SIGNIFICANCE OF THE BLACK LINE WITHIN OIL PALM TISSUE DECAYED BY *GANODERMA BONINENSE*

Keywords: Black line;*Ganoderma boninense*;Fungal;Mycelium

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A histological study was conducted on the black line found in stem tissue of oil palm infected with Ganoderma. *The line was of fungal origin and formed by the action of a single mycelium. Deeply embedded within the line were masses of swollen hyphal cells which form resting structures. These structures might be critical for the prolonged survival of the pathogen within infected oil palm tissue buried in soil.*

INTRODUCTION

The presence of narrow black lines in wood decayed by wood-rotting fungi has been reported by several authors (Campbell, 1933 and 1934; Lopez-Real, 1975). A list compiled by Lopez-Real (in Lopez-Real, 1975) indicates that 44 species of basidiomycetes and 10 species of ascomycetes have been reported to form these lines and several factors have been said to be responsible. Campbell (1933) cited the following: (1) the antagonism of the mycelia of two different fungi occupying the same substratum, (2) the action of a single mycelium of certain wood-rotting fungi, and (3) the production of wound gum stimulated by (a) natural wounding, (b) parasitic invasion by fungi or bacteria, and (c) desiccation.

The terminology used to describe these black lines varies in reports from different authors. The term 'zone lines' has frequently been used (Campbell, 1933 and 1934) but it does not take into consideration the specific factor responsible for the formation of the lines. To avoid this confusion Lopez-Real (1975) suggested that the term 'pseudosclerotia' be used to emphasize specifically the basic fungal origin of the black lines. His laboratory investigations on the formation of these lines by *Armillaria mellea* and *Stereum hirsutum* revealed that with both fungi the characteris-

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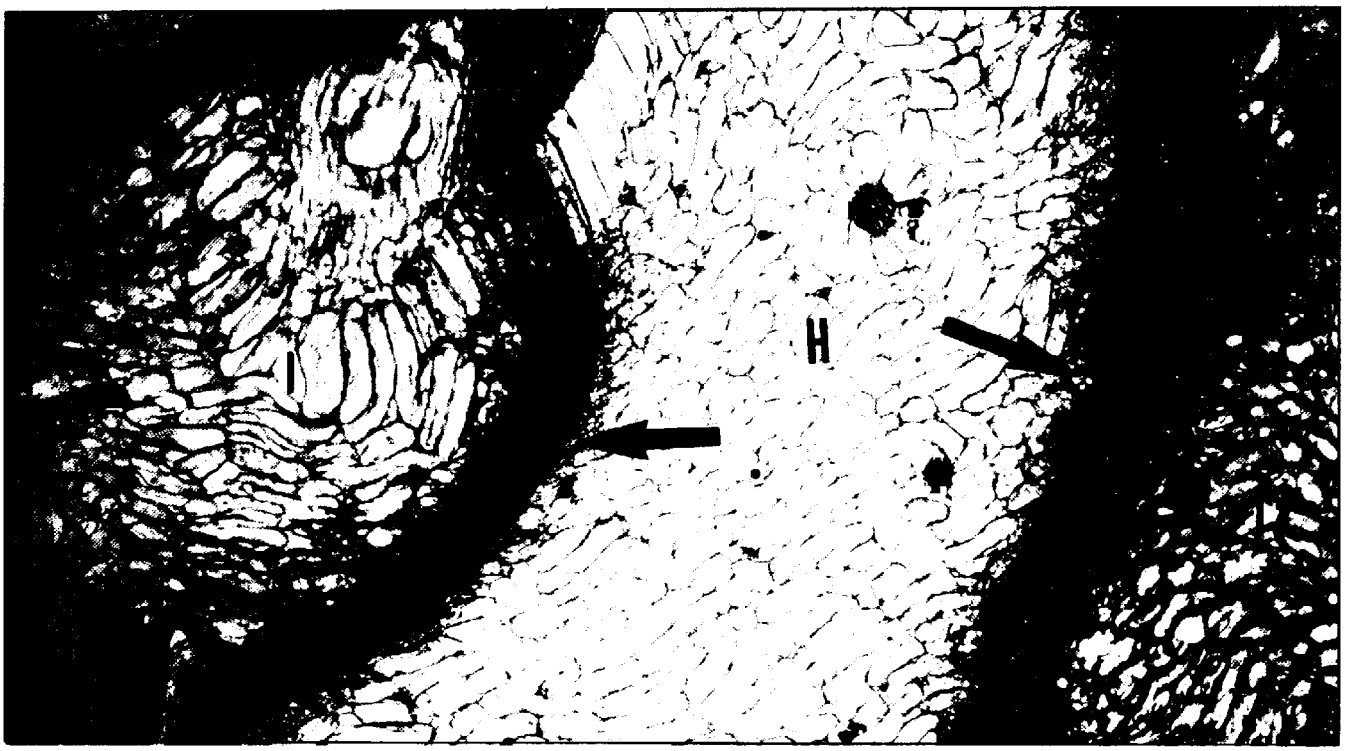

Figure 1.

