CHARACTERIZATION OF PARAMETERS FOR THE DEVELOPMENT OF AN EARLY SCREENING TEST FOR BASAL STEM ROT TOLERANCE IN OIL PALM PROGENIES

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ABSTRACT

*Basal stem rot (BSR) of oil palm (*Elaeis guineensis *Jacq.) is caused by* Ganoderma boninense*, and is one of the most commercially devastating diseases in Southeast Asia. So far, cultural practices, combined to some extent with biological control, have been considered as the best approach for controlling the disease. However, in recent years, sources of genetic resistance and susceptibility have been identified in field trials, leading to the consideration of a genetic approach as an integrated component in controlling the disease. To develop this approach, an early screening test at the nursery or pre-nursery stage is needed, based on artificial inoculation and correlated with field observations. The success of nursery seedling artificial inoculation relies on a set of parameters such as defining the inoculum potential like the aggressiveness of* G. boninense *isolates, the incubation period of pre-infected rubberwood blocks (RWB), the ratio between the size of pre-infected RWBs and the volume of soil for infection, and the quality of nursery or pre-nursery shade as pre-disposing factors. When this set of parameters was optimized, disease symptoms were observed three months after the inoculation of germinated seeds. This avoided the transfer of seedlings from the pre-nursery to the main nursery, minimized transplanting shock and root damage, and also reduced the time taken for the screening test and the nursery area required. This early inoculation enabled the discrimination of isolates according to their aggressiveness, expressed by the quantification of external and internal disease symptoms and by using a standardized scoring scale. Development of this method will lead to the selection of isolates to be used in screening for resistance or tolerance to BSR. Preliminary results seemed to show that it was possible to distinguish the degree of susceptibility of progenies inoculated artificially at the germinated seed stage or when transferred to polybags. A good correlation was obtained between those two stages, suggesting that the level of resistance was conserved irrespective of the physiological stage used for screening in the nursery. However, it is important to bear in mind that these results need to be confirmed and correlated with field observations under natural infection conditions.*

Keywords: *Ganoderma*, basal stem rot, oil palm, early screening test, inoculum potential.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.), the leading source of vegetable oil with 31.5 million tonnes produced in 2003, is subject to parasitic threats in each of its main growing zones. The leading production zone is now in Southeast Asia, mainly in Indonesia, where oil palm growing began in the second decade of the 20th century, and in Malaysia, where its cultivation is more recent.

The major obstacle to crop sustainability in this zone is a disease, basal stem rot (BSR), which irremediably ends in palm death. Although Sanderson *et al.* (2000) strongly suggested that spores are involved in the epidemiology, BSR is mainly considered as a soil-borne disease, spreading through root-to-root contact or infected debris. The causal fungus belongs to the genus *Ganoderma,* of which several species appear to be incriminated (Idris *et al.*, 2000). Although the taxonomy of the genus *Ganoderma* remains confused (Miller *et al.*, 1999), it now seems to be accepted (Ho and Nawawi, 1985; Khairudin *et al.*, 1991) that *Ganoderma boninense* is the predominant species responsible for disease occurrence and development.

BSR can cause considerable damage. The most seriously affected zones lie in north Sumatra, in Indonesia, and along the western coastal zones of Peninsular Malaysia (Singh, 1991; Ariffin and Idris, 2002). Indeed, it is not rare to find that all the palms in a given plot that are still standing at the end of the cropping cycle (25 years) are in fact infected by *Ganoderma*. Such infection occurs with the appearance of fruiting bodies at the base of the stem, together variety of symptoms ranging from unopened spears, to more or less total yellowing of the crown, and, in many cases, the appearance of deep cracks at the base of the stem.

It is commonly accepted that disease incidence worsens over successive replanting, due to continual inoculum development: massive palm, infection at the end of a cropping cycle clearly jeopardizes the phytosanitary condition of the field for any replanting. Consequently, the shakes are high, given that the fourth cropping cycle is well under way in north Sumatra, whilst the third cropping cycle is developing in Malaysia.

Cultural practices, notably involving elimination and sanitation measures, help to slow down the spread of the disease, but they are often based on costly logistics. They cannot meet the demands of integrated disease control, even when combined with biological control tools (biofungicides, mycorrhizae) whose efficiency and durability are currently being studied (Abdullah *et al.*, 2003).

Previous observations (Akbar *et al.*, 1971) indicate that variations in susceptibility to BSR could be detected within the main gene pools represented in the world's commercial oil palm, while very little attention was paid to the genetic aspects of integrated control until recently. Sources of resistance and of susceptibility were found in field trials in north Sumatra, by de Franqueville *et al.* (2001), subsequently confirmed by Durand-Gasselin *et al.* (2005). Field observations have proved to be coherent, given the genetic and statistical designs on which the trials were based.

These results suggest that available genetic resources can be used to improve the level of resistance in planting material for oil palm growers in BSR risk zones.

