HISTOPATHOLOGY OF ROOTS, STEMS AND LEAVES OF BLACK PEPPER PLANTS INFECTED WITH *NECTRIA HAEMATOCOCCA* F. SP. *PIPERIS* AND *PHYTOPHTHORA PALMIVORA*[1]

FRANCISCO C.O. FREIRE[2] & JOHN BRIDGE[3]

[2] EMBRAPA, CPATU – Caixa Postal 48, 66000 – Belém-Pará – Brazil
[3]Commonwealth Institute Of Parasitology, 395 A Hatfield Road,

(Accepted for publication at 20/08/85)

ABSTRACT

FREIRE, F.C.O. & BRIDGE, J. Histopathology of roots, stems and leaves of black pepper plants infected with *Nectria haematococca* f. sp. *piperis* and *Phytophthora palmivora*. Fitopatol. bras. 11:131-150. 1986.

Comparative histological observations were made on uninjured and artificially injured roots, stems and leaves of black pepper seedlings, cultivar Singapura, infected with *Nectria haematococca* f. sp. *piperis* and *Phytophthora palmivora*. Germtubes formed from conidia of *N. haematococca* f. sp. *piperis* penetrated uninjured roots of black pepper preferentially at the root tips and at points formed by the emergence of lateral roots. Fungal penetration in artificially injured roots was quicker compared to uninjured ones. Penetration of uninjured black pepper roots by germtubes of encysted zoospores of *P. palmivora* occurred on the whole root extremities but preferentially at the region of elongation just behind the root tips. Artificially injured roots were invaded and colonized quicker than uninjured ones. Once within the roots hyphae of both fungi branched and grew intercellularly and intracellularly. Neither *N. haematococca* f. sp. *piperis* nor *P. palmivora* formed appressorium or appressorium-like structures before root penetration. Xylem vessels completely obstructed by a "gum-like substance" were observed in roots colonized by both fungi. Simultaneous penetration and colonization of uninjured and artificially injured roots of black pepper seedlings by *N. haematococca* f. sp. *piperis* and *P. palmivora* presented the same pattern of infection as when both fungi were inoculated singly. Colonization of root tissues was not even. Frequently infective hyphae of *P. palmivora* were observed prevailing over hyphae of *N. haematococca* f. sp. *piperis*. Germtubes from conidia of *N. haematococca* f. sp. *piperis* and from encysted zoospores of *P. palmivora* did not penetrate uninjured stems of black pepper seedlings at the internodal region. Artificially injured stems, however, were quickly invaded and colonized by both fungi but stem infection progressed rather slowly compared with root infection. The presence of a "gum-like substance" and "tylose-like structures" were frequently observed in the xylem vessels of stems heavily infected with both fungi. Leaves of black pepper seedlings, either uninjured or artificially injured, were easily invaded and colonized by *N. haematococca* f. sp. *piperis* and *P. palmivora*. When all leaf tissues were completely disarranged a "gum-like material" plugging xylem vessels was commonly observed.

[1]Portion of a Ph.D. thesis submitted by the senior author to London University.

RESUMO

Histopatologia de raízes, caules e folhas de plantas de pimenta-do-reino infectadas com *Nectria haematococca* f. sp. *piperis* e *Phytophthora palmivora*

Observações histológicas comparativas foram realizadas em raízes, caules e folhas de plântulas de pimenta-do-reino, cultivar Singapura, intactas ou mecanicamente feridas, e inoculadas com *Nectria haematococca* f. sp. *piperis* e *Phytophthora palmivora.* Os tubos germinativos formados a partir de conídios de *N. haematococca* f. sp. *piperis* penetraram as raízes intactas de pimenta-do-reino preferencialmente nas extremidades radiculares e nas regiões de emergência das raízes laterais. A penetração do fungo em raízes mecanicamente feridas foi mais rápida em comparação com a penetração em raízes intactas. A penetração de raízes intactas de pimenta-do-reino pelos tubos germinativos de zoósporos encistados de *P. palmivora* ocorreu sobre toda a extensão das extremidades radiculares mas, preferencialmente, na região de alongamento, exatamente atrás das extremidades radiculares. Raízes mecanicamente feridas foram invadidas e colonizadas mais rapidamente por *P. palmivora* que raízes intactas. Quando estabelecidas dentro das raízes, hifas tanto de *N. haematococca* f. sp. *piperis* quanto de *P. palmivora* se ramificaram e cresceram intercelularmente e intracelularmente. Nem *N. haematococca* f. sp. *piperis* nem *P. palmivora* formaram apressório antes de penetrarem as raízes de pimenta-do-reino. Vasos do xilema completamente obstruídos por uma substância aparentemente gelatinosa foram observados em raízes colonizadas pelos dois fungos. A invasão e a colonização de raízes de pimenta-do-reino, intactas e mecanicamente feridas, inoculadas simultaneamente com *N. haematococca* f. sp. *piperis* e *P. palmivora,* apresentaram o mesmo padrão de infecção de quando os fungos foram inoculados isoladamente. A colonização dos tecidos radiculares, entretanto, não foi uniforme. Frequentemente hifas de *P. palmivora* foram observadas ocupando uma maior área de tecidos infectados em comparação com hifas de *N. haematococca* f. sp. *piperis.* Tubos germinativos de conídios de *N. haematococca* f. sp. *piperis* e de zoósporos encistados de *P. palmivora* não penetraram caules intactos de plântulas de pimenta-do-reino na região dos entrenós. Caules mecanicamente feridos, contudo, foram rapidamente invadidos e colonizados por ambos os fungos. Muito embora os fungos tenham invadido e colonizado rapidamente os caules mecanicamente feridos, o processo infeccioso como um todo foi mais lento, comparado à invasão e colonização radiculares. A presença de uma substância aparentemente gelatinosa e de estruturas semelhantes a tiloses obstruindo os vasos do xilema foram comumente observadas em caules extremamente infectados por ambos os fungos. Folhas de plântulas de pimenta-do-reino, intactas ou mecanicamente feridas, foram facilmente invadidas e colonizadas por *N. haematococca* f. sp. *piperis* e *P. palmivora*. Em tecidos foliares totalmente colonizados pelos dois fungos a ocorrência de uma substância aparentemente gelatinosa obstruindo os vasos do xilema foliar foi frequentemente observada.

INTRODUCTION

Nectria haematococca f. sp. *piperis* (*Fusarium solani* f. sp. *piperis*) is undoubtedly the most destructive pathogen of black pepper (*Piper nigrum* L.) in Brazil. Firstly recorded as a root and foot rot pathogen, *N. haematococca* f. sp. *piperis* was later found causing a serious blight in branches of black pepper (Albuquerque, 1968). The disease is so destructive, either as root and foot rot or blight, that the production life of black pepper plants in the Amazonian region has been shortened from approximately 15 years to no more than 8 years. The farmers have to have, in the same plantation, vines of different ages in order to cope with the losses due to the death of older plants. Moreover, they usually move out seeking for new areas to establish new plantations, increasing their expenses and diminishing their profits (Albuquerquer & Conduru, 1971; Homma, 1981).

N. haematococca f. sp. *piperis* is a relatively new pathogen of black pepper and almost nothing is known about its actual pathological effects in roots of this plant. The first information provided by Freire & Santos (1978) reports that the fungus can invade and colonize root tissues quickly after inoculation, suggesting a powerful toxic activity. Histological observations carried out by Fukutomi et al. (1981a; 1981b) in stems and roots of black pepper plants naturally infected with *N. haematococca* f. sp. *piperis* revealed the presence of fungal hyphae in vessels and cortical parenchymatous cells. Vessels cells appeared completely blockaded with gelatinous cementing material.

Observations on the histopathological changes caused by the parasitism of *Phytophthora palmivora* in roots of black pepper plants have been provided by Alconero et al. (1972). Zoospores invaded roots six hours after inoculation and progressed inside the root tissues either intercellularly or intracellularly. Only nine hours following the inoculation, fungal hyphae were observed deep in the cortex. Twelve hours after zoospore invasion, tissue maceration was evident in some roots.

