular elements by a "gum-like material" in roots infected with this nematode, although a marked lytic activity of *R. similis* in roots of citrus has been reported by DuCharme (1959). He also suggested that the tunnels and cavities in the root could have been caused through enzymes released by the nematodes, such as cellulase, pectinase and protease. Occurence of amylase, pectinase, cellulase, invertase, protease and chitinase have been confirmed in saliva of plant parasitic nematodes (Lee & Atkinson, 1976). In addition to these findings, enzymes such as hydrolases, cellulase, invertase and pectinase have been identified in *R. similis* (Giebel, 1982) and could be responsible for the initiation of the process leading to the obstruction of the xylem vessels of black pepper roots as observed in this work.

Venkitesan & Setty (1977) carried out histopathological studies in roots of black pepper, cultivar Kalluvali, and reported that *R. similis* did not affect stelar portions, but these workers did not mention how many days they conducted the histopathological observations. It is likely that they did not detect the presence of *R. similis* in the vascular tissues of black pepper roots because they did not allow the nematodes sufficient time to explore all the root tissues. Other aspects mentioned by these workers, such as root invasion, cortex damage and oviposition in the necrotic cortex by *R. similis* were observed in our work.

In roots of banana, Blake (1966) found that *R. similis* did not enter the stele through the passage cells of the endodermis. He suggested that in banana roots the endodermis probably prevents *R. similis* from entering the stele from the cortex. He reported lysis of cells and formation of tunnels and cavities in banana roots, as a result of the nematode parasitism.

The possible involvement of secondary organisms in roots of black pepper, cultivar Singapura, associated with *R. similis,* cannot be discarded. As the nematodes were not surface sterilized but only rinsed in sterilized distilled water, and seedlings were watered with normal tap water, secondary pathogens such as bacteria could have penetrated the tissues upon which *R. similis* fed taking advantage of the wounds as entry avenues, and of any possible nutrients released during the nematode feeding process. Fungal hyphae were never observed in histological sections of black pepper roots inoculated with *R. similis* singly.

The present histopathological study shows that, irrespective of any other possible factor involved during the infectious process, *R. similis* proved to be extremely harmful to black pepper plants, as demonstrated in histological sections of roots examined during the different stages of parasitism. Some differences between results reported by various workers can be explained by the utilization of different isolates or races of *R. similis,* differences in host morphology or even to the use of gnotobiotics conditions under which the experiments were performed.

LITERATURE CITED

BISESSAR, S. Plant parasitic nematodes of crops in Guyana. PANS 15: 74-77. 1969.

BLAKE, C.D. The histological changes in banana roots caused by *Radopholus similis* and *Helicotylenchus multicinctus*. Nematologica 12: 129-137. 1966.

DuCHARME, E.P. Morphogenesis and histopathology of lesions induced on citrus roots by *Radopholus similis*. Phytopathology 49: 388-395. 1959.

GIEBEL, J. Mechanism of resistance to plant nematodes. Annual Review of Phytopathology 20: 257-279. 1982.

JOHANSEN, D.A. Plant Microtechnique. McGraw Hill Book Co. Inc., New York, 523 pp. 1940.

LEE, D.L. & ATKINSON, H.J. Physiology of Nematodes. The Macmillan Press Limited, London, U.K., 215 pp. 1976.

LOOS, C.A. Symptom expression of Fusarium wilt disease of the Gross Mitchel banana in the presence of *Radopholus similis* (Cobb, 1893) Thorne, 1949 and *Meloidogyne incognita acrita* Chitwood, 1949. Proceedings of the Helminthological Society of Wahington 26: 103-111. 1959.

O'BANNON, J.H.; MYERS, R.F. & FEDER, W.A. A comparative histological study of citrus varieties tolerant to and susceptible to the burrowing nematode (*Radopholus similis*) (Abstr.). Nematologica 13: 147-148. 1967.

O'BANNON, J.W. Worldwide dissemination of *Radopholus similis* and its importance in crop production. Journal of Nematology 9: 16-25. 1977.

VENKITESAN, T.S. & SETTY, K.G.H. Pathogenicity of *Radopholus similis* to black pepper (*Piper nigrum*). Indian Journal of Nematology 7: 17-26. 1977.

VILSONI, F.; McCLURE, M.A. & BUTLER, L.D. Occurence, host range and histopathology of *Radopholus similis* in ginger (*Zingiber officinale*). Plant Disease Reporter 60: 417-420. 1976.