However, the breeding process takes a very long time to develop if it only relies on field trials, which are sometimes difficult to interpret depending on the phytosanitary condition of the previous cycle. In the absence of corrobating phenotypic data, use of molecular tools would be largely premature. It is therefore essential to develop an early screening test involving artificial inoculation of the pathogen, making it possible to distinguish rapidly between sources of susceptibility and sources of resistance in the palms to the disease.

Developing such a tool is a complex matter, due to the nature and variability of the pathogen (Miller *et al.*, 1999; Pilotti *et al.*, 2002). Nevertheless, some encouraging results based on 23 crosses were obtained by Idris *et al.* (2004), but the aim of the current research is to develop a test that can compare a large number of crosses, at the earliest stage possible, for which results are significantly correlated to those obtained on mature palms under natural field contamination conditions.

Several parameters govern the success of such tests and the aim of this paper is to scrutinize those that are important in the initiation of disease symptoms at an early stage (*Figure 1*).

The choice of isolates according to their aggressiveness, inoculum preparation, the volume of inoculum, the quality of shade under which incubation of the disease occurs are all aspects that need to be taken into consideration. Therefore, these parameters cannot be studied separately due to a strong relationship between them.

MATERIAL AND METHODS

General Principles

Planting material. The planting material used in this work came from seed production and was not, at this stage, derived from mating designs specifically intended to study possible sources of resistance or susceptibility, and to compare them with those detected under natural contamination conditions.

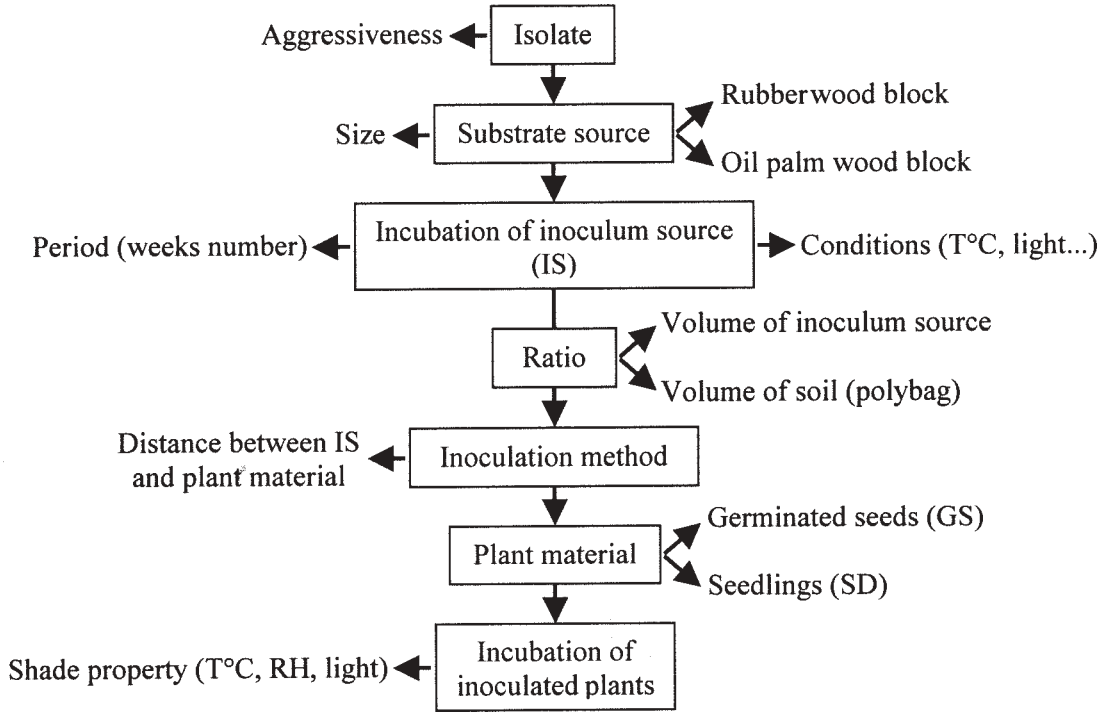

Figure 1. Identification of the major parameters involved in the Ganoderma *artificial inoculation method in the nursery which need to be improved and standardized.*

This material was inoculated either by planting germinated seeds in pre-nursery bags containing the inoculum, or by transplanting three-month-old seedlings in nursery bags containing the inoculum.

The distance between the planting material used and the inoculum source was standardized at 5 cm, irrespective of bag or inoculum size.

Ganoderma boninense *isolates.* Ten dikaryotic isolates of *Ganoderma boninense* were used in the experiments and are listed in *Table 1*.