Although some research has been undertaken on the histopathological effects of *N. haematococca* f. sp. *piperis* and *P. palmivora* in roots of black pepper, many aspects of their behaviour with this plant are still poorly understood. This work was done in an attempt to interpret the pathological changes during the different stages of infection of these organisms, either alone or interacting on black pepper plants.

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 uprooted and their root systems washed for 10 minutes in running tap water and twice in sterilized distilled water. Seedlings were placed in 9 cm diameter plastic Petri dishes and their roots covered with 60 g of "40 – 100 mesh" acid washed silica sand, one seedling per plate. Before placing them in the plastic Petri dishes, some seedlings had their roots gently scratched with a heat sterilized blade or the roots were punctured with a heat sterilized needle. Some uninjured seedlings were also inoculated with both fungi to compare the mechanism of tissue invasion and colonization. Inoculations were achieved by pouring into each plate, separately, 10 ml of a conidial suspension (100,000 conidia/ml) of *N. haematococca* f. sp. *piperis* or 10 ml of a zoospore suspension (10,000 zoospores/ml) of *P. palmivora*. For simulatenous inoculation of roots of black pepper each seedling was inoculated with 5 ml of a conidial suspension of *N. haematococca* f. sp. *piperis* (200,000 conidia/ml) and 5 ml of a zoospore suspension of *P. palmivora* (20,000 zoospores/ml). After inoculation, enough sterilized distilled water was added to each plate to keep a suitable moisture content for both plants and fungi. Plates were kept on glasshouse benches with temperatures ranging from 22ºC to 36ºC.

For histopathological observations of individual infection, root samples were taken 6 and 12 hours, and 1, 2, 3 and 4 days after inoculation. In case of simultaneous inoculation, root samples were collected 6 and 12 hours, and 1 – 4 days following inoculation with both fungi. For inoculation of stems and leaves, five month-old black pepper seedlings were thoroughly washed in sterilized distilled water, placed in 14 cm diameter glass Petri dishes and their root systems covered with 100 g of acid washed sand. Cotton wool soaked with distilled water was placed in each plate to keep a high humidity content. Stems were wounded by scratching their surface with a heat sterilized blade or puncturing them with a heat sterilized needle. Detached young leaves of black pepper seedlings were wounded in the same way and placed in Petri dishes with cotton wool soaked with distilled water. For inoculation, drops of a conidial suspension of *N. haematococca* f. sp. *piperis* (1,000,000 conidia/ml) or a zoospore suspension of *P. palmivora* (100,000 zoospores/ml) were poured on to injured and uninjured stems and leaves. Detached leaves were inoculated on both sides. Plates were kept under the same conditions as previously described. Stem samples were taken 6, 12 and 24 hours, and 2, 4, 6, 8 and 10 days after inoculation with *N. haematococca* f. sp. *piperis* and *P. palmivora*. Leaf samples were collected 6, 12, 24 and 48 hours following inoculation with both fungi.

Roots, stems and leaves of infected and noninfected plants were cut into small pieces and fixed in F.A.A. for 24 hours. All material was then serially dehydrated through an ethyl alcohol series, embedded in paraffin wax (melting point 56°C), sectioned (12 – 15 μ m) 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). In order to achieve a better contrast between fungal hyphae and root tissues, sections from root samples were stained according to Conn (1936).

The possibility that *N. haematococca* f. sp. *piperis* and *P. palmivora* may produce substances *in vitro* that are inhibitory to each other was assessed by growing the two fungi together on 5 Petri dishes each of peptone-potato dextrose agar medium (Peptone 10 g; PDA Oxoid – Oxoid Limited 39 g; distilled water 1,000 ml) and V-8 juice agar medium (Campbell's V-8 juice 200 ml; $CaCO_3$ 3 g; agar-agar 15 g; distilled water 800 ml). Each plate was inoculated with two 7 mm diameter discs of 8 and 5 day-old cultures of *N. haematococca* f. sp. *piperis* and *P. palmivora* grown on peptone-potato dextrose agar and V-8 juice agar, respectively. Discs were obtained from the growing edge of each fungal culture with the aid of a heat sterilized cork borer and placed approximately 1.5 cm apart in opposite sides of each Petri dish. Plates were kept on benches, under artificial cool white illumination for 16 hours in each 24 hours cycle, at an intensity of 4950 lux at culture level, where temperatures ranged from 23°C to 32°C. Mycelial growth was observed each two days over a period of 10 days.

RESULTS

Root infection

Histological sections prepared six hours after inoculation showed that penetration of *N. haematococca* f. sp. *piperis* in uninjured roots of black pepper was preferentially at the root tips and at points formed by the emergence of lateral roots, but sometimes at any point of the root epidermis a few millimeters behind the root tip. The fungus formed a germtube that penetrated between two epidermal cells, or more frequntly, directly through the epidermal root surface. Formation of appressoria before penetration was never observed. Twelve hours following inoculation, some hyphae were seen in the outer layers of the cortex. By 24 hours, hyphae had branched and grew intercellularly and intracellularly in the cortex, usually in parallel strands. The fungus colonized and destroyed the root cortex rapidly as shown in sections prepared 48 and 72 hours following inoculation. By the fourth day of infection, the whole cortex appeared completely disorganized and, in such roots, hyphae were the sole structures between the epidermis and vascular tissues, which were also invaded and colonized. In some cases, hyphae branched and formed knot-like masses in the outer cortex. Such masses, however, had branched and spread through the root tissues colonizing them in the same manner. Macroconidia and microconidia were frequently produced in the outer cortex, always associated with hyphae segments. When cortical cells were completely collapsed many chlamydospores of one and two cells and different sizes were seen inside the outer cortex. Chlamydospores were also observed outside the roots, usually formed from conidia that did not penetrate.

Colonization of injured roots of black pepper by *N. haematococca* f. sp. *piperis* showed the same pattern of infection as for uninjured roots. Once inside the root tissues the fungus behaved essentially in the same manner, but invasion and colonization were quicker than for uninjured roots and only six hours after inoculation conidia were observed penetrating through the wounds by forming a long germtube (Figure 1).

In histological sections prepared four days following inoculation, many xylem vessels appeared totally obstructed by a "gum-like substance".

Zoospores of *P. palmivora* were observed encysting on the whole root extremities of black pepper seedlings but preferentially at the region of elongation, just behind the root tips. Six hours after inoculation, zoospores were seen penetrating the epidermal root surface between two cells or directly through the cell wall, without forming any visible appressorium or appressorium-like structure. During the onset of penetration some germinated zoospores were pushed away from root surface by the germtubes.

Twelve hours following inoculation infective hyphae had branched and grown intercellularly and intracellularly. Twenty-four hours after inoculation hyphae were well established deep in the cortex. Within 48 hours extensive hyphal growth was observed reaching the endodermis, phloem and xylem vessels. At this stage of infection many epidermal and cortical cells had already collapsed. By 72 hours, the root tissues were completely colonized and hyphae were seen growing inside the xylem vessels, initially longitudinally but later some branching occurred and adjacent tracheids were also invaded. Growth from one cell into another cell seemed to have occurred through direct penetration of the cell wall. Four days following inoculation, root tissues were totally macerated and conspicuous strands of hyphae were the main structures in the cortex (Figure 2). No chlamydospores or fruiting bodies were observed within the infected tissues.

Artificially injured roots of black pepper were colonized and destroyed by *P. palmivora* in basically the same way as uninjured roots. Zoospores showed a positive response towards wounds and only 6 hours after inoculation many of them were observed trying to encyst inside the root wounds.

Infected tissues of injured roots were completely disintegrated 72 hours after inoculation, sooner than injured roots. Plugging of xylem vessels with a "gum-like material" was frequently observed.