Transverse section of infected stem showing the black line (arrows) separating the healthy (H) from infected (I) tissues (X93)

Figure 2.

Transverse section of infected stem showing the normal fungal hyphae (F) with clamp connections (arrows) in cells away from the black line (X930)

tic stages involved are: (1) hyphal proliferation, (2) hyphal swelling and aggregation, and (3) pigmentation.

In oil palm stem tissue decayed by *Ganoderma boninense* the presence of the black lines is evident. This study was initiated to investigate the origin of these lines and to explore their possible significance in disease development and spread.

MATERIALS AND METHODS

Samples for this study were collected from twenty-year old palms naturally infected with *Ganoderma* and growing in peat soil at Jalan Kebun, Klang. Stem tissues containing the black lines were sampled immediately behind the fruiting bodies of *Ganoderma* and cut into cubes measuring approximately 8 x 8 x 10 mm. These cubes were transferred to the selective medium for *Fomes annosus* 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 with the pure culture of *Ganoderma* were consistently isolated from diseased stem with this medium.

Samples were prepared for histological staining following the method described by Johansen (1940) and sectioned at 12 - 20 µm using a 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 either Johansen Quadruple Stain (Johansen, 1940) or with rhodamine B/methyl green (Pearce, 1984). Stained sections were mounted using DPX.

RESULTS

In sections stained with rhodamine B/methyl green the black lines appeared red suggesting that they were highly lignified. This staining technique also gave a good contrast between diseased tissue, which appeared green, and healthy tissue, which stained pinkish red *(Figure 1)*. The black line seemed to demarcate the healthy from diseased tissues. However, this technique failed to make individual hypha from the tissue samples readily observable.

Individual hypha could be easily discerned in the infected portion of samples treated by the Johansen Quadruple staining technique. Each individual hypha assumed the normal shape and size with clear evidence of the presence of clamp connections in infected cells away from the black line *(Figure 2)*. Nearer to the black line, the hyphae began to swell and seemed to have deposited additional cell wall material. Swelling of the hyphae occurred both at intercalary and terminal ends forming structures somewhat resembling 'chlamydospores' *(Figure 3)*. Within the black line the swelling of hyphae became more pronounced and the cell wall assumed a thicker appearance *(Figure 4)*. Bubble cells, presumably of host origin, intermingled with the swollen hyphal cells occurring within the black line. Although the black line formed a demarcation between healthy and diseased tissue, a few fungal hyphae could be observed growing from the black line into the healthy stem tissue.

DISCUSSION

This study reveals that the black line observed in the stem of oil palm infected with *Ganoderma* is caused by a single mycelium and thus emphasizes the fungal origin of its formation. This observation is analogous to that described on wood decayed by other fungi such as *A. mellea* (Campbell, 1934; Lopez-Real, 1975), *S. hirsutum* (Lopez-Real, 1975), and *Xylaria polymorpha* (Campbell, 1933). The presence of fungal hyphae almost exclusively on one side of the black line precludes the possibility of a dual infection involving another fungus and clearly indicates that *Ganoderma* is the only one present. Clear evidence of fungal structures within the black line suggests that it is not solely due to the deposition of wound gums as a result of wounding or parasitic invasion.

Events leading to the formation of the black line in the stem of a naturally infected palm are much like those described in a laboratory study on the black lines formed by *A. mellea*