TABLE 1. LIST OF *Ganoderma* ISOLATES AND THEIR ORIGIN

Isolates	Estate	Tissue
J	Bangun Bandar	Fruiting body
GI2	Tanah Gambus	Fruiting body
980264	Dolok	Infected stem
980533	Dolok	Infected stem
GI4	Tanah Gambus	Fruiting body
9810511	Dolok	Infected stem
D	Bangun Bandar	Fruiting body
GI3	Tanah Gambus	Fruiting body
R55P3	Dolok	Infected stem
A	Tanah Gambus	Infected stem

Inoculum. Preliminary trials confirmed that inoculation by a fungus culture in liquid medium or a culture on a sawdust-based substrate were not effective, even though substantial progress has been made in culturing *Ganoderma boninense* (Breton *et al.*, 2005a). The work described here is based on artificial inoculation using rubberwood blocks (RWB). RWBs were boiled for 6 hr, then autoclaved for 2 hr at 120°C in plastic bags containing 100 ml of potato agar (PA). After one night, the RWBs were each inoculated with four fragments of a 15-day-old *G. boninense* culture. The bags containing the RWBs were left to incubate in the dark at 27°C for varying lengths of time depending on the experiments. The size of the RWBs also varied depending on the type of experiment and the development stage at which the planting material was inoculated. The RWB incubation time depended on fungus penetration and the resulting colonization of tissues, but was generally between 12 and 16 weeks.

Symptom recording. Symptoms were recorded monthly, recording the numbers of plants displaying foliar symptoms and/or fruiting bodies. At the end of the trial, between 25 and 30 weeks after inoculation depending on how symptoms developed, the seedlings were dissected by making two longitudinal cuts in the bole and the severity of internal symptoms was assessed by a visual estimation of the proportion of tissues damaged by *G. boninense*. The estimation

was based on the following scale:

(0) healthy: no internal rot
(1) up to 20% rotting of bole tissues
(2) from 20 to 50% internal rotting
(3) over 50% internal rotting
(4) total rotting of bole tissues along with total desiccation of the plant

The results, infection percentages or symptom severity, were subjected to an analysis of variance and a Tukey test (p=0.05).

Trial conditions. Several exploratory trials were set up under various shade conditions: natural shades (D and E), semi-natural shades (B and C), artificial shade (A) or unshaded. Light and temperature were monitored under natural or artificial shade as recorded in *Figure 2*. The degrees of shade tested were called A to E.

Specific Trials Conducted

Ratio between the volume of inoculum and the volume of soil to be inoculated. A trial comparing the ratio between the volume of inoculum and the volume of soil to be inoculated was set up under natural shade. It comprised five treatments with four replicates of 10 plants inoculated at the germinated seed stage in pre-nursery bags (15 x 21 cm) with a single isolate (9805033). These treatments corresponded to the following ratios: i) 14%, ii) 7%, iii) 4%, iv) 2% and v) 0.7%.

Comparison of isolates and of their aggressiveness. Two trials comparing isolate aggressiveness were set up six months apart: one at the germinated seed stage, the other at the three-leafed seedling stage. Seven isolates were common to both trials, which were set-up under semi-natural shade. The host plant gene pool of each trial was similar randomly selected and of unknown susceptibility.

- Trial at the germinated seed stage: four replicates of 10 seeds per isolate
- Trial at the three-leaf stage: four replicates of 10 plants per isolate
- seven common isolates: 9810511, 9805033, 9808264, D, J, G12, G14
- Ratio of inoculum volume: soil volume: 5%

Effect of root injury prior to inoculation. A trial was conducted under semi-natural shade at the three-leaf stage. A single isolate (9805033) was used to inoculate seedlings from five specific crosses, for which the resistance level of the parental palms were unknown. There were four replicates of 10 seedlings per treatment (with or without injury). Wounds were made by cutting the roots 5 cm from the bole when the plants were transferred to nursery polybags with an inoculum:soil ratio of around 5%.

Physiological stage comparison. The trial was conducted under semi-natural shade using different progenies at two physiological stages, germinated seed and six-month-old seedlings. A single isolate (A) of *Ganoderma* was used to inoculate planting materials from seven crosses. Four replicates of 10 seedlings or germinated seeds per treatment were used with an inoculum: soil ratio of around 5%. It is important to point out that this trial was carried out at the same time as certain parameters of the screening method were being standardized.

RESULTS

Shade Effect

The effect of shading on disease development was discovered empirically. It was found that for similar inoculation conditions, disease development kinetics and the number of seedlings displaying symptoms varied considerably from one type of shade to the next: no symptoms without shade, few symptoms under artificial shade, rapid and substantial development of the disease under natural shade (*Figure 2*).

An indicative trial, in which cultures of *G. boninense* were placed in petri dishes under aluminium foil, on a bamboo pole under different shade conditions, showed, nine days after incubation, that *in vitro* mycelium growth was significantly less without shade than for any of the other treatments (*Figure 3*). It is important to note that shades B and C were similar in composition, but were located in different areas.

Figure 4 shows variations in the percentage of infections recorded in simultaneous trials conducted on seedlings at the same physiological stage, derived from similar gene pools and inoculated with the same isolate of *G. boninense*.