Simultaneous inoculation with *N. haematococca* f. sp. *piperis* and *P. palmivora* on uninjured roots of black pepper seedlings, presented the same pattern of infection as when both fungi were inoculated singly. *P. palmivora* was more competitive than *N. haematococca* f. sp. *piperis* in exploiting root tissues, as germtubes from zoospores successfully penetrated roots only 6 hours following inoculation. By this time, germtube from conidia of *N. haematococca* f. sp.

piperis were in contact with root epidermal cells but penetration was only actually completed 12 hours after inoculation. Both fungi penetrated roots by forming germtubes. No appressorium or appressorim-like structures were seen before or during penetration. Once inside the root cortex, both fungi progressed intercellularly and intracellularly, but colonization of root tissues was not even in the 1 – 4 days following inoculation.

Sometimes, in parts of heavily infected roots, hyphae of *N. haematococca* f. sp. *piperis* seemed to predominate. Most frequently, however, infective hyphae of *P. palmivora* were observed prevailing over hyphae of *N. haematococca* f. sp. *piperis.* By four days, all root tissues were macerated and fungal hyphae were usually seen invading xylem vessels.

Penetration and colonization of artificially injured roots of black pepper by *N. haematococca* f. sp. *piperis* and *P. palmivora* were similar to their infection when inoculated singly on artificially injured roots. The only difference was that root necrosis in injured roots developed more rapidly and, just two days after inoculation, all root tissues were collapsed and disorganized. Xylem vessels completely plugged with a "gum-like substance" was a common feature.

When both fungi were inoculated on V-8 juice agar medium *N. haematococca* f. sp. *piperis* showed initially a normal mycelial growth and conidial production but was later totally engulfed by the more rapid growth of *P. palmivora.* Production of sporangia by *P. palmivora* was markedly enhanced at the zone where the two colonies contacted, compared to sporangial production at areas away from the contact point. Also, some of the sporangia formed at the zone of contact between the two fungi were abnormally long and narrow, sometimes measuring 120 x 16 μ m, in contrast with the usual average sporangial size of this isolate of *P. palmivora* on V-8 juice agar medium (44 x 30 μ m). Frequently, these abnormal sporangia had one or more constrictions. Presence of sporangia of apparent normal size but exhibiting two conspicuous papillae were also observed.

On peptone-potato dextrose agar medium, at the zone where the two colonies met, production of conidia by *N. haematococca* f. sp. *piperis* was again normal, but *P. palmivora* did not produce sporangia when cultures were examined 7 days after transferring both fungi to the media surface. At the point of contact between the two fungi, hyphae and knot-like masses of *P. palmivora* were thicker and with amber-brown colour initially when stained in lactophenol cotton blue but turning into dark-brown later, contrasting with the normal blue of these structure from other areas of the culture medium.

On V-8 juice agar medium, the hyphae of *N. haematococca* f. sp. *piperis* and *P. palmivora* after establishing contact, grew without inhibiting each other's growth and intermingled freely. The same was observed when both fungi grew on peptone-potato dextrose agar medium. On this culture medium, however, growth of *P. palmivora* was apparently inhibited later when the diameter of *N. haematococca* f. sp. *piperis* increased, and grew into the areas already occupied by hyphae of *P. palmivora.*

Stem infection

Germtubes from conidia of *N. haematococca* f. sp. *piperis* were not able to penetrate and colonize uninjured stems of black pepper seedlings at the internodal region. In a few instances, infection started at the basal parts or joints of the internodes, but how the fungus penetrated was not observed. Colonization of stem tissues was slow and even 10 days after inoculation most of the infective hyphae were still in the cortex. External symptoms were hardly visible in most of the samples examined.

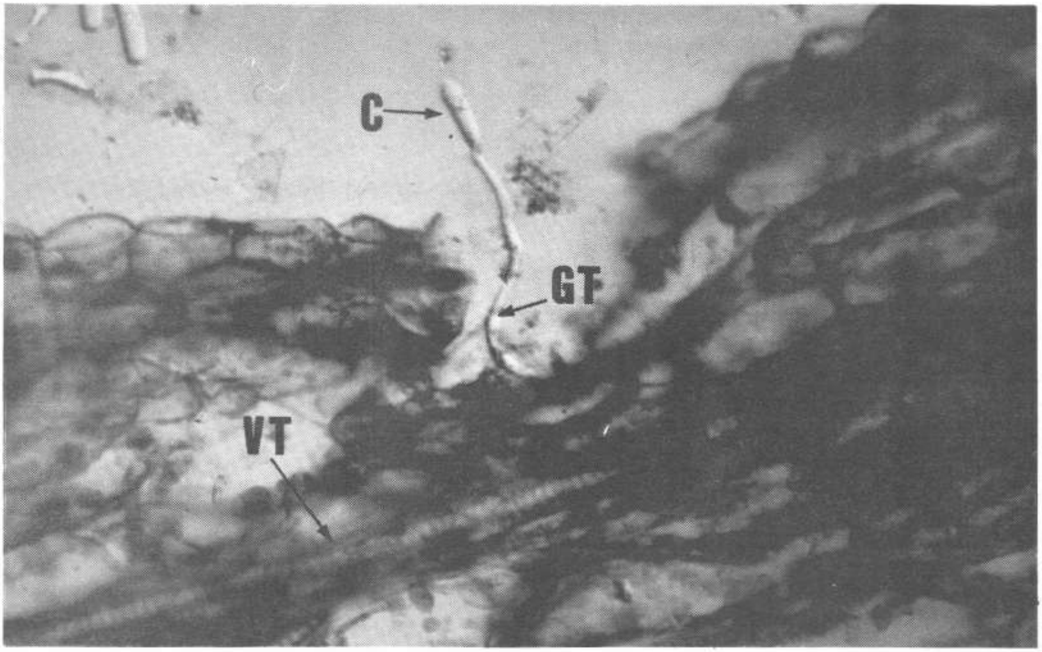

Figure 1. L.S. Penetration of artificially injured black pepper root by a conidium of *N. haematococca* f. sp. *piperis* six hours after inoculation. C – conidium; GT – germtube; VT – vascular tissues

Penetration of artificially injured stems by *N. haematococca* f. sp. *piperis* occurred actually 12 hours after inoculation, although many conidia had been observed with their germtubes in contact with wounded tissues six hours following inoculation. Conidia did not form true appressoria but just little swellings at the end of the germtubes. The injured internodal areas were easily invaded and tissues readily colonized. Twenty-four hours following inoculation, hyphae had branched and grew intercellularly and intracellularly within the cortex. Fungal colonization progressed rather slowly compared with root infection. Fungal hyphae generally progressed more rapidly longitudinally to the axis of the stem through the cortex. After 2, 4, 6 and 8 days following inoculation hyphae of *N. haematococca* f. sp. *piperis* had reached the endodermis and vascular tissues. By 10 days, many cortical cells had collapsed. In several histological sections, hyphae were observed growing in just one area of the stem while other areas nearby remained apparently unaffected.

The presence of a "gum-like substance" plugging some xylem vessels elements was a common feature in heavily infected stems (Figure 3). Tylose-like structures were frequently observed in the xylem vessels of infected stems but their identity was not confirmed.

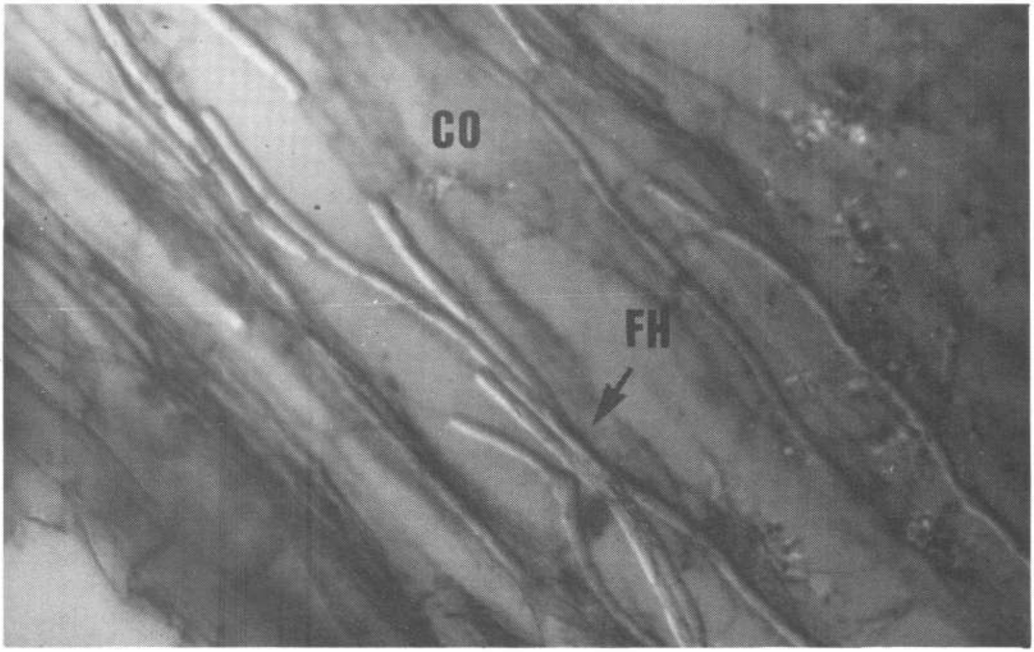

Figure 2. L.S. Hyphae of *P. palmivora* in black pepper root. FH – fungal hyphae; CO – cortex

