Mycopathologia (2005) 159: 101–107

Studies on oil palm trunks as sources of infection in the field

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Abstract

Diseases of oil palm caused by *Ganoderma boninense* are of major economic importance in much of South-East Asia. This paper describes results from an ongoing field trial concerning the spread of the pathogen from artificially inoculated trunks used to simulate spread from windrowed trunks. Three planting distances for bait seedlings revealed that the closer the seedling was planted to the source of inoculum the sooner it succumbed to the disease. However, infection only occurred when the trunks were mounded (covered with soil), and seedlings planted around uncovered trunks (at any distance) have showed no symptoms of disease to date. Isolates are being collected from infected plants and molecular analysis is being undertaken to give more information on the spread of the pathogen.

Key words: debris, disease-spread, *Ganoderma*, oil palm

Introduction

Basal stem rot (BSR) caused by *Ganoderma boninense* remains the most significant constraint to sustainable oil palm production in South East Asia with significant yield losses through direct loss of the stand, reduced yield of diseased palms and the requirement for earlier replanting. Debris left in the field from the previous crop is a very important source of infection [1, 2] and much of the management of the disease involves the removal of as much of this debris as possible, particularly at replanting time [3].

Oil palm can also become infected with BSR following conversion after rubber as is common in North Sumatra, and generally this occurs about 10–14 years after planting [4]. Hasan and Turner [1] and Flood et al. [5] reported that the times of greatest practical significance for the control of *Ganoderma* in oil palm are likely to be (1) soon after planting when debris from previous plantings (windrowed trunks and stumps) remain in the ground and (2) later in the planting cycle, when the roots come into contact with inoculum from this debris left in the ground.

The current trial was set up to investigate infection of seedlings from windrowed palms in more detail. Preliminary molecular analysis had indicated that infected stumps are direct sources of infection to bait seedlings [2] but few samples were available for analysis. In the current trial, more replicates, both in terms of sources of infection and numbers of seedlings available for sampling, were initiated. In addition, trunks were artificially inoculated with a known isolate in an attempt to standardise the inoculum in each treatment. Different planting distances were used between the trunks (inoculum sources) and the bait seedlings, in order to assess the time taken for infection to spread from windrowed trunks to seedlings in the field and hence, provide practical recommendations on planting distance from windrows for estate managers at replanting time.

The effect of covering trunks with soil (to simulate what happens in the field when oil palm material is left in the ground) as compared to oil

palm debris placed on the surface of the ground at replanting was also investigated so that practical recommendations on the treatment of oil palm debris at replanting could be made.

Materials and methods

Field methods

A pilot study was set up to determine if it were possible to artificially inoculate oil palm trunks and thus, have a standard inoculum for infection of bait seedlings and for molecular analysis. Treatment 1 was a felled healthy palm and Treatment 2, a felled poisoned (100 ml Gramoxone/palm) healthy palm. Rubber wood dowels ($2 \times 2 \times 6$ cm) were inoculated with a single known *G. boninense* isolate (2 mm^3) taken from the active edge of a culture obtained from the brackets of the pathogen. When fully colonised (about 9 weeks), the rubber dowels were inserted into the oil palm tissues at 0.5 m intervals along the length of the trunk and at intervals around the circumference (Figure 1). As a control, healthy palms were inoculated with rubber dowels that had not been colonised by *Ganoderma* (Treatment 3). After 6 months, the palms were cut to reveal the extent of rotting. Visual comparison of the rates of colonisation showed that all inoculated palms had been colonised as evidenced by a rot from which *Ganoderma* could be re-isolated, but felled palms were colonised more quickly if they had been poisoned. Consequently, poisoned palm trunks were used for the main trial. Similar rotting was not observed in healthy un-inoculated trunks (Treatment 3).

For the main trial, 27-year old oil palms were cut down and their trunks artificially inoculated with the same isolate of *Ganoderma* as described previously. The site chosen for the trial was Batu Lokong, Begerpang Estate, and at the time of the start of the trial (July 1998) this consisted of standing rubber trees. The oil palm trunks were transported into the area from outside.

The experimental design was a split plot design, with main plots comparing mounded and un-mounded oil palm trunks, and sub-plot treatments for comparing three different planting distances of the seedlings from the trunks. Inoculated trunks were either covered with soil (Treatment 1) or were left on the surface (Treatment 2). Six-month-old seedlings were then planted 0.5 m away from the trunk (A), 1.0 m away (B) or 1.5 m away (C). Five replicate trunks were used for each treatment with six seedlings planted around each trunk (Figure 2).

