Plant Pathology Bulletin 1: 147-152, 1992

Basal Stem Rot of Oil Palm Caused by *Ganoderma boninense*

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Accepted for publication: September, 23, 1992

ABSTRACT

Lim, T. K., Chung, G. F., and Ko, W. H. 1992. Basal stem rot of oil palm caused by *Ganoderma boninense*. Plant Pathol. Bull. 1:147-152.

The novel and rapid techniques involving root and frond inoculations was developed for establishing the causal relationship between *Ganoderma boninense* and the basal stem rot of oil palm. Using both techniques, *G. boninense* isolated from naturally diseased oil palm and grown in a wheat-oat grain medium was found to be pathogenic, while *G. lucidum*, a medicinal isolate and *G. philippii*, isolated from red root disease of rubber were not pathogenic and not involved in the disease etiology. Symptoms of necrosis on the roots and fronds appeared 4-6 weeks after inoculation, and the causal fungus was reisolated from diseased tissues fulfilling Koch's postulates.

Key words: Basal stem rot, oil palm, *Ganoderma boninense*, pathogenicity, Malaysia.

INTRODUCTION

Wilting associated with basal stem rot (Figs. 1, 2, 3 & 4) is currently the most devastating disease attacking oil palm (*Elaeis guineensis* Jacf.) in Malaysia and Indonesia. Sporophores of *Ganoderma boninense* Pat. are commonly found growing on the basal portion of the infected palm (Figs. 5 & 6). When basal stems of the diseased trees were sectioned longitudinally, the infected tissues were found to be yellowish brown (Fig. 7) with brown lines or dark brown lines (Fig. 8). This disease has also been reported to occur in Africa-Ghana, Nigeria, Zaire, Cameroun, San Tome & Principe, Angola, Northern Rhodesia and Tanzania, and in Oceania-Papua New Guinea (14). Recently, the disease has also been observed in Honduras, Central America (3).

In Malaysia, the disease is particularly rife on oil palm grown on coastal and riverine alluvial soils, devastating more than 80% of plantings by the time they are mid-way through their economic life of about 25 years and resulting in more than 50% losses (14). The catastrophic impact of the disease is also being felt on palms grown in peat soils and low lying inland soils prone to water-logging and incidence of the disease is rising among palms less than 10 years old replanted from virgin jungle, rubber, pineapple, coconut and oil palm (2).

The etiological agent of the disease had been a controversial issue. Turner (14) listed 16 species of *Ganoderma* being associated with basal stem rot of oil palm, among which 7 species viz., *G. applanatum* (Pers.) Pat., *G. boninense*, *G. chalceum* (Cooke) Steyaert, *G. lucidum* (W. curt. et fr.) Karst, *G. miniatocinctum* Steyaert, *G. pseudoferreum* (Wakef.) Overh. & Steinmann, and *G. tornatum* (Pers.) Bres. were reported from Peninsular Malaysia. Steyaert (10) identified 6 species in Peninsular Malaysia, *i.e. G. boninense*, *G. miniatocinctum*, *G. chalceum*, *G. tornatum*, *G. zonatum* Murrill and *G. xylonoides* Steyaert. Recently Ho and Nawawi (4) reported that *G. boninense* was the major pathogen based on association rather than on pathogenicity studies. Using an inoculum size of 750 cm^2 of naturally diseased oil palm tissues to inoculate seedlings, Navaratnam and Chee (8) obtained positive infection of 15 of the 22 seedlings inoculated. However, using similar technique, numerous attempts by other workers failed to reproduce their results (Ramasamy, unpublished). In Nigeria, attempts to establish the pathogenicity of *G. zonatum* by binding roots to bags of inoculum, binding sporophores to stem or inoculating stems with spores of sporophores failed to achieve positive infection (9). Moreover, the use of a nonaxenic culture by Navaratnam and Chee (8) did not adhere to Koch's postulates. Because of the lack of such valid proof to establish pathogenicity conclusively, many researchers and oil palm growers felt that *Ganoderma* could be of secondary importance in the disease etiology and that other predisposing factors could play a greater role (Swinburne, unpublished). There is certainly a need to determine the exact etiology of this devastating disease.