Few germtubes of *P. palmivora* zoospores were observed trying to penetrate the uninjured surface of stems of black pepper seedlings at the internodal region. None of the germtubes were seen actually penetrating and few zoospores were pushed away from the stem surface by germtubes. The basal parts of the internodes were not invaded and colonized by *P. palmivora* during the 10 – day period of histopathological observations. No appressorium or appressorium-like structures were formed by the zoospores.

Penetration of artificially injured stems by germtubes of *P. palmivora* zoospores was striking only 6 hours after inoculation. Great numbers of zoospores grouped around the wounds, with their germtubes showing a marked positive response towards wounds. Normally zoospores did not form appressoria-like structures before penetrating the injured stem surface. In a few cases, however, some zoospores did form strong appressoria before penetrating wounded stems. In these cases, appressoria were not directed towards the wounds but were formed on intact parts of the stem surface, near the injured areas. Twelve hours after inoculation, infective hyphae of *P. palmivora* branched and invaded epidermal and cortical cells. Twenty-four hours following inoculation hyphae were well established intracellularly and intercellularly within the cortex and some epidermal cells had collapsed.

Two, four, six and eight days following inoculation, extensive colonization of cortex and endodermis occurred. Within 10 days, infective hyphae were observed colonizing vascular tissues. Sometimes hyphae penetrated xylem vessels, generally advancing longitudinally but later with some branching occurring, adjacent tracheids were also invaded. Plugging of xylem vessels with a "gum like material" was always visible. Xylem vessels partially obstructed with tylose-like structures were frequently detected.

Leaf infection

Six hours after inoculation germtubes of *N. haematococca* f. sp. *piperis* conidia were observed to have germinated and apparently begun penetrating uninjured epidermal surface of leaves of black pepper seedlings at the bases of globular structures, probably glandular hairs, on both surfaces of the leaves, but in a few instances penetration occurred directly through the epidermal cell wall. Conidia did not form appressoria or appressoria-like structures before penetrating. Twelve and twenty-four hours following inoculation, extensive hyphae development was visible in cells of the multiple epidermal layer, progressing both intercellularly and intracellularly. Forty-eight hours after inoculation, in heavily infected leaves, hyphae had invaded spongy mesophyll and palisade tissues. At this stage of infection all tissues were colonized and collapsed. Xylem vessel elements were observed partially plugged with a "gum-like substance".

Penetration of artificially injured leaves of black pepper seedlings by germtubes of *N. haematococca* f. sp. *piperis* conidia was greatly increased compared to uninjured leaves. As many as four conidia were observed penetrating through the same wound, exhibiting a marked growth response towards the injured surface only six hours after inoculation. Subsequent advance and colonization of injured leaf tissues by the pathogen were similar to infection in uninjured leaves. Twenty-four hours after inoculation all tissues had been invaded. Histological sections prepared 48 hours following inoculation showed that all the leaf tissues were completely disarranged.

In the sixth hour after inoculation germinating tubes produced by zoospores of *P. palmivora* were observed penetrating between epidermal cells but sometimes penetration occurred directly through the cell wall. A few germtubes were also seen penetrating at the bases of glandular hairs. No appressoria or appressoria-like structures were formed by zoospores before penetration. Twelve hours following inoculation thick hyphae were seen growing within the multiple epidermal layer, usually colonizing the tissues intercellularly. Epidermal cells were frequently found dead within 12 hours at the point of penetration by the germtubes (Figure 4). Hyphae also invaded and colonized tissues intracellularly and hypertrophied nuclei and nucleoli were observed in epidermal cells near the point of penetration. Twenty-four hours after inoculation fungal hyphae were deep within the spongy mesophyll and palisade tissues. Fortyeight hours after inoculation all leaf tissues were colonized and collapsed. A "gum-like material" plugging xylem vessels was commonly observed.

Six hours after inoculation of artificially injured leaves of black pepper, zoospores of *P. palmivora* had aggregated and germinated, directing their germtubes towards the wounds. Once more no appressoria or appressoria-like structures were formed by the zoospores before or during penetration. Further growth and colonization by the pathogen within leaf tissues showed the same pattern of infection for uninjured leaves, although cell collapse and extensive colonization had occurred ealier in injured leaves, just 24 hours following inoculation.

DISCUSSION

Root infection

The efficient penetration of uninjured black pepper roots by conidia of *N. haematococca* f. sp. *piperis,* directly through the root epidermis only 6 hours following inoculation, and its successful colonization of all root tissues, indicate the high pathogenicity of this fungus to black pepper plants. The rapid necrotic process and cell collapse suggests a powerful action of enzymes and toxins produced by the fungus during pathogenesis. Production of enzymes and toxins by species of *Fusarium* have been reported by many workers (Brian et al, 1961; Garrett, 1970; Aizenberg, 1978; Schippers & Gams, 1979; Baker et al, 1981).

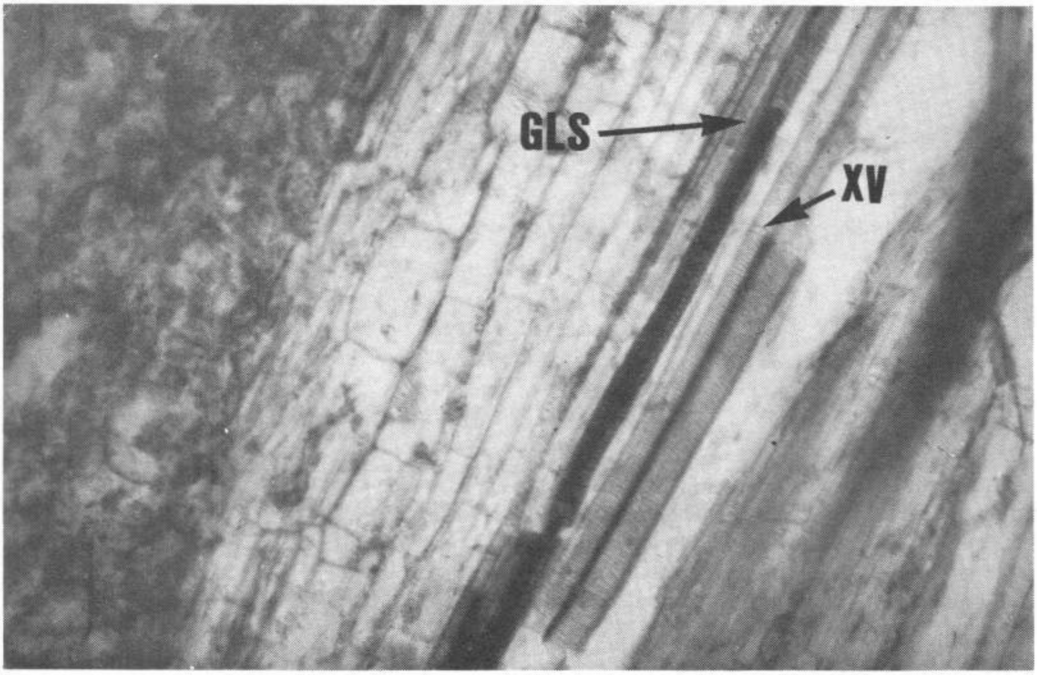

Figure 3. L.S. Stem of black pepper seedling infected by *N. haematococca* f. sp. *piperis.* Note xylem vessel obstructed with a "gum-like substance". XV – xylem vessel; GLS – "gum-like substance".