The trial is ongoing and has been monitored regularly. The mounded treatments were regularly checked and recovered with soil as required. As seedling palms showed symptoms, they were harvested and attempts were made to re-isolate the pathogen by plating onto *Ganoderma* Selective Medium [6] after surface sterilisation with 70% alcohol for 2 min.

In addition, every 6 months, the trunks were monitored with regard to their decay. This was assessed by dividing the trunks into 10 parts each representing 10% of the trunk volume. A metal rod pushed into that part of the trunk gave a measure

Figure 1. Dowels were inserted into the oil palm tissues at 0.5 m intervals along the length of the trunk and at intervals around the circumference.

Figure 2. Five replicate trunks were used for each treatment with six seedlings planted around each trunk.

of the decay and the mean percentage decay for each treatment was calculated.

Molecular methods

Small (2 mm^3) blocks of mycelium from *Ganoderma* isolates from the trial were transferred onto Malt Agar plates and grown at 25 °C for 7 days. Subsequently, a 2 mm^3 block was taken from the edge of the colonies, transferred into a 250 ml flask containing 60 ml of GYM liquid media and grown for 5 days at 25 °C, at 200 rpm on an orbital incubator. The cultures were collected by filtration, the agar blocks were removed and the mycelium was washed with distilled water before freeze-drying.

DNA was extracted by grinding the freeze dried mycelia to a fine powder, under liquid nitrogen, in a pestle and mortar. Fifty milligrams of ground mycelium was used for DNA extraction following the method described by Raeder and Broda [7]. DNA was re-suspended in 100 μl of TE buffer and purified using a Wizard DNA Clean-Up System (Promega) following the manufacturers instructions.

Polymerase chain reaction (PCR) amplification of the Internal Transcribed Spacer (ITS) region was undertaken using primers ITS 1 and 4 [8]. PCR was performed in a reaction volume of 50 μl using 0.5 μl of each primer (Sigma), 5 μl of 10× buffer, 2.5 U of *Tth* enzyme (HT Biotechnology) 200 μm of each dNTP (Gibco) and 4 μl of diluted (10^{-2}) template DNA. Amplification products were separated on a 1.5% agarose gel in 0.5× TBE buffer and stained in ethidium bromide (0.5 μg/ml).

Inter simple sequence repeat-PCR (ISSR) was performed with primer ISSR-TGT as described by Grunig et al. [9] at an annealing temperature of 46 °C for 1 min. Amplification reactions were carried out in 20 μl volumes containing 0.5 μl primer (Sigma), 200 μm of each dNTP (Gibco), 0.02 Units *Taq* DNA polymerase, 1.5 mM $MgCl_2$ (Sigma) and 2 μl of template DNA. Amplification products were separated on a 1.5% agarose gel in 0.5× TBE buffer and stained in ethidium bromide (0.5 μg/ml).

Statistical analysis

Gel images were examined and analysed by creating a binary matrix of the presence or absence of bands. This matrix was then analysed using principal co-ordinate analysis based on standardised Euclidean distance using MVSP package (Kovach Computing, Anglesey).

Results

To date, no seedlings have become infected in any of the treatments around un-mounded trunks

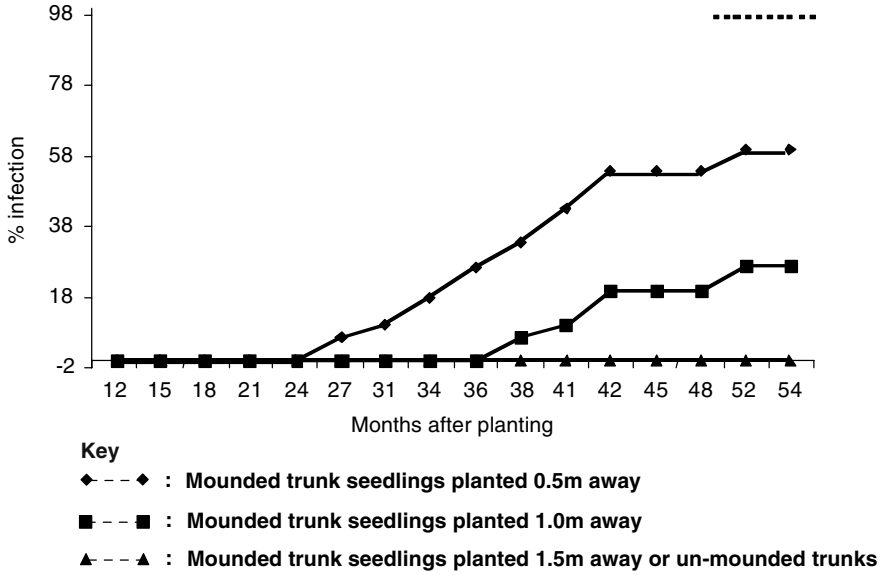

Figure 3. Infection of seedlings planted at different distances from trunks.