Shade A was not efficient for rapid induction of *Ganoderma* disease symptoms, as compared with shades B-C and E. Shade A displayed high light intensity leading to a higher temperature than shades B-C and E (*Figure 2*). The inhibition of *in vitro* mycelium growth observed under shade A seemed to be related to the temperature recorded under that shade regime. These results may explain the low symptom expression observed under shade A. Consequently, the temperature effect under the tested degrees of shade was not the only major factor involved in the efficiency of disease expression. The temperature recorded under shades B-C was slightly higher than that under shade E (*Figure 2*). Shade E therefore demonstrated a slightly lower disease expression efficiency than shades B-C (*Figure 4*). However, according to our observations, the differences recorded in these cases might also have been due to better relative humidity under shades

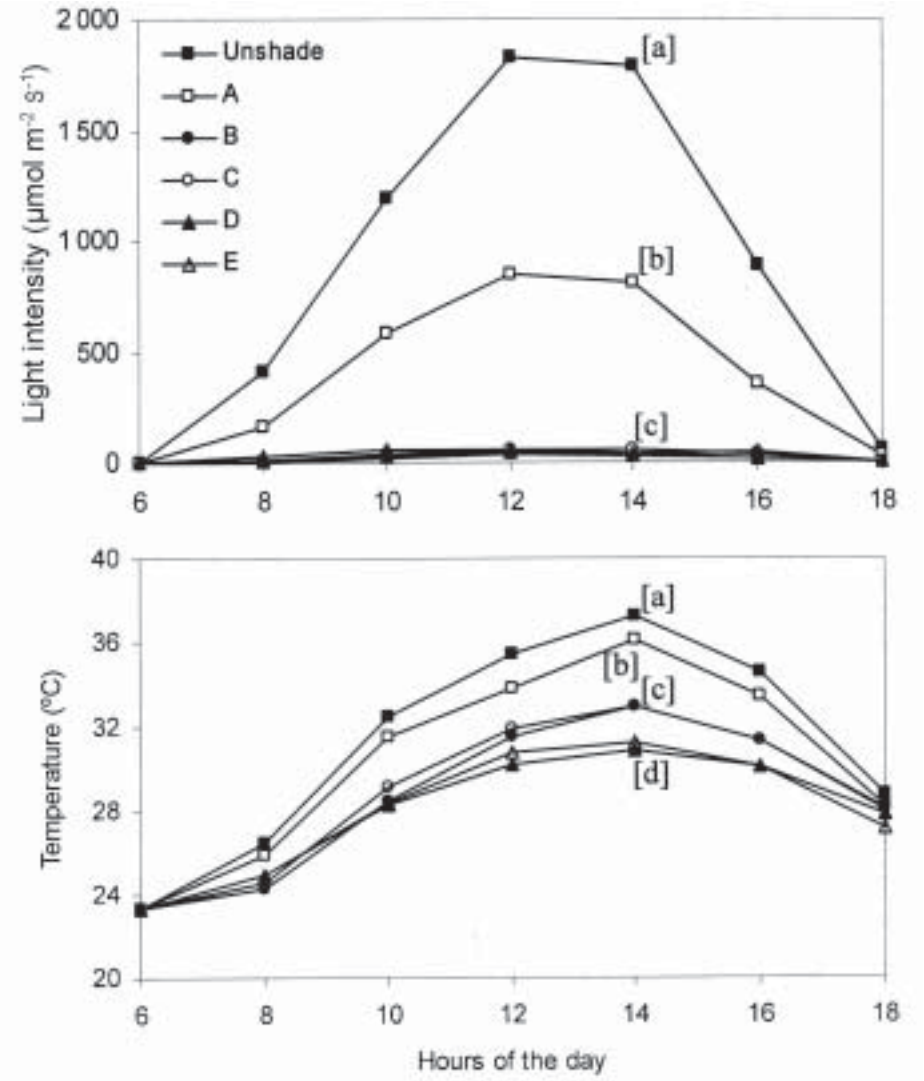

Figure 2. Characterization of shade in terms of light and temperature. Curves with a common letter are not statistically significant by Tukey test at p=0.005 (2.00 pm data).

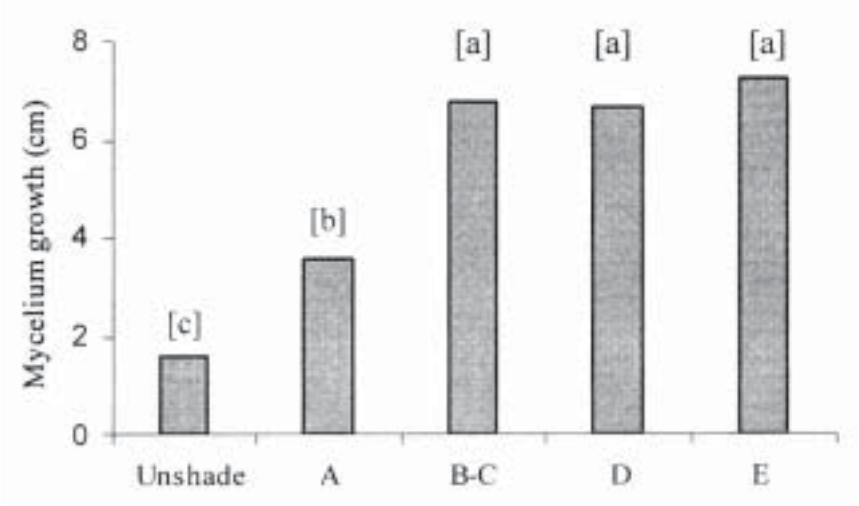

Figure 3. Characterization of in vitro *mycelium growth nine days after incubation under different shade conditions. Bars with a common letter are not significantly different at p=0.005 with Tukey test.*

