HISTOPATHOLOGICAL STUDIES ON COLONIZATION OF OIL PALM ROOT BY *Ganoderma boninense*

Histopathological investigations on roots naturally diseased by infection with Ganoderma boninense *suggest that the fungus is a vascular pathogen. Infection of the xylem vessels preceded the appearance of the pathogen in surrounding cells. The pathogen caused severe plugging of the vessels, which may account for the symptoms of impairment of water uptake in infected palms. No evidence of a host reaction by formation of tyloses to restrict fungal movement was observed in infected vessels. The pathogen was not tissue specific and was present in all tissue types at the advanced stage of pathogenesis.*

Keywords: *Ganoderma boninense*, Basal stem rot, Histology

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INTRODUCTION

The most serious disease of oil palm in Malaysia is the basal stem rot caused by species of *Ganoderma*. The disease has also been reported in Indonesia, Ghana, Zaire, Nigeria, Cameroon, Angola, Sàn Tomé and Principe, Tanzania and Rhodesia (Turner, 1981), and recently in Honduras (Chincilla and Richardson, 1987). Studies by Ho and Nawawi (1985) established that *Ganoderma boninense* is the causal organism responsible for the disease in Malaysia.

The occurrence, symptomatology, aetiology and economic importance of the disease have been described by Turner (1981). The disease is characterized by the appearance of multiple spear leaves and the presence of fruiting bodies of the pathogen on the stem base close to the soil level. The fungus spreads within

infected roots up into the trunk. Once inside the trunk it grows radially, and eventually produces numerous fruiting bodies (Turner, 1966). Information on the host-pathogen interaction at the cellular level is lacking; hence this study was initiated to determine the mode of colonization of oil palm root by *Ganoderma boninense*.

MATERIALS AND METHODS

The root samples for this study were collected from twenty-year old palms naturally infected with *Ganoderma* growing in peat soil at Jalan Kebun, Klang, Malaysia. On severely infected palms the presence of numerous fruiting bodies of *Ganoderma* was evident at the palm bases. Roots were collected from such palms on the same side as the fruiting bodies were growing. Portions of root samples were transferred to the *Fomes annosus* selective medium of Kuhlman and Hendrix (1962) with the following modifications: 300 ppm streptomycin, 100 ppm chloramphenicol, 136 ppm ridomil and 285 ppm PCNB. Cultures of a basidiomycete with clear evidence of clamp connections and identical to the pure culture of *G. boninense* were consistently isolated from diseased roots and stems on this medium.

Samples were also prepared for histological staining following the method described by *Johansen* (1940) and sectioned at 12-20 µm using Leitz 1512 rotary microtome. Sections were placed on clean glass slides with several drops of Haupt's adhesive and 4% formalin, dried at 38°C – 40°C, then stained with Johansen Quadruple Stain (1940). Stained sections were mounted on DPX.

RESULTS

The anatomy of roots from healthy palms was as described by Purvis (1956) and Ho *et al.* (1985).

In roots infected with *Ganoderma* there was disintegration of thin-walled ground parenchyma cells inside the endodermis. Later, these cells were completely disintegrated, leading to the formation of cavities (*Figure 1*). This situation eventually resulted in complete

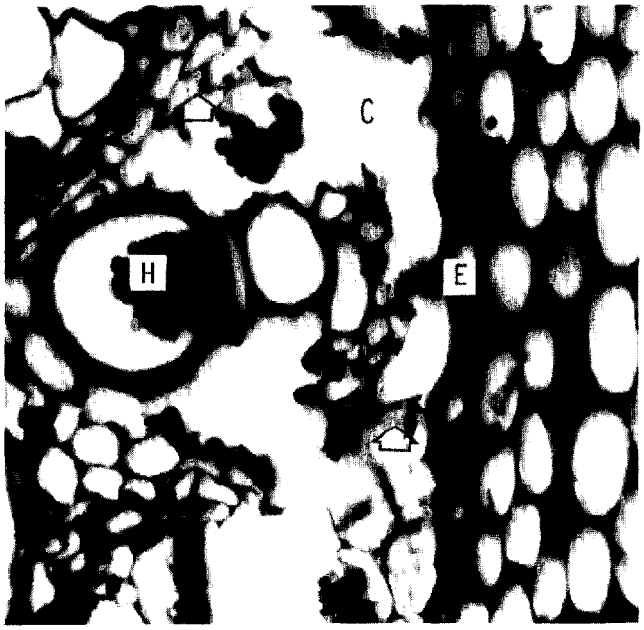

Figure 1. Transverse section of an infected root showing cavities (C) formed by disintegration of ground parenchyma and pericycle cells (arrows), hypae (H) in vessel element, and intact endodermal cells (E) (X372).