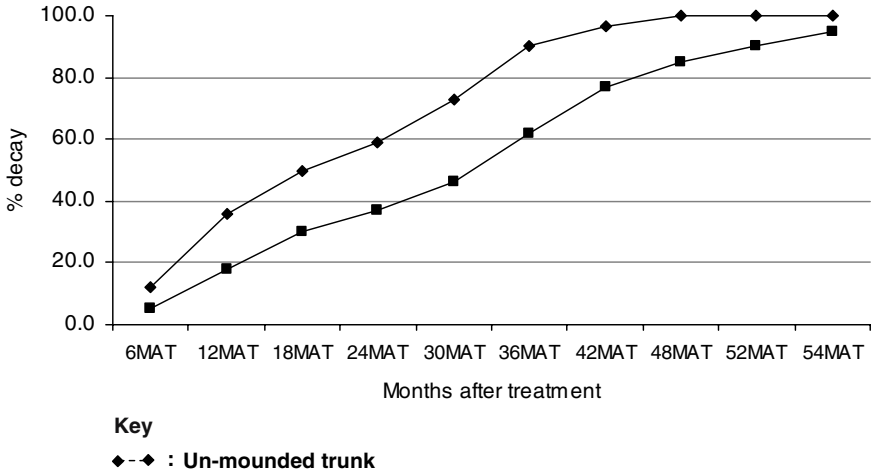

Figure 4. Decay of trunks used in mounded and un-mounded treatments.

(Figure 3) despite the fact that the trunks themselves were extensively colonised by the pathogen. Also, no seedlings planted 1.5 m from the inoculated, mounded trunks have yet shown any symptoms of infection (54 months after planting). However, in the treatments where the trunks were mounded and the seedlings planted 0.5 m away from the trunks (Treatment A) 60% of seedlings have become infected and 27% of seedlings have become infected where the seedlings were planted 1.0 m away from the trunks (Treatment B). Seedlings planted 0.5 m from the mounded trunks began to die after 27 months whilst those planted 1.0 m away began to succumb after 38 months. The infection rate started to plateau around 42 months (Figure 3) when the mounded trunks were about 80% decomposed (Figure 4). The un-mounded trunks had completely decomposed by 48 months.

As part of the ongoing trial, isolates are being collected from various infected seedlings for molecular analysis. The map of the trial indicating the position of diseased seedlings is presented in Figure 5. Molecular analysis using ITS primers was used to distinguish any non-*Ganoderma* isolates and these were discarded from further analysis.

Principal co-ordinate analysis (Figure 6) of the IISR band patterns of isolates recovered from treatments (mounded trunks) A5 and A3 to date

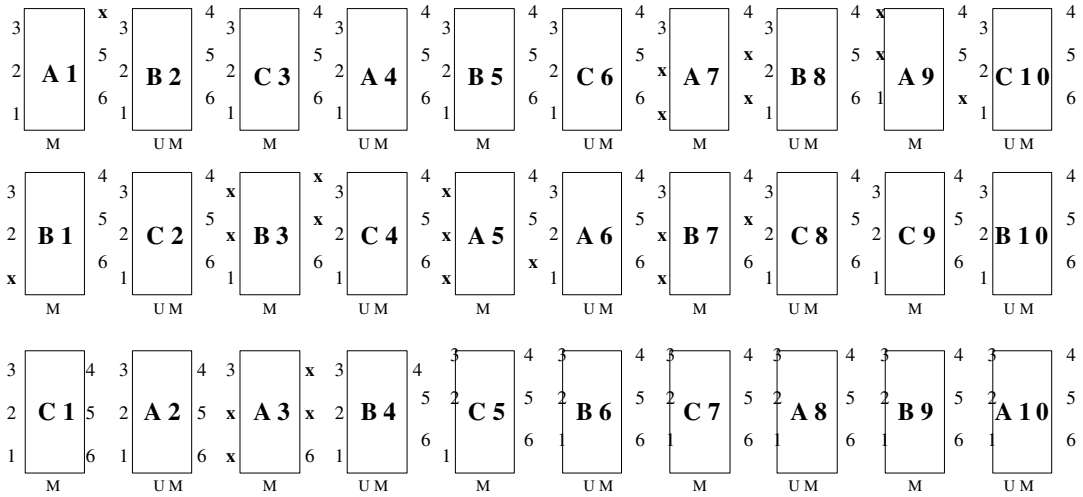

Figure 5. Map of the trial showing diseased palms and the isolates collected to date.