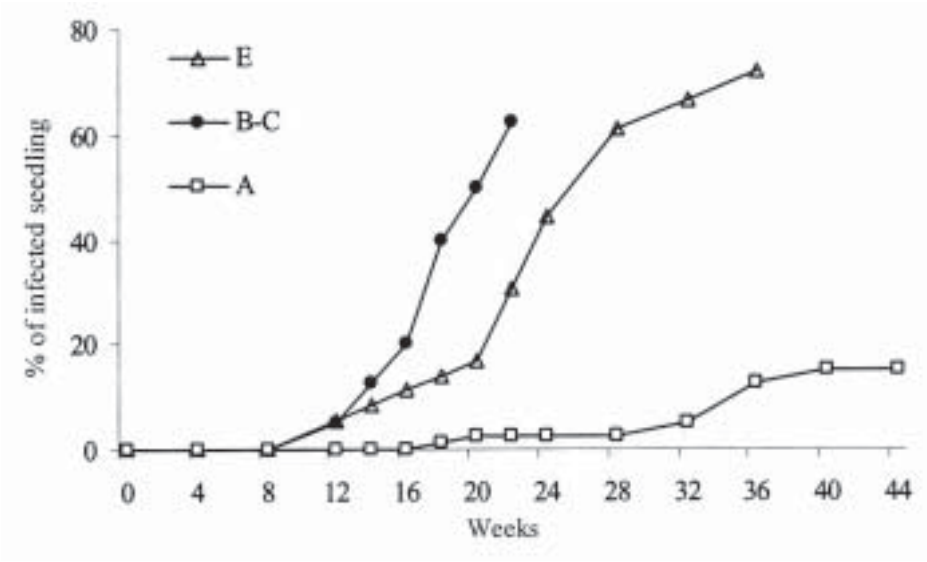

Figure 4. Effect of shade on disease expression.

B-C than under shade E. The same observation can be made for shade D (data not shown). Shade B or C were characterized by a temperature and relative humidity which did not inhibit mycelium growth and seemed to favour the process of seedling infection.

Ganoderma is a soil-borne fungus which the development dependent on soil temperature rather than ambient temperature. Therefore, the difference of ambient temperature recorded under the tested shades has a significant impact on the temperature inside the nursery polybag, leading, for the best shade, to decrease of several degrees (up to 10°C) the temperature of the soil in comparison with unshade condition (data not shown).

Inoculum Source (rubberwood block) Incubation Time

The inoculum source incubation time was linked to the volume of the RWB used. Under our experimental conditions, four incubation times were tested (8, 12, 16 and 20 weeks). After incubation, some blocks were cut to observe mycelium penetration inside the wood in relation to their *pathogenicity* recorded later on infected seedlings. Eight weeks after incubation, mycelium development was still on the surface of the RWB. The percentage of infected seedlings was around 5% and non-reproducible, 28 weeks after seedling inoculation (data not shown). Twelve to 20 weeks after incubation, mycelium could be seen inside the RWBs. These three incubation times were efficient for inducing reproducible disease symptoms, but RWBs after 20 weeks incubation gave the highest percentage of infected seedling (*Figure 5*). It should be noted that no significant difference was observed between efficacy of RWBs after 12 and 16 weeks incubation. RWBs after 20 weeks of incubation seemed to produce a too strong an inoculum potential unsuitable for progeny discrimination, as compared with 12 and 16 weeks (*Figure 6*).

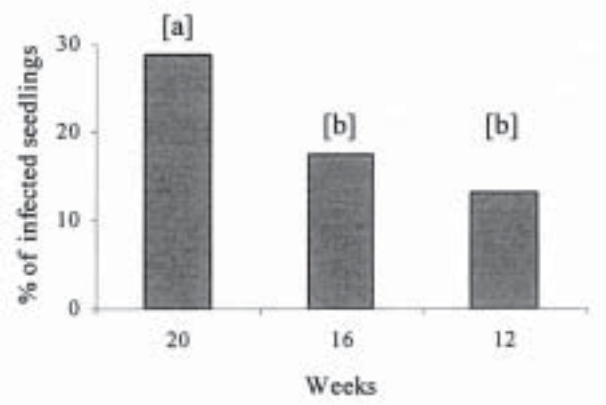

Figure 5. Effect of the inoculum source (RWB) incubation time on the percentage of infected seedlings. Bars with a common letter are not significantly different at p=0.005 with Tukey test.