N. haematococca f. sp. *piperis,* in contrast to wilt fungi, does not establish itself in particular areas of the root, but invades and exploits all types of parenchyma tissues. Occasionally, in cases of heavily infected roots, hyphae were observed within the xylem vessels. The main cause of xylem vessel obstruction, however, was the presence of a "gum-like material" which was never observed in xylem vessels of healthy roots. The mechanism of plant wilt diseases and the factors involved have been discussed by Green (1981).

The histopathological observations from the present study concerning the root invasion and tissue colonization of black pepper roots by *N. haematococca* f. sp. *piperis* corroborate previous reports by Fukutomi et al. (1981a; 1981b) and by many workers for the histopathology of other host plants infected with *Fusarium* spp. (Chatterjee, 1958; Toussoun &Snyder, 1961; Chi et al, 1964; Alconero, 1968; Siddiqui & Halisky, 1968; Abawi & Lorbeer, 1971).

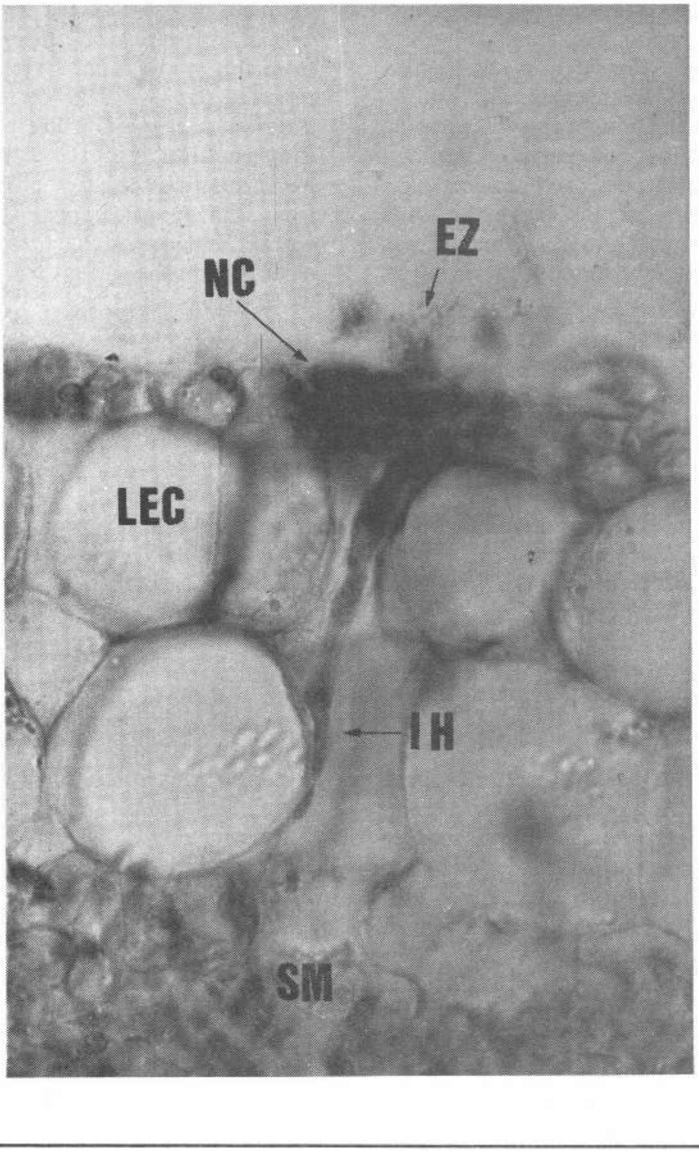

Figure 4. L.S. Infective hyphae of *P. palmivora* between cells of the lower epidermis of black pepper leaf 12 hours after inoculation. IH – infective hypha; EZ – "empty" zoospore. NC – necrotic cells; SM – spongy mesophyll; LEC – lower epidermal cells.

Invasion and colonization of injured root tissues of black pepper by *N. haematococca* f. sp. *piperis*, apart from being more rapidly achieved, was similar to those for uninjured roots. Considering that high numbers of roots are mechanically injured by normal growth through the soil scraping and rubbing against soil particles, also by invasion of nematodes and insects, the direct penetration of black pepper roots by *N. haematococca* f. sp. *piperis* is not probably so important in nature as invasion through wounds.

Although penetration and colonization of uninjured roots of black pepper by *P. palmivora* were quite rapid processes, no appressorium or appressorim-like structures were formed before or during the penetration, suggesting that such an organ is not critical for root invasion by the fungus. Despite the importance of mechanical pressure has been suggested as a necessary part of the process by pathogens to gain entry to roots, more recently the tendency is to accept that enzymatic cell wall hydrolysis and mechanical pressure are combined and simultaneous actions prior to invasion (Tippett et al, 1976).

The efficient tissue maceration caused by *P. palmivora* in roots of black pepper, demonstrates the fungus ability to produce enzymes and toxins capable of destroying and disorganizing plant tissues. Many workers have reported the occurrence of enzymes and toxins in *P. palmivora* and other species of this genus (Ravisé & Trique, 1972; Siew, 1973; Breiman & Barash, 1981; Prasad et al, 1981). The use of toxins of *P. palmivora* in the screening for resistance in black pepper plants against this fungus has been reported by Siew (1973).

In roots of black pepper *P. palmivora* did not show any particular preference for types of tissues. All root tissues were successfully colonized, without an apparent selectivity. Xylem vessels were occasionally invaded and occupied by fungal hyphae. Plugging of xylem vessels with a "gum-like material" was a common feature in roots of black pepper infected with *P. palmivora*. The histopathological observations obtained from this study are in agreement with those reported by Alconero et al. (1972) for invasion and colonization of black pepper roots, cultivar Kal-balancotta, by *P. palmivora*, but haustoria-like projections formed from the fungal hyphae inside the root cortex, as observed by these workers, were never seen in the present study.

The reaction of *P. palmivora* zoospores in injured roots of black pepper directing their germtubes towards the wounds was striking. After invading wounded roots, however, the fungus behaved the same way and colonized root tissues showing the same pattern of infection. Maceration and collapse of injured tissues occurred earlier than for uninjured roots. The preference of *P. palmivora* for wounded tissues of black pepper was also reported by Leather (1967) in Jamaica.

When inoculated simultaneously on uninjured roots of black pepper, *N. haematococca* f. sp. *piperis* and *P. palmivora* invaded and colonized root tissues showing the same pattern of infection as when they were inoculated singly. In simultaneous infection, *P. palmivora* again revealed its marked ability to rapidly invade the root epidermis 6 hours after inoculation. *N. haematococca* f. sp. *piperis* also initiated root invasion at the same time but when it actually reached the outer cortical layers, 12 hours following inoculation, infective hyphae of *P. palmivora* were already well established in the root cortex. Invasion and colonization of root tissues by both fungi were not synchronous, so that each fungus could be detected predominating in different parts of the root system. When they shared the same site for invasion and colonization, then *P. palmivora* was clearly at an advantage as it spread throughout the root tissues more rapidly than *N. haema-*

tococca f. sp. *piperis.* Both fungi, nevertheless, were observed exploiting the same site of the root tissues, indicating that the possible production of any enzymes and toxins by the two fungi do not affect each other. Infective hyphae of *P. palmivora* were more frequently seen that hyphae of *N. haematococca* f. sp. *piperis,* perhaps because *P. palmivora* hyphae usually .appear thicker than those of *N. haematococca* f. sp. *piperis* and therefore are more easily observed within root tissues.