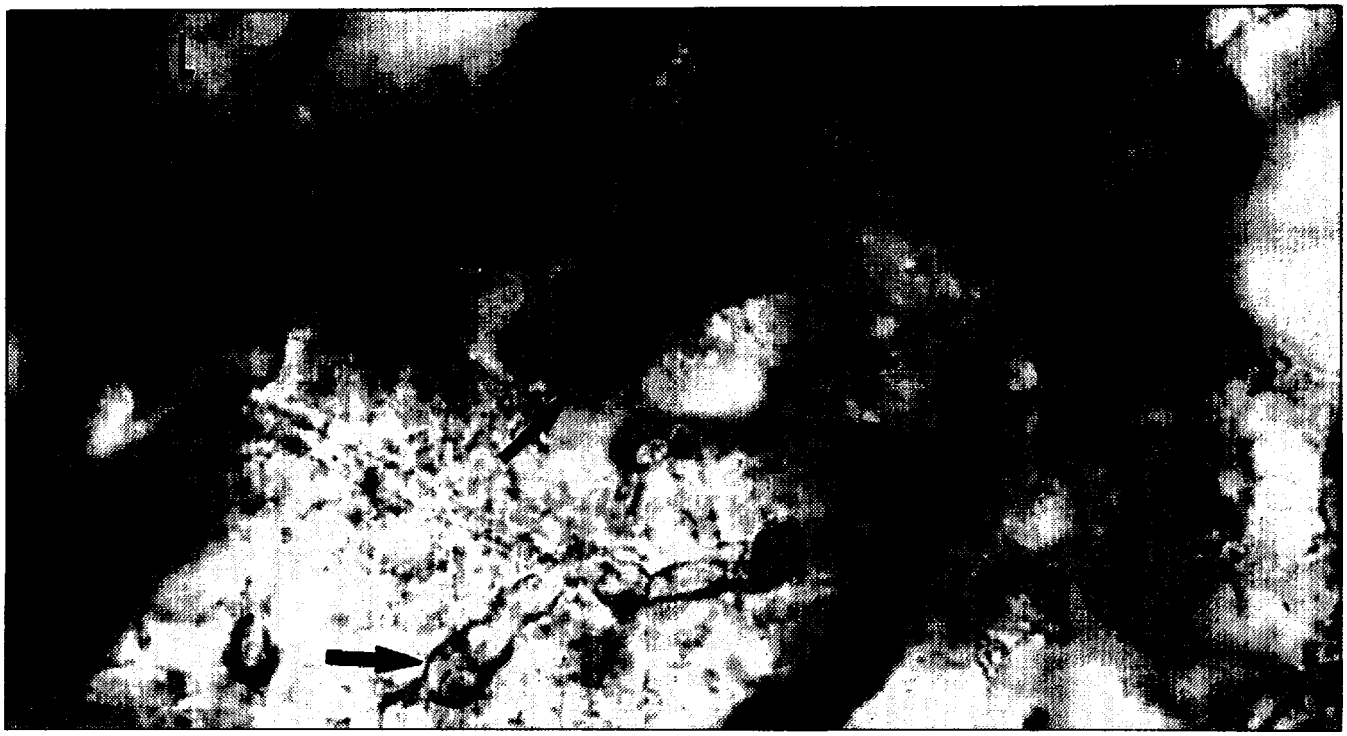

Figure 3.

Transverse section of infected stem showing formation of resting structures (arrows) in cells adjacent to the black line (BL)

Figure 4.

Transverse section of infected stem showing thick walled resting structures (arrows) in cells within the black line (X930)

and *S. hirsutum* (Lopez-Real, 1975). Hyphal proliferation was observed in infected cells away from the black line; hyphal swelling occurred in cells adjacent to the line; aggregation and pigmentation of hyphae occurred within the line. The intermingling of bubble cells and swollen fungal hyphae within the line provides evidence of the involvement of both the host and the pathogen in its formation.

It is of interest to note the formation of 'chlamydospore-like' structures in cells adjacent to and within the black line. Although we have never observed chlamydospores of *Ganoderma* formed in artificial media, studies on *G. lucidum*, a pathogen of areca nut, have revealed the formation of chlamydospores on malt agar medium (Venkatarayan, 1935). *Ganoderma* is known to survive in infected woody remains buried in soil and to cause infection when the area is planted with oil palm (Turner, 1981). If the fungus were to exist as free hyphae within the infected woody remains there is every possibility that the naturally occurring soil antagonists such as *Trichoderma, Aspergillus*, and *Penicillium* would easily replace the pathogen (Varghese, 1975). The ability of the pathogen to form resting structures deeply embedded in the host's tissue suggests a mechanism for long-term survival of the pathogen in soil. Although the period of survival of *Ganoderma* in infected host tissue buried in soil has never been documented, the authors' experience has revealed a number of cases where the incidence of *Ganoderma* was high in oil palm plantings where the previous crops were non-hosts for the *Ganoderma* pathogenic to oil palm, such as pineapple and rubber. With a disease of similar aetiology, caused by *Phellinus weirii* attacking conifers, infective material remained viable for at least 50 years in the soil associated with remains of roots and stems (Hansen, 1979).

One perplexing problem connected with studies of *Ganoderma* on oil palm is the constant failure to cause disease through artificial inoculation. Attempts to inoculate oil palm seedlings using either naturally diseased tissue or pure cultures of *Ganoderma* have not yielded successful results that can be regarded as conclusive (Navaratnam, 1961; Navaratnam and Chee, 1965). Various inoculation techniques carried out by the present authors also gave negative results. None of these inoculation experiments took into consideration the mechanism of *Ganoderma* survival in the soil. It is very likely that infected tissues used as inocula contained free *Ganoderma* hyphae which were easily replaced by antagonists once introduced into the soil. With the present evidence that the pathogen forms resting structures within the black line, fresh inoculation attempts should be made, including the black line in the infected block to be used as inoculum.

The technique of clean clearing during replanting have been advocated as a measure to reduce incidence of *Ganoderma* in subsequent planting (Turner, 1965). The rationale behind this recommendation is to shred the infected bole and roots into small pieces and consequently reduce the inoculum potential of these woody remains. However, despite the adoption of this technique the incidence of *Ganoderma* still remains high (Singh, 1984). Again in this case the role played by the black line for the long term survival of *Ganoderma* should be evaluated. It is possible that the inoculum potential does not depend on the size of the infected woody remains, but more on the presence or absence of resting structures embedded within the line.

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