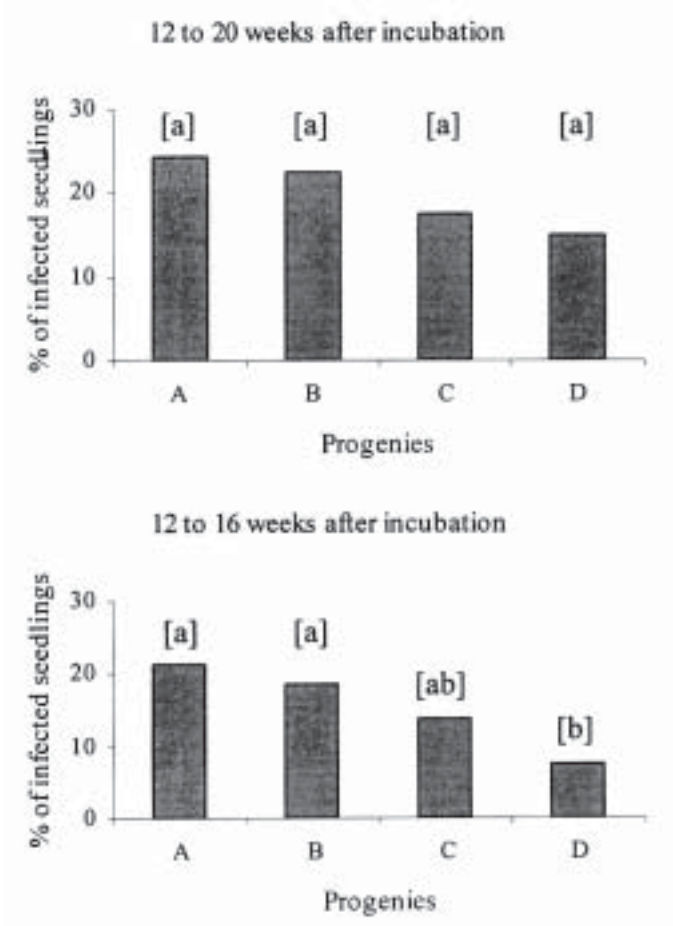

Figure 6. Discrimination between progenies depending on the incubation time of the inoculum source (RWB). Bars with a common letter are not significantly different at p=0.005 with Tukey test.

Ratio of the Inoculum Volume to the Volume of Soil to be Inoculated

Figure 7 shows that the infection rate logically depended on the ratio of the volume of the inoculum to the volume of soil to be inoculated. The lowest ratios (0.7% and 2%) induced few or no symptoms. The highest ratio (14%) may not have been discriminatory enough due to its excessively high inoculum potential. The intermediate ratios, between 4% and 7%, appeared to form an acceptable compromise, though that needs to be confirmed.

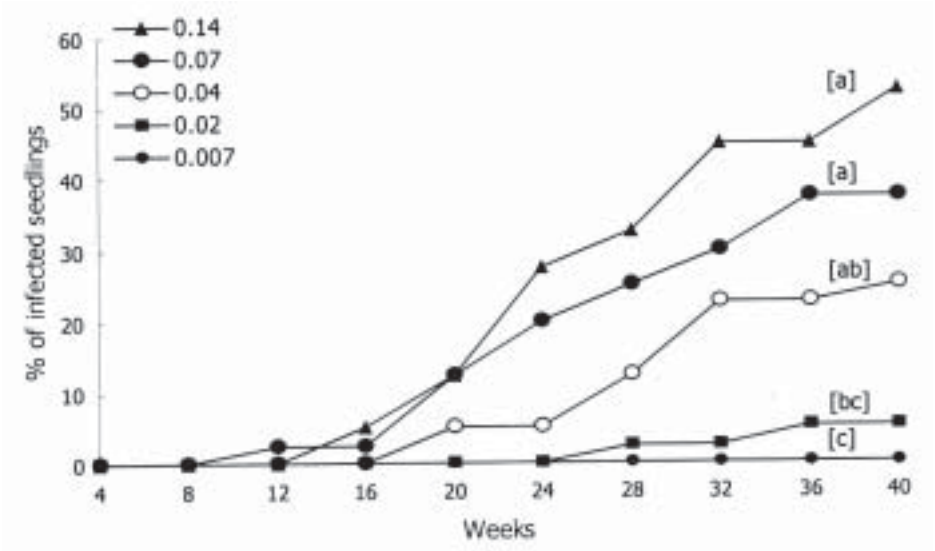

Figure 7. Effect of the inoculum: soil ratio on disease expression. Curves with a common letter are not statistically significant by Tukey test at p=0.005.

Another important parameter inside the polybag was the distance between the source of inoculum and the planted material (germinated seed or seedling). When germinated seeds were inoculated, a difference of 5 cm could lead to a significant difference of 40% of infected seedlings, 20 weeks after inoculation. Standardization of that distance seems to be necessary to decrease this high variability which could have a considerable impact on the results of a progeny test.