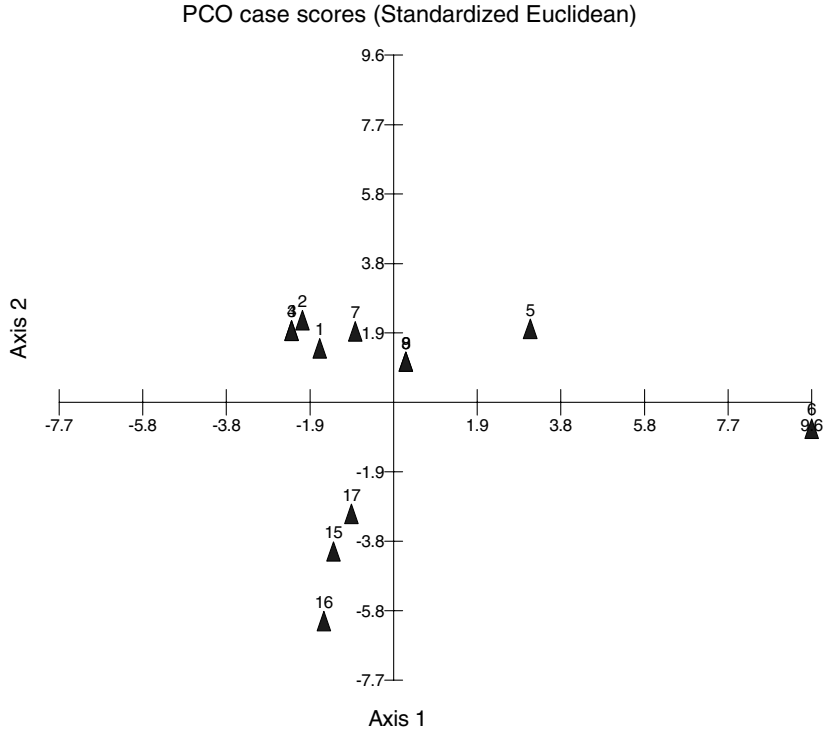

Figure 6. Principle co-ordinate analysis of ISSR study.

showed that all isolates from inside infected seedlings (isolates 2–4) around trunk A3 were nearly identical to the original isolate inoculated into the trunk (isolate 1) and so it is likely that the same genet (*sensu Rayner*) was present in the trunk and in the infected seedlings. However, one isolate (5) from the root of seedling 5, planted around the A3 trunk was unique from all isolates so far examined (Figure 6) as was isolate 6 taken from a fruit body growing on the A3 trunk.

With trunk A5, one isolate (7) from infected seedling 2 was very similar to the original isolate and isolates from the other four infected seedlings had similar profiles to each other and to that of a fruit body growing on the trunk, however this was different to the original inoculum. This second group of isolates had some bands in common with the 'original' group and other bands were different.

Discussion

Debris from previous crops of oil palm represents significant disease sources for oil palm seedlings at replanting [1, 2]. In second generation oil palms and under normal plantation practice at Lonsum, the old palms are pushed over and the boles removed from the ground. The trunks and boles are generally pushed into alternative old planting rows and two rows of young palms planted in between, approximately 4 m apart and 3 m from the windrowed debris. A few early deaths of the seedlings due to *Ganoderma* are generally observed in the first few years (but not generally exceeding one per hectare). Little infection is seen until about 10–14 years after planting when serious losses due to *Ganoderma* start to appear. However, if normal plantation practice is not followed and boles are left in the ground up to 25% of palms may become infected within 6 years. It appears that the occasional early deaths are due to seedlings being wrongly planted much closer to the windrows than the recommended 3 m. This is much less likely to happen if smaller windrows are placed in every old planting row – a new practice being adopted by Lonsum.

The results in this paper demonstrate that infection can occur if planting is within 1 m of infected material that has been covered by soil.