The sole report concerning the possible interaction between *N. haematococca* f. sp. *piperis* and *P. palmivora* on black pepper plants was provided by Albuquerque & Conduru (1971). These workers suggested that *P. palmivora* assists the penetration of *N. haematococca* f. sp. *piperis.* In the present histopathological study, only the simultaneous infection involving these two fungi was investigated, but it seems doubtful that a heavy infection of *P. palmivora* would help *N. haematococca* f. sp. *piperis* to invade and colonized the root tissues. The relationship between these two fungi within black pepper roots did not appear to be antagonistic, at least during the period of histopathological observations. It seems that it is more a question of which fungus invades first and establishes itself in the appropriate place. Absence of apparent antagonistic effects between *N. haematococca* f. sp. *piperis* and *P. palmivora* in black pepper roots was rather different compared with their growth on culture media. Although the initial contact between the two colonies on either media was apparently nromal, signs of an antagonistic relationship appeared during later stages. After covering completely the *N. haematococca* f. sp. *piperis* colony on V-8 juice agar, *P. palmivora* showed an increase in production of sporangia at the area of contact between the two colonies. On peptone-potato dextrose agar, where *N. haematococca* f. sp. *piperis* inhibited slightly the growth of *P. palmivora,* the colour and the thickness of *P. palmivora* mycelium changed markedly. These alterations, according to Porter (1924), are typical examples of antagonistic effects. In spite of these aspects being apparent *in vitro* they were not detected *in vivo,* probably because the inhibitory or defense mechanism of the black pepper plants influenced the antagonistic balance between *N. haematococca* f. sp. *piperis* and *P. palmivora.* Other physiological processes or the substrate itself may also have caused some dilution or breakdown of any possible toxic substance produced by the fungi so that antagonistic effects were not detected during the period in which the histological sections were prepared.

Perry (1959), studying the simultaneous infection of *Fusarium solani* f. *pisi* and *Fusarium oxysporum* f. *pisi* on pea roots, did not find any inhibition effect between the two fungi on artificial media, in the soil, or in the rhizosphere, but noticed a marked inhibition of *F. oxysporum* f. *pisi* by *F. solani* f. *pisi* within the host tissues. He concluded that the infection and collapse of root cortex by *F. solani* f. *pisi* acted antagonistically on *F. oxysporum* f. *pisi* which has to pass through the cortex to reach the vascular tissues. *N. haematococca* f. sp. *piperis* and *P. palmivora* differ from the above two fungi in having habit of destroying all root tissues without any selectivity for colonization sites, which makes the competition between them more difficult to assess during pathogenesis. The destructive manner of tissue colonization exhibited by both fungi, apparently involving the production of powerful toxins and enzymes, seems to characterize typical macerative and/or toxicogenic* actions.

The simultaneous presence of *P. palmivora, F. solani* and other fungi in roots of black pepper, under natural conditions, has been reported by Holliday & Mowatt (1963) and Leather (1967). Fukutomi et al. (1981c) also reported the simultaneous presence of *F. solani* f. sp. *piperis* and *Colletotrichum* sp. in roots of black pepper growing under natural conditions in the Brazilian Amazonian region. Other simultaneous infections involving species of *Fusarium* with different species of fungi in roots of host plants have been reported by various workers (Kehr et al, 1962; Kerr, 1963; Escobar et al, 1967; Burke et al, 1969; Pierre & Wilkinson, 1970; Warren & Kommedahl, 1973; Kraft, 1978; Pieczarka & Abawi, 1978; Krupa & Dommergues, 1979; Sippell & Hall, 1982).

Interaction of *N. hametococca* f. sp. *piperis* and *P. palmivora* in artificially injured roots of black pepper was similar to the interaction of these two fungi in uninjured roots. The only difference was that injured root were always invaded and colonized more rapidly compared with uninjured ones. Plugging of xylem vessels with a "gum-like substance" and tissue necrosis and maceration were also more striking in roots colonized by the two pathogens than when each fungus was inoculated alone.

Stem infection

The inability of *N. haematococca* f. sp. *piperis* to penetrate uninjured stems of black pepper seedlings at the internodal region seems to be related to physical or chemical barriers, such as thicker cuticle and waxes. In many fungi, such obstacles stimulate spores to form appressorium or appressorium-like structures to overcome these barriers (Emmett & Parbery, 1975). *N. haematococca* f. sp. *piperis* did not form any visible mechanical structure, but only normal germtubes. Sometimes the fungus invaded through the basal area of the internodal region but how the conidia penetrated was not seen.

Fungal infection in these cases progressed rather slowly and with visible symptoms only at the basal point of the internodes. Infection of basal areas of black pepper stems by *N. haematococca* f. sp. *piperis* was reported by Albuquerque et al. (1976). They suggested that these areas of the stem are the most suitable for fungal invasion. Infection of joints of black pepper stems by *N. haematococca* f. sp. *piperis* may be related to the presence of intercalary meristems. In young stems of black pepper these meristematics tissues are responsible for production of new leaves and branches, although this ability is lost in mature stems. The possible presence of thinner cuticle and the absence of wax in these areas may help conidia to invade and colonize stem tissues. Presence of intercalary meristems is well know in monocotyledon particularly grasses (Esau, 1965).

The formation of swellings at the end of apparently normal germtubes of conidia of *N. haematococca* f. sp. *piperis* was occcasional. These swellings cannot be defined as mechanical structures for penetration as their formation was erratic and usually they were never formed before penetration of roots and leaves. Moreover, in many other histological sections, conidia were seen penetrating injured stems without forming any hyphal swelling.

In several cases, when *N. haematococca* f. sp. *piperis* invaded injured stems of black pepper, infective hyphae remained colonizing tissues in some areas of the stem while other parts were totally free of infection. In such situations, external symptoms were not visible for the 10 day period of histopathological observations. This seems to corroborate the situation suggested by Albuquerque & Conduru (1971), that *N. haematococca* f. sp. *piperis* can remain undetected inside stem cutting tissues, in

* The term macerative pathogen is used in this work to define a fungus which utilizes enzymes to destroy the host tissues. Toxicogenic pathogen, on the other hand, is a fungus which kills the host tissues by toxins in advance of invasion by hyphae (Krupa & Dommergues, 1979).

the form of hyphal segments, making possible its dissemination to new areas as stem cuttings are the only profitable way to propagate black pepper in new plantations.

In stems heavily infected with *N. haematococca* f. sp. *piperis*, clogging of xylem vessels was always observed in histological sections, suggesting that obstruction of vascular elements with a "gum-like material" is the main reason for the wilt symptoms in diseased plants. Fukutomi et al. (1981a) also observed xylem vessels of black pepper stems completely blockaded with a gelatinous cementing material. They concluded that wilt symptoms showed by such infected plants were caused by the obstruction of xylem vessels which hindered the normal suply of water and nutrients. Wood (1967) and Garrett (1970) suggested that vascular obstruction by gels was caused by fungal degradation of xylem cell walls by enzymes from the pathogens, chiefly pectinases. Other possible explanations for vascular plugging have also been discussed by these authors.

The possible presence of physical and chemical barriers, such as thicker cuticle and waxes, seemed to have prevented *P. palmivora* zoospores from penetrating uninjured surface of black pepper stems. A few zoospores germinated and were observed trying to penetrate but were raised from the stem surface. A similar phenomenon was reported by Marks & Mitchell (1971) during invasion of *P. megasperma* zoospores in roots of alfalfa. These workers suggested that the zoospores lost adhesion before the germtubes had entered the root, or the germtubes were distorted or deflected at the onset of penetration. No appressorium or appressorim-like structures were formed by zoospores of *P. palmivora*. Infection at the basal part of internodes did not occur.

On injured stems, zoospores of *P. palmivora* presented a striking response germinating and directing uniformly their germtubes towards wounds. The attraction exerted by the injuries upon the zoospores seems to have been caused by substances released from the wounds. There are now evidences showing that zoospores can detect compounds diffusing from their hosts. Bimpong & Clerk (1970) demonstrated chemotaxis in zoospores of *P. palmivora* to extract of cocoa pod. Other workers have reported chemotaxis in zoospores of different species of *Phytophthora* (Goode, 1956; Dukes & Apple, 1961; Zentmyer, 1961; Turner, 1963; Ho & Hickman, 1967).