Comparison of the Isolates and their Aggressiveness

Significant differences existed between the isolates, whether they were inoculated at the germinated seed stage (*Figure 8*) or at the three-leaf stage (*Figure 9*). These differences were evident either when the results were expressed as a percentage of diseased seedlings or when they were expressed in terms of the severity of internal symptoms. It should also be noted that a very good correlation existed between isolate classifications in the two independent experiments ($r^2 = 0.85$, *Figure 10*).

The severity of internal symptoms also showed significant differences in relation to isolate aggressiveness (*Figure 11*). The results showed a positive correlation between the isolate pathogenicity recorded when the percentage of infection was considered (*Figures 8* and *9*) and their aggressiveness estimated by quantifying the symptoms (*Figure 10*). It is important to point out

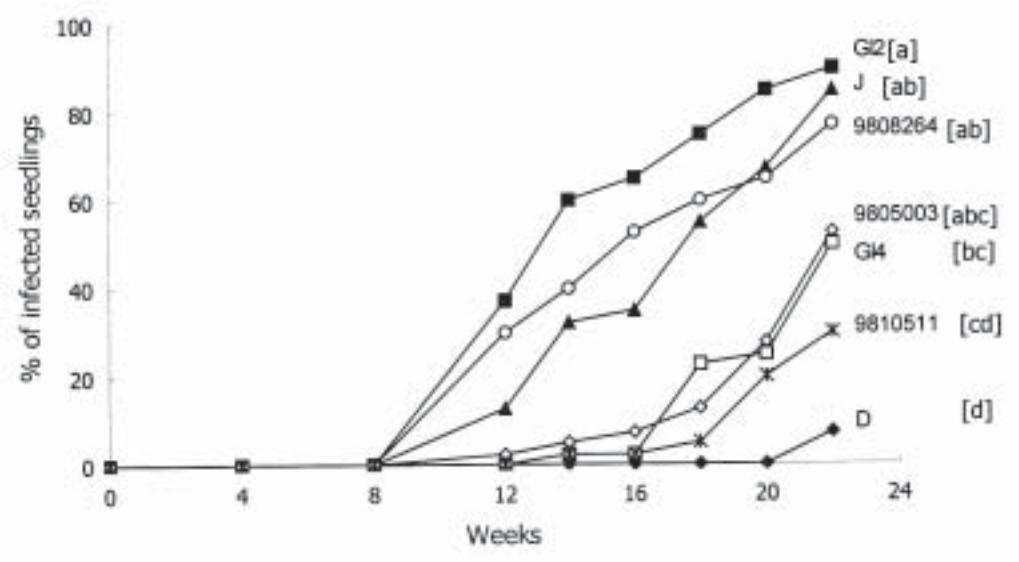

Figure 8. Characterization of isolate aggressiveness after inoculation at the germinated seed stage. Curves with a common letter are not statistically significant by Tukey test at p=0.005 (data 22 weeks).

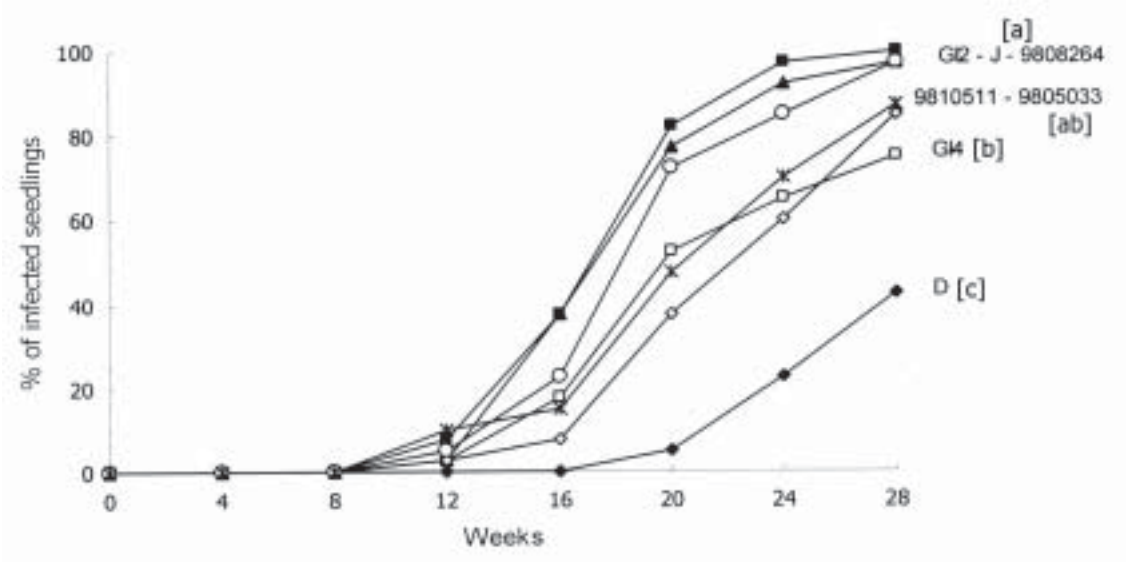

Figure 9. Characterization of isolate aggressiveness after inoculation at the three-leaf stage. Curves with a common letter are not statistically significant by Tukey test at p=0.005 (data 28 weeks).