Figure 2. Transverse section of infected root showing hyphae (arrows) in pericycle cells (P) and endodermis (E). There was a complete dissolution of ground parenchyma cells leading to the formation of cavities (C) (X930).

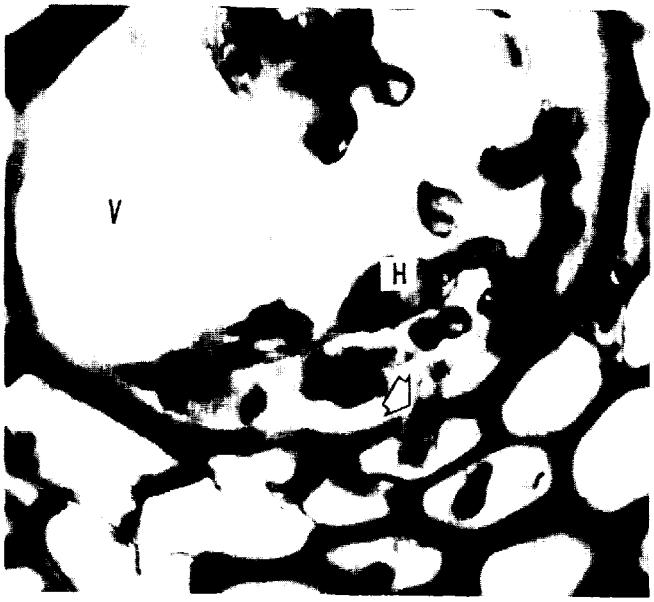

Figure 3. Transverse section of infected root showing hyphae (H) in xylem vessel (V). Hyphae were observed penetrating between the vessel wall and surrounding cells (arrow) (X372).

Figure 4. Transverse section of infected root showing hyphae (arrow) in inner pith region (X372).

Figure 5. Longitudinal section of infected root showing plugging of the xylem vessel with the fungal hyphae (H), and movement of the hyphae into adjacent vessel elements (arrows) (X930).

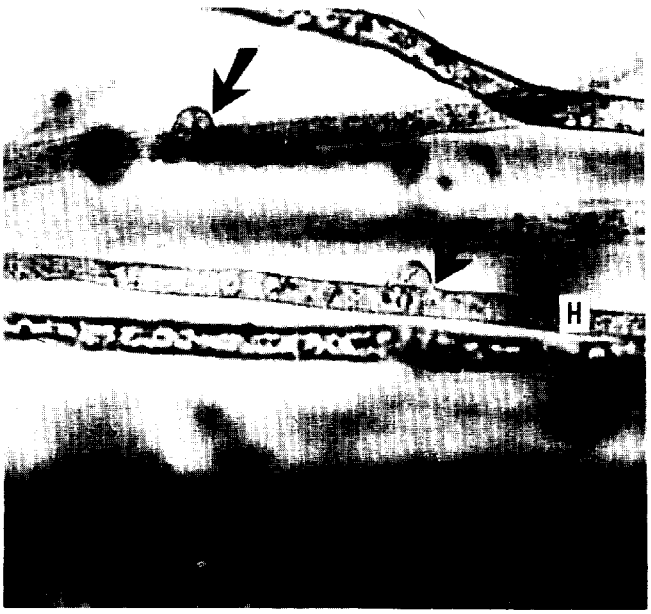

Figure 6. Longitudinal section of infected root showing hyphae (H) with clamp connections (arrows) in xylem vessel (X930).

detachment of the stele from the cortex at the endodermis. Most of the cells of the pericycle, immediately adjacent to the endodermis, had also disintegrated, leaving the endodermis intact and acting as a barrier to restrict further movement of the fungus into the cortical region (*Figure 1*). In those few pericycle cells that remained, the walls had begun to disintegrate and fungal hyphae could be observed growing inside and at the same time penetrating into contiguous cells (*Figure 2*). The endodermis, which is highly lignified, remained intact when the rest of the adjacent thin-walled parenchyma cells had already disintegrated. At a later stage, the fungus eventually penetrated the endodermis at some points (*Figure 2*) and from there made its way into the inner cortex.