This agrees with the above observations that in commercial fields appreciable infection during the first 10–14 years is only observed if oil palm debris is not removed from the soil and also agrees with the conclusions of Flood et al. [2]. These authors concluded that young palms would succumb quickly when exposed to large amounts of infected debris close to their planting points. Seedlings planted 0.5 m away from inoculated mounded trunks began to show signs of infection after 27 months while those planted 1.0 m away began to succumb after 38 months. Thus, distance from inoculum is an important factor in determining when a young palm begins to show symptoms but another important factor would appear to be if the infected debris is left on the surface of the ground. To date (nearly 5 years after the establishment of the trial) no seedlings have become infected in any of the treatments where trunks were placed on the ground surface (un-mounded). This would appear to add further weight to the field observations that BSR is seen earlier and the incidence higher when debris from previous plantings is left in the ground.

Un-mounded trunks were clearly colonised by the pathogen and it will be interesting to monitor the situation now that these trunks have fully decomposed. Presumably, the pathogen has now completely utilised the nutrients in the un-mounded trunks and may be entering a soil phase and seeking new hosts (new sources of nutrients) as suggested by Flood et al. [5]. In which case, the baited seedlings around un-mounded trunks should could be expected to show symptoms in the next few years. Interestingly, if we consider the average distance from replanted seedlings to windrows is 3 m (using current practice) and based on the timing of the onset of symptoms in this trial, then we could predict that infection would be seen in the field between 10 and 14 years after replanting. This agrees with what is actually observed in the Lonsum Estates.

Mounded trunks did not decay as quickly as the un-mounded treatments and covering with soil may have protected the trunks and pathogen from competition and external weathering. In contrast, un-mounded trunks would have been subject to prevailing weather conditions, which would have softened the oil palm tissue and may have made it more amenable to degradation by other micro-organisms. Thus, the *Ganoderma* inoculum in these un-mounded trunks could have been subjected to natural competition and antagonism from these micro-organisms and the actual inoculum level of *Ganoderma* is low. Alternatively, covering with soil might have been thought to encourage competitive soil micro-organisms and reduced inoculum available for seedling infection. The trial is being monitoring and over the next year or so, the fate of baited seedlings around these un-mounded trunks may give clarification of the situation.

To date, the trial would appear to validate current plantation practice of not leaving oil palm debris in the ground at replanting. Thus, practical recommendations to the estate managers would include (1) ensuring that replanted seedlings are not planted close to previous stand debris on replanting and that (2) this debris should not be left in the ground. Consequently, as much material as practically possible should be removed from the ground at replanting.

Preliminary molecular analysis [2] supported the assumption that infected stumps are direct sources of infection to bait seedlings [1] but few samples were available for those analyses. In this current trial, more replicates (both in terms of sources of infection and numbers of seedlings available for sampling) were included in the experimental plan to ensure more molecular analysis could be done. The results to date indicate that trunks can represent direct sources of infection. For example, in trunk A3, molecular analysis suggested that the same genet was present inside infected seedlings as that that had originally been inoculated into the trunks. This would suggest that the trunk was a direct source of infection. Recent analysis of Trunk 7 (not shown) also showed that an isolate from seedling 6 was identical to the original isolate. However, the population dynamics of the pathogen appear complicated. An isolate from a root of one of the infected seedlings gave unique ISSR band patterns, as did an isolate from a bracket growing on the trunk.

The ISSR band patterns from isolates from infected seedlings around Trunk 5 showed that one isolate from infected seedling (2) was very similar to the original isolate inoculated into the trunk. Also, four other infected seedlings contained isolates of the same genet as a fruit body growing on the trunk which would also indicate that the source of infection was the trunk, however in this case this genet was different to the original inoculum.

The four isolates from trunk A5 that had similar ISSR band patterns also had some bands in common with the original inoculum although other bands were different. This could suggest that segregation and recombination had occurred in these trunks between the inoculum and another isolates (or isolates) possibly already present in the trunk when it was cut down. A survey of all of the fruit bodies that appeared on all the trunks would have given a clearer picture of the original *Ganoderma* populations in the trunks at the start of the trial, however this was not conducted and therefore it is difficult to arrive at definite conclusions about the population dynamics. Nevertheless, in the seedlings examined to date, it would appear that the trunks do constitute a direct source of infection.

Acknowledgements

The authors would like to thank P.T.P.P. LONDON SUMATRA [LONSUM] INDONESIA Tbk and Dr. Hugh L Foster (Director of Bah Lias Research Station) for helpful discussions and for permission to publish this work.

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