Fig. 1. A diseased field with wilting oil palm trees caused by Ganoderma basal stem rot.
Fig. 2. A diseased field with about 90% of oil palm trees wiped out by Ganoderma basal stem rot.

In this paper we report for the first time a novel, reproducible and quick method to establish the pathogenicity of *G. boninense* as the pathogen in mature field palms and seedlings.

MATERIALS AND METHODS

Inoculum preparation

A 2-week-old malt agar culture of *G. boninense* isolated from a naturally diseased oil palm in Banting, Selangor, designated Gb-07 was used to inoculate a wheat-oat medium (1) consisting of 10 g whole wheat grains, 10 g whole oat grains and 10 ml distilled water. The fungus was grown in this substrate for 10-14 days at 28 C and used as inoculum for the pathogenicity tests unless otherwise stated.

Pathogenicity test on roots

The rhizosphere soil around two healthy 15-year-old palms (Dura X Pisifera Hybrid) near Ebor Research was carefully removed to expose the roots. Soil particles adhering to roots were washed off and intact, unsevered, healthy roots of approximately 5-7 mm in diameter were selected. The roots were surface-disinfected with 75% ethanol and gently scratched longitudinally (5 mm long) at a distance of 20-30 cm from the base of the trunk. About 10 *Ganoderma* seeded wheat-oat grains were placed on the injured portion of the root and the inoculum was then wrapped with Parafilm and further secured with cellophane. After inoculation, the roots were tagged with raffia strings at the proximal end near the trunk and the roots were covered over with the soil. For the check treatment, roots were similarly inoculated with autoclaved fungus-free grains. To determine the time required for infection, the soil was carefully removed to expose the roots and the inoculum was removed after 4, 6, 8 and 10 weeks of inoculation. Each treatment consisted of 12 replicates, 6 from each palm. Reisolations from artificially induced diseased roots and healthy roots from the check were made on malt agar after surface sterilization of the root pieces in 75% ethanol for 3 min followed by 0.25% sodium hypochlorite for 10 min.

The above procedures were repeated in additional testes using *G. boninense* (Gb-07 and Gb-09) grown in wheat-oat grains and rice grains (10 g whole rice grains and 10 ml distilled water); a wheat-oat grain inoculum of