An interesting aspect during the penetration of injured stems of black pepper by *P. palmivora* was the occasional formation of appressoria by some zoospores. Such appressoria were not formed to penetrate through the wounds because they occurred on the intact stem surface near the wounds. As formation of appressoria was never observed during inoculation of *P. palmivora* zoospores on uninjured stems of black pepper, it is likely that their formation in this particular situation was influenced by the presence of a hard surface together with the release of a chemical stimulus from the wounds. Formation of appressoria by plant pathogenic fungi has been discussed by Emmett & Parbery (1975).

Xylem vessels of black pepper stems heavily infected with *P. palmivora* always appeared plugged with a "gum-like material". Powers (1954), studying the mechanism of wilting in tobacco plants infected with *P. parasitica* var. *nicotianae*, concluded that obstruction of xylem vessels of stems with a "gum-like substance" was the main cause for the wilting symptoms. He also suggested that the material plugging xylem vessels is a product of decomposition of host cells invaded by the pathogen. Clogging of xylem vessels in stems of black pepper infected with *P. palmivora* seems to be caused by the same mechanism.

Leaf infection

The successful penetration by conidia of *N. haematococca* f. sp. *piperis* on both surfaces of uninjured young leaves of black pepper contrasts with their inability to invade uninjured stems of black pepper seedlings in the internodal region. It is likely that young black pepper leaves do not possess strong physical or chemical barriers which may be present on the stems preventing conidia from penetrating. The slight preference exhibited by conidia of *N. haematococca* f. sp. *piperis* to penetrate at the base of glandular structures (probably glandular hairs) on both surfaces of the leaves, suggests that, in these particular areas, foliar cuticle is thinner than elsewhere or absent so that penetration is an easier process. Also, the bases of such structures could be points of exudation for any plant substance, which could act as a chemical stimulus for the fungal germtubes.

On injured leaves invasion was a more rapid process, with several conidia germinating towards the same wound, indicating a positive response to some substances released from the wounds. As conidia of *N. haematococca* f. sp. *piperis* are non-motile structures, they sometimes formed long germtubes directed towards the source of stimulus. After invading wounded leaves of black pepper, infective hyphae spread rapidly, colonizing all foliar tissues. Forty-eight hours following inoculation a marked necrosis was visible on the infected leaves. Necrosis of injured leaves of black pepper, caused by inoculation with *N. haematococca* f. sp. *piperis,* was reported by Albuquerque & Ferraz (1976).

Following colonization and collapse of vascular parenchyma, due to invasion with *N. haematococca* f. sp. *piperis,* foliar infection led to the occlusion of xylem vessels by a "gum-like material". Similar observations were reported by Stuehling & Nelson (1981) for leaves of chrysanthemum infected wth *F. oxysporum* f. sp. *chrysanthemi.* These workers suggested that vascular plugging was caused by the accumulation of large amounts of cell by-products. Cell destruction and foliar tissue maceration caused by *N. haematococca* f. sp. *piperis* resemble those in the roots and stems of black pepper seedlings.

The extremely rapid infection process of *P. palmivora* zoospores on black pepper leaves may be related to the age of the leaves. Only young leaves were inoculated and fungal invasion and colonization were always successful. Probably the pathogenesis in older leaves would be slower. Indeed, Holliday & Mowatt (1963) reported that mature leaves of nine black pepper cultivars presented higher resistance to *P. palmivora* than young ones; this resistance being in part broken down by wounding. Leather (1967) also found that young leaves of black pepper were more easily infected with *P. palmivora* than older leaves. Turner (1969) reported more susceptibility in yound leaves of black pepper to *P. palmivora,* compared to mature leaves. The same worker observed that more lesions developed in the lower surface of the leaves than on the upper surface. The greater susceptibility of young black pepper leaves to *P. palmivora* suggests the presence of a much thinner cuticle, compared to older leaves, so that the germtubes of *P. palmivora* zoospores can penetrate young leaves more readily. As foliar cuticle seems to be thinner on the lower surface of black pepper leaves than on the upper surface, this could explain the more successful penetration by germtubes of *P. palmivora* zoospores through the lower surface.

Penetration and colonization of artificially injured leaves were even easier than on uninjured leaves, as the zoospores showed a positive chemotaxis towards the wounds, penetrating leaves efficiently 6 hours after inoculation. All foliar tissues collapsed earlier, compared to uninjured foliar tissues. "Gum-like material" obstructing xylem vessels in midvein and lateral veins was clearly visible 48 hours following inoculation. It is likely that vascular plugging is also responsible for the flaccidness of leaves of black pepper infected with *P. palmivora*.

LITERATURE CITED

ABAWI, G.S. & LORBEER, J.W. Pathological histology of four onion cultivars infected by *Fusarium oxysporum* f. sp. cepae. Phytopathology 61: 1164-1169. 1971.

AIZENBERG, V.L. Pectin hydrolysis by *Fusarium* Link species. Mikrobiologicheskii Zhurnal 40: 582-589. 1978.

ALBUQUERQUE, F.C. Pimenta-do-reino. **In** "Livro anual de Agricultura" (Ministério da Agricultura, Brasília), p. 215-223. 1968.

ALBUQUERQUE, F.C. & CONDURU, J.M.P. Cultura da pimenta-do-reino na região Amazonica. Belém, IPEAN. Série Fitotecnica, Vol. 2, 149 pp. 1971.

ALBUQUERQUE, F.C. & FERRAZ, S. Caracteristicas morfologicas e fisiologicas de *Nectria haematococca* f. sp. *piperis* e sua patogenicidade a pimenta-do-reino (*Piper nigrum* L.). Experientae 22: 133-151. 1976.

ALBUQUERQUE, F.C.; FERRAZ, S. & SEDIYMA, C.S. Influência da tecnica de inoculação e da concentracao de esporos na patogenicidade de *Nectria haematococca* f. sp. *piperis* sobre pimenta-do-reino. Experientae 22: 165-174. 1976.

ALCONERO, R. Infection and development of *Fusarium oxysporum* f. sp. *vanillae* in vanilla roots. Phytopathology 58: 1281-1283. 1968.

ALCONERO, R.; ALBUQUERQUE, F.C.; ALMEYDA, N. & SANTIAGO, A.G. Phytophthora foot rot of black pepper in Brazil and Puerto Rico. Phytopathology 62: 144-148. 1972.

BAKER, R.A.; TATUM, J.H. & NEMEC, S. Jr. Toxin production by *Fusarium solani* from fibrous roots of blight-diseased citrus. Phytopathology 71: 951-954. 1981.

BIMPONG, C.E. & CLERK, G.C. Motility and chemotaxis in zoospores of *Phytophthora palmivora* Butl. Annals of Botany 34: 617-624. 1970.

BREIMAN, A. & BARASH, I. Partial characterization of phytotoxic compounds in culture filtrates of *Phytophthora citrophthora*. Phytopathologische Zeitschrift 122: 1-9. 1981.

BRIAN, P.W.; DAWKINS, A.W.; GROVE, J.F.; HEMMING, H.G.; LOWE, D. & NORRIS, G.L.F. Phytotoxic compounds produced by *Fusarium equiseti*. Journal of Experimental Botany 12: 1-12. 1961.

BURKE, D.W.; HAGEDORN, D.J. & MITCHELL, J.E. *Aphanomyces* and *Fusarium* root rot of peas with partial vs. total exposure of roots to infested soil. Phytopathology 59: 1261-1266. 1969.

CHATTERJEE, P. The bean root rot complex of Idaho. Phytopathology 48: 197-200. 1958.

CHI, C.C.; CHILDERS, W.R. & HANSON, E. J. Penetration and subsequent development of three Fusarium species in alfalfa and red clover. Phytopathology 54: 434-437. 1964.

CONN, H.J. Biological Stains, W.F. Humphrey Press Inc., Geneva and New York, 276 pp. 1936.