Fungal hyphae were also seen in the xylem elements. Infection of the main vessel revealed profuse fungal growth with deposition of dark staining materials (*Figure 3*). The wall of the vessel did not stain with normal intensity and there were breaks along the wall. Fungal hyphae were observed penetrating the cell wall into the adjacent sclerenchyma cells surrounding the vessels. There was collapse and complete dissolution of some of the walls of infected sclerenchyma cells. From here the pathogen made further ingress into the inner pith region where the fungal hyphae grew both intra- and intercellularly (*Figure 4*). In some of the infected xylem, complete plugging of the vessel occurred because of abundant hyphal growth (*Figure 5*). There was no evidence of the formation of tyloses by the host to restrict fungal movement within the infected vessels. Further spread into adjacent xylem elements took place through pit pairs. Clear evidence of the presence of clamp connections on the fungal hyphae was seen in some of the infected vessels (*Figure 6*). Much as in the xylem, the phloem and the cells surrounding it were also infected (*Figure 7*). Fungal hyphae and deposition of dark staining materials caused occlusion of some of the phloem cells.

DISCUSSION

The symptoms of palms infected with *Ganoderma* in the field are reminiscent of those caused by impairment of water uptake

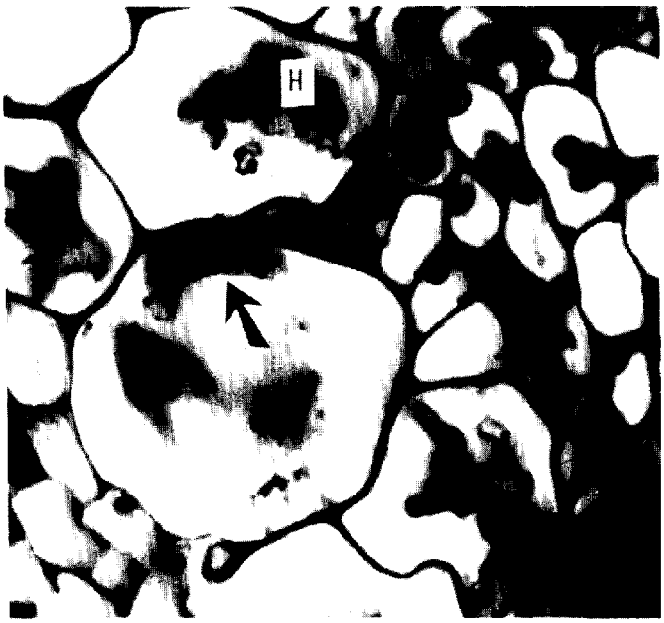

Figure 7. Transverse section of infected root showing fungal hyphae (H) and other deposits (arrows) in phloem (X930).

(Turner, 1981). The present study suggests that the basal stem rot of oil palm is a vascular disease: infection of healthy tissues by *Ganoderma* seemed to have occurred through movement of the pathogen within infected phloem and xylem vessels. This conclusion is based on observation of samples taken from regions of root at the initial stage of infection which showed profuse hyphal growth within the vessel elements with little growth observed elsewhere. Only after the vessels were substantially colonized, with evidence of cell-wall deterioration, were hyphae observed to penetrate the walls and begin the process of infection of surrounding cells. Once outside the vessels, the fungus caused rapid dissolution of the thin-walled ground parenchyma cells, only to be restricted in growth on contact with the highly lignified endodermal layer. That too eventually gave way to fungal penetration and thus exposed the cortical region to fungal attack. While the damage to the ground parenchyma cells was severe the attack on the pith cells was not very rapid. Though fungal hyphae were also observed in the pith, cell collapse and overall damage to this region were somewhat restricted by the thick-walled sclerenchyma cells.

This study was conducted on a naturally diseased palm in the field because of the constant failure to induce disease on nursery seedlings artificially. Nevertheless, investigations are continuing using various techniques of artificial inoculation with the hope of obtaining a better understanding of the process of infection of oil palm by *Ganoderma*.

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REFERENCES

CHINCHILLA C and RICHARDSON DL (1987). Four potentially destructive diseases of the oil palm in Central America. *Proc. of 1987 Int. O.P/P.O Conf – Agriculture*, Kuala Lumpur, Palm Oil Research Institute of Malaysia. 468 – 470.

HO YW and NAWAWI A (1985). *Ganoderma boninense* Pat. from basal stem rot of oil palm (*Elaeis guineensis*) in Peninsular Malaysia. *Pertanika, 8*, 425 – 428.

JOHANSEN DA (1940). *Plant Microtechnique*. McGraw Hill, New York, 523 pp.

KUHLMAN EG and HENDRIX Jr. FF (1962). A selective medium for the isolation of *Fomes annosus*. *Phytopath., 52*, 1310 – 1311.

PURVIS C (1956). The root system of the oil palm: its distribution, morphology and anatomy. *J W Afric. Inst. Oil Palm Res., 1*, 61 – 82.

TURNER PD (1966). Infection of oil palm by *Ganoderma* in Malaya. *Oléagineux, 21*, 73 – 76.

TURNER PD (1981). Oil Palm Diseases and Disorders. Incorporated Society of Planters, Kuala Lumpur, 280 pp.