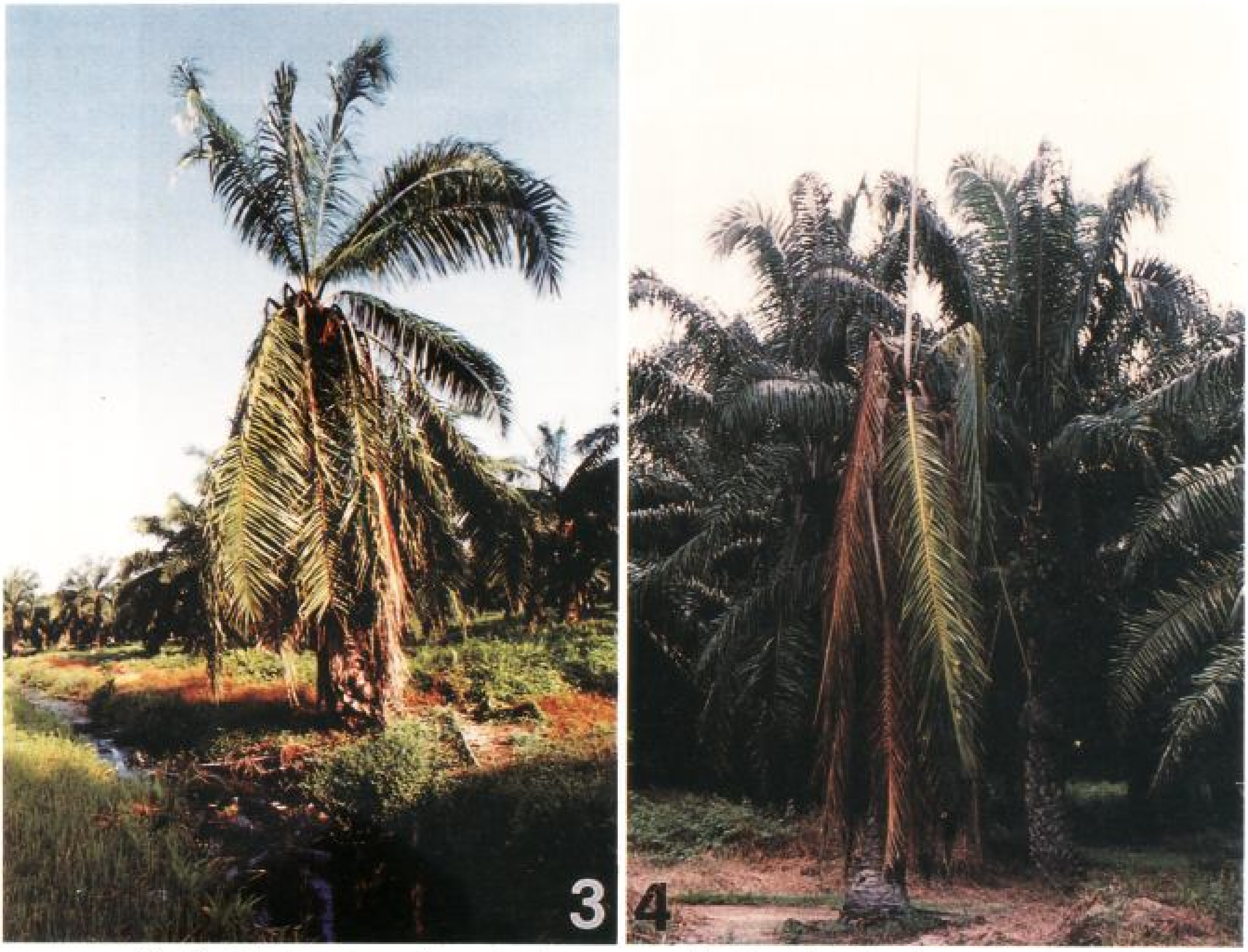

Fig. 3. Initial symptoms of an oil palm tree caused by Ganoderma basal stem rot.
Fig. 4. Advanced symptoms of an oil palm tree caused by Ganoderma basal stem rot.