DUKES, P.D. & APPLE, J.L. Chemotaxis of zoospores of *Phytophthora parasitica* var. *nicotianae* by plant roots and certain chemical solutions. Phytopathology 3: 195-197. 1961.

ESAU, K. Plant Anatomy. John Willey & Sons, Inc., London, U.K., 767 pp. 1965.

ESCOBAR, C.; BEUTE, M.K. & LOCKWOOD, J.L. Possible importance of *Pythium* in root rot of peas. Phytopathology 57: 1149-1151. 1967.

EMMETT, R.W. & PARBERY, D.G. Appressoria. Annual Review of Phytopathology 13: 147-167. 1975.

FREIRE, F.C.O. & SANTOS, A.V.P. Histopathology of the roots of pepper (*Piper nigrum* L.) parasitized by *Meloidogyne incognita* and *Fusarium solani* f. sp. *piperis.* Resumos dos trabalhos cienticos e conferenciais, III Reunião Brasileira de Nematologia, Sociedade Brasileira de Nematologia, Escola Superior de Agricultura, Mossoro, 1978. Mossoro, R.N. Colecao Mossoroense Vol. 62, 79. 1978.

FUKUTOMI, M.; HIRAKATA, K. & HAMADA, M. Studies on the stem rot and root diseases of black pepper: (4) Anatomical observations on the distribution of the hyphae of the pathogenic fungus in invading tissues and the blockade of vessels cells. XIV Congresso da Sociedade Brasileira de Fitopatologia, Porto Alegre, 1981. Programas e Resumos, p. 139. 1981.

FUKUTOMI, M.; HIRAKATA, K. &HAMADA, M. Electron microscope studies on the infection of stem and root rot diseases caused by *Fusarium solani* in black-pepper . VIII Coloquio da Sociedade Brasileira de Microscopia Eletronica, Rio de Janeiro, 1981. Resumos, p. 46. 1981.

FUKUTOMI, M.; HIRAKATA, K. & HAMADA, H. Studies on the stem rot and root rot disease of black-peppers: (2) Isolation of the pathogenic fungus with five selective isolation media and pathogenicity of isolates to stems and roots. XIV Congresso da Sociedade Brasileira de Fitopatologia, Porto Alegre, 1981. Programas e Resumos, p. 148. 1981.

GARRETT, S.D. Pathogenic Root-Infecting Fungi. Cambridge University Press, 294 pp. 1970.

GOODE, P.M. Infection of strawberry roots by zoospores of *Phytophthora fragariae.* Transactions of the British Mycological Society 39: 367-377. 1956.

GREEN, R.J. Jr. An overview. In "Fungal Wilt Diseases of Plants" (Mace, M.M., Bell, A.A. and Beckman, C.H., Eds.), p. 1-24. Academic Press, London, U.K. 1981.

HO, H.H. & KICKMAN, C.J. Factors governing zoospore responses of *Phytophthora menasperma* var. sojae to plant roots. Canadian Journal of Botany 45: 1983-1994. 1967.

HOLLIDAY, P. & MOWATT, W.P. Foot rot of *Piper nigrum (Phytophthora palmivora).* Commonwealth Mycological Institute, Kew, England, Phytopathological Paper No. 5, 62 pp. 1963.

HOMMA, A.K.O. Oferta e demanda de pimenta-do-reino a nivel mundial; perspectivas para o Brasil. Belém, EMBRAPA/CPATU, 29 pp. 1981.

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

KEHR, A.E.; O'BRIEN, M.J. & DAVIS, E.W. Pathogenicity of *Fusarium oxysporum* f. sp. *cepae and its interaction* with *Phyrenochaeta terrestris* on onion. Euphytica 11: 197-208. 1962.

KERR, A. The root rot – *Fusarium* wilt complex of peas. Australian Journal of Biological Science 16: 55-69. 1963.

KRAFT, J.M. Effects of root rot pathogens on *Fusarium* wilt of peas. Plant Disease Reporter 62: 216-221. 1978.

KRUPA, S.V. & DOMMERGUES, Y.R. Ecology of Root Pathogens. Elsevier Scientific Publishing Company, Amsterdam, 281 pp. 1979.

LEATHER, R.I. The occurrence of a *Phytophthora* root and leaf disease of black pepper in Jamaica. F.A.O. Plant Protection Bulletin 15: 15-16. 1967.

MARKS, G.C. & MITCHELL, J.E. Penetration and infection of alfalfa roots by *Phytophthora megasperma* and the pathological anatomy of infected roots. Canadian Journal of Botany 49: 63-67. 1971.

PERRY, D.A. Studies on the mechanism underlying the reduction of pea wilt by *Fusarium solani* f. *pisi.* Transactions of the British Mycological Society 42: 388-396. 1959.

PIECZARKA, D.J. & ABAWI, G.S. Effect of interaction between *Fusarium*, *Pythium* and *Rhizoctonia* on the severity of bean root rot. Phytopathology 68: 403-408. 1978.

PIERRE, R.E. & WILKINSON, R.E. Histopathological relationship of *Fusarium* and *Thielaviopsis* with bean. Phytopathology 60: 821-824. 1970.

PORTER, C.L. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. American Journal of Botany 11: 168-188. 1924.

POWERS, H.R. Jr. The mechanism of wilting in tobacco plants affected by black shank. Phytopathology 44: 513-521. 1954.

PRADAD, G.; MEHROTRA, R.S. & SRIVASTAVA, S.N. Influence of temperature on production and activity of pectolytic and cellulotytic enzymes by *Phytophthora parasitica* var. *nicotianae in vitro*. Phytophthora Newsletter No. 9, 32-33. 1981.

RAVISÉ, A. & TRIQUE, B. Determination des enzymes pectinolytiques de deux souches *Phytophthora* de Bary. Variations d'activite dans les tissus des plantules de tomate en relation avec les genomes de resistance. Agronomie Tropicale 27: 751-762. 1972.

SCHIPPERS, B. & GAMS, W. Soil-borne Plant Pathogens. Academic Press, London, U.K., 686 pp. 1979.

SIDDIQUI, W.M. & HALISKY, P.M. Histopathological studies of red clover roots infected by *Fusarium roseum.* Phytopathology 58: 874-875. 1968.

SIEW, L.B. The use of toxin for the screening of black pepper for root rot resistance. MARDI Research Bulletin 1: 10-14. 1973.

SIPPELL, D.W. & HALL, R. Effects of *Fusarium solani phaseoli, Pythium ultimum* and *F. oxysporum* on yield components of white bean. Canadian Journal of Plant Pathology 4: 54-58. 1982.

STUEHLING, B.A. & NELSON, P.E. Histopathology of the apical leaves of a susceptible chrysanthemum cultivar infected with *Fusarium oxysporum* f. sp. *chrysanthemi.* Phytopathology 71: 1152-1155. 1981.

TIPPETT, J.T.; HOLLAND, A.A.; MARKS, G.C. & O'BRIEN, T.P. Penetration of *Phytophthora cinnamomi* into disease tolerant and susceptible eucalypts. Archives of Microbiology 108: 231-242. 1976.

TOUSSOUN, T.A. & SNYDER, W.C. Germination of chlamydospores of *Fusarium solani* f. *phaseoli* in unsterilized soils. Phytopathology 51: 620-623. 1961.

TURNER, P.D. Influence of root exudate of cococa and other plants on spore development of *Phytophthora palmivora.* Phytopathology 53: 1337-1339. 1963.

TURNER, G.J. Leaf lesions associated with foot rot of *Piper nigrum* and *P. betle* caused by *Phytophthora palmivora.* Transactions of the British Mycological Society 53: 407-415. 1969.

WARREN, H.L. & KOMMEDAHL, T. Prevalence and pathogenicity to corn of *Fusarium* species from corn roots, rhizosphere, residues and soil. Phytopathology 63: 1288-1290. 1973.

WOOD, R.K.S. Physiological Plant Pathology. Blackwell Scientific Publications, Oxford and Edinburgh, 570 pp. 1967.

ZENTMYER, G.A. Chemotaxis of zoospores for root exudates. Science 133: 1595-1596. 1961.