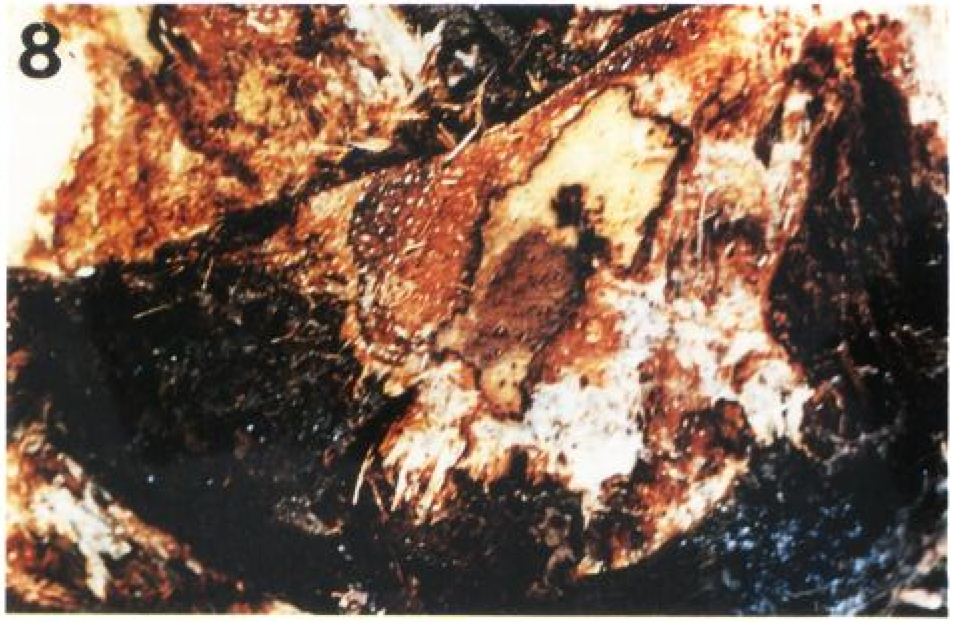

Fig. 5. Spoirophores of *Ganoderma boninense* growing on basal portion of an infected oil palm tree.
Fig. 6. Closse up view of *Ganoderma boninense* fruiting bodies.
Fig. 7 & 8. Symptoms on cut surface of basal stem of an oil palm tree affected by Ganoderma basal stem rot.

G. lucidum (G1-02), a medicinal isolate obtained from a commercial mushroom grower in Kluang, Johore; a wheat-oat grain inoculum of *G. philippii* (Bres. & Henn.) Bres. (= *G. pseusoferreum*), isolated from red root disease of rubber and fungus free wheat-oat grains and rice grains to serve as blank checks.

Pathogenicity tests on frond rachises

Fifteen months old palms in polybags were used. The adaxial surface of the green rachis 120 cm away from the main stem axis was disinfected with 75% ethanol and lightly injured by scratching (5 mm) with a sterilized needle. The wound was then inoculated with *Ganoderma* seeded wheat-oat grains and secured with Parafilm and cellophane as described above. To facilitate inoculation operations, the five pinnae at each side of the rachis to be inoculated were removed. For the check treatments, the rachis was inoculated with fungus-free grains. Assessment for infection was carried out after 4, 6, 8 and 10 weeks of incubation and reisolations were made as for the root tests. Each treatment consisted of 10 rachises.

RESULTS

All oil palm roots inoculated with isolate Gb-07 of *G. boninense* showed signs of positive infection when assessed after four weeks. Symptoms discerned were softening of the outer skin of the roots and browning and necrosis of the underlying root tissues when the tough, outer skin is removed. The symptoms were identical to those observed in naturally infected roots of oil palm. After six to eight weeks, the root necrosis extended to 8-11 cm (mean 9 cm) towards the trunk axis. In some cases, necrosis extended into the trunk axis after 10 weeks. *G. boninense* was reisolated from artificially infected roots. Roots similarly inoculated with fungus-free wheat-oat grains remained healthy and the underlying root tissues were pale yellowish-white in colour.

In the other test, isolates of Gb-07 and Gb-09 of *G. boninense* grown in wheat-oat grains gave 100% infection of oil palm roots after 10 weeks. However, roots inoculated with *G. lucidum* and *G. philippii* grown in wheat-oat grains did not developed any disease symptoms (Table 1). Roots similarly inoculated with fungus-free wheat-oat grains used as the control also remained healthy.

In the frond test, isolates Gb-07 and Gb-09 of *G. boninense* gave 100% infection (Table 1) and disease could be seen four to six weeks after inoculation. The fungus caused both an external and internal discoloration and necrosis of the rachises. Internally, necrosis can be seen as a blackish brown decay of the rachis tissues extending outwards and downwards from the inoculation site on the adaxial rachis surface. *G. boninense* was reisolated from artificially infected rachises. Rachises inoculated with *G. lucidum* or *G. philippii* or with fungus free wheat-oat grains remained healthy.

TABLE 1. Comparison of pathogenicity of three species of *Ganoderma* grown in wheat-oat grains on roots and rachises of oil palm

Species & isolates	Disease incidence (%)[1]	
	Root	Rachis
G. boninense		
Gb-07	100	100
Gb-09	100	100
G. lucidum	0	0
G. philippii	0	0
Control	0	0

1. Ten roots or 10 rachises were used for each treatment.

When oil palm roots and rachises were inoculated with isolate Gb-07 and Gb-09 of *G. boninense* grown in rice grains, only 50 to 70% infection was obtained after 10 weeks. Moreover the disease was less severe than that caused by the same fungus grown in wheat-oat grains.

DISCUSSION

The results of the pathogenicity tests satisfied Koch's postulates, proving conclusively that *G. boninense* is the pathogen and is of primary significance in the etiology of the disease. The pathogen can attack both roots and fronds. Turner (12) after examination of numerous palms exhibiting various stages of infection and disease development, concluded that basal stem rot was primarily transmitted through the roots in contact with an inoculum source. Our results tended to substantiate his observations that roots could be the main foci of infection. The results also showed that injured fronds (rachis) could contract infection when in contact with a suitable inoculum source.

Our results indicated that the purity and quality of a monoaxenic inoculum source was more critical for infection than the quantity of inoculum. The lack of an uncontaminated, pure monoaxenic inoculum could be the prime reason for the failures of numerous workers to prove pathogenicity of *G. boninense* employing the technique advanced by Navaratnam and Chee (8). Although, the latter obtained some positive results, the use of a non-monoaxenic inoculum source (diseased palm tissues) did not fulfill Koch's postulates.

Naturally diseased tissues as well as sporophores of *Ganoderma* were rapidly overgrown and overwhelmed by secondary microorganisms such as various species of *Trichoderma, Penicillium, Gliocladium, Aspergillus,* and other soil fungi (7).

The results also indicated that the medicinal isolate of *G. lucidum,* and *G. philippii* isolated from red root disease of rubber are not pathogenic to oil palm and not involved in the etiology of basal stem rot. The *Ganoderma* species associated with basal stem rot was first identified as *G.*

lucidum by Wakefield (13) in West Africa and by Thompson (11) in Malaysia.

With this novel, quick and reproducible technique, it is now possible to determine the pathogenicity and cross-pathogenicity of other *G. boninense* isolates and *Ganoderma* species from other hosts and to determine the host range of *G. boninense.* This method should also be suitable for the screening of oil palm varieties and hybrids for resistance to basal stem rot. Additionally, the technique provides a rapid method to screen for the in vivo efficacy of chemicals which normally necessitates one-half to two years before any indication of effectiveness can be realized (5,6), and also paves the way for the study of the uptake and behaviour of chemicals in the oil palm seedlings.

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摘 要

林東貴[1]、曾日輝[2]、柯文雄[3]. 1992. *Ganoderma boninense* 引起油椰子之莖基腐病. 植病會刊 1:147-152. (1. 馬來西亞農業大學植物保護系， 2. 馬來西亞 Sime Darby Plantations, Ebor Research, 3. 美國夏威夷大學植病系)

本試驗發展出接種油椰子(油棕)之根部與莖基部的新方法，能使 *Ganoderma boninense* 表現病原性，快速誘發莖基腐病。利用此種接種技術，分離自自然界油椰子病株上的 *Ganoderma boninense* 菌株，經培養於小麥一燕麥基質供爲接種源，被證實對油椰子具有病原性。然而，*Ganoderma lucidum*（一藥用菌株）與 *Ganoderma philippii*（分離自罹患紅根病(Red root disease)之橡膠菌株）則不具病原性，因而此兩菌與油椰子莖基腐病之病因無關。依上述方法行人工接種4～6星期後，接種植株之根部與莖基部出現壞疽病徵，相同之病菌均可自發病組織上再分離得到一依柯霍氏法則以確定病害之病因。

關鍵字：莖基腐病，油椰子(油棕)，*Ganoderma boninense*, 病原性，馬來西亞。