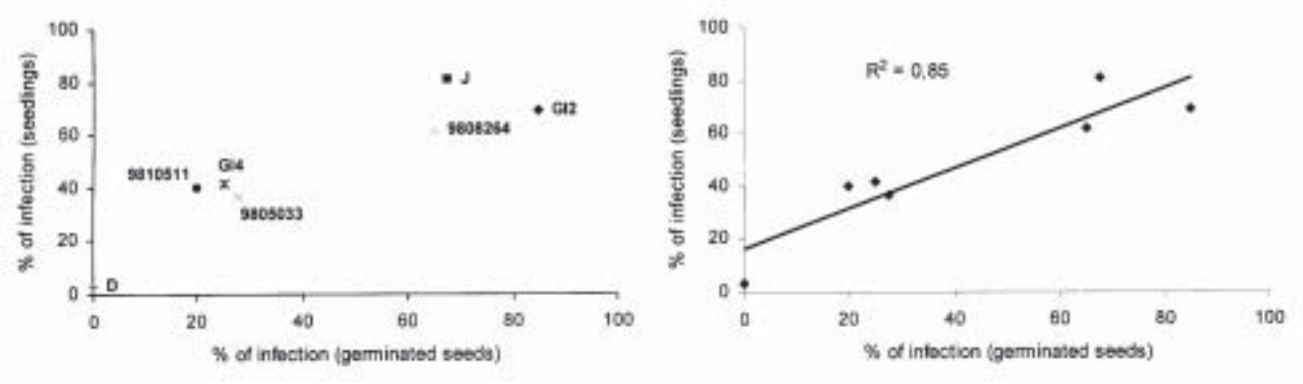

Figure 10. Relationship between germinated seed stage results and three-leaf stage results (20 weeks after inoculation).

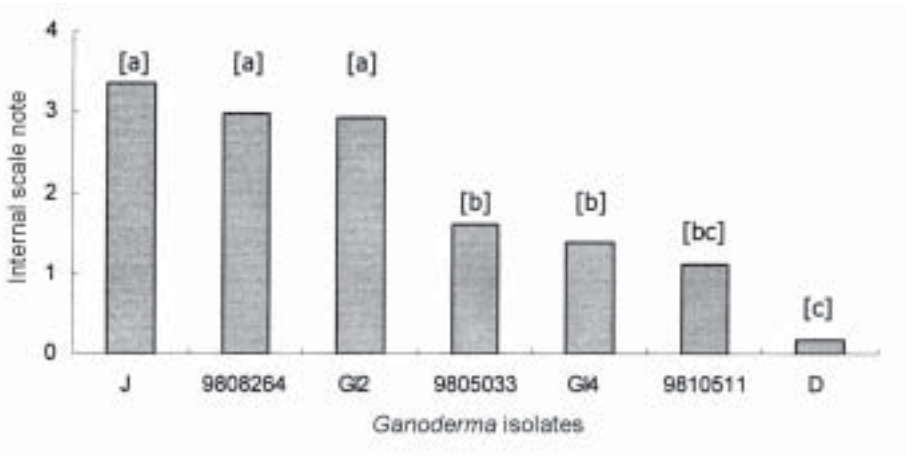

Figure 11. Severity of internal symptoms depending on Ganoderma *isolates. Bars with a common letter are not significantly different at p=0.005 with Tukey test.*

that using the percentage of infection, which took the number of individuals more into account, made it possible to estimate the pathogenicity of the isolates (qualitative component of pathogenicity), *i.e.* their ability to infect the host plant and induce disease symptoms independently from their intensity. On the other hand, quantification of symptoms using an appropriate recording scale made it possible to estimate the aggressiveness of the isolates (quantitative component of the pathogenicity) via the intensity of damage caused by the pathogen. Under our experimental conditions, we were able to detect a positive correlation between the pathogenicity and the aggressiveness of the isolates used in the previous experiments. However, further experiments should make it possible to define the most discriminatory recording method for screening oil palm progenies in response to *Ganoderma* challenge inoculations.

Effect of Root Injury Prior to Inoculation

Figure 12 shows that root injury speeded up the appearance of disease symptoms. There were no interactions between wounds and the performance of the crosses, which did not differ from each other, irrespective of the scenario. Any interaction will be studied more closely once a known susceptibility range is available.

Preliminary Results of Screening Using Germinated Seed *vs.* Six-Month-Old Seedling Stages

The results showed that it was possible to distinguish between progenies using our inoculation method as regards their different levels of susceptibility to *Ganoderma* (*Figure 13*). A positive correlation between the two physiological stages of inoculation was observed. These preliminary results seem to indicate that the genetic background involved in the tolerance/susceptibility to *Ganoderma* is conserved for the two physiological stages tested.

DISCUSSION AND CONCLUSIONS

The results presented in this paper strongly suggest that variation in or more of the parameters of environmental pre-disposing factors and inoculum potential greatly influence success or failure of oil palm inoculations with *Ganoderma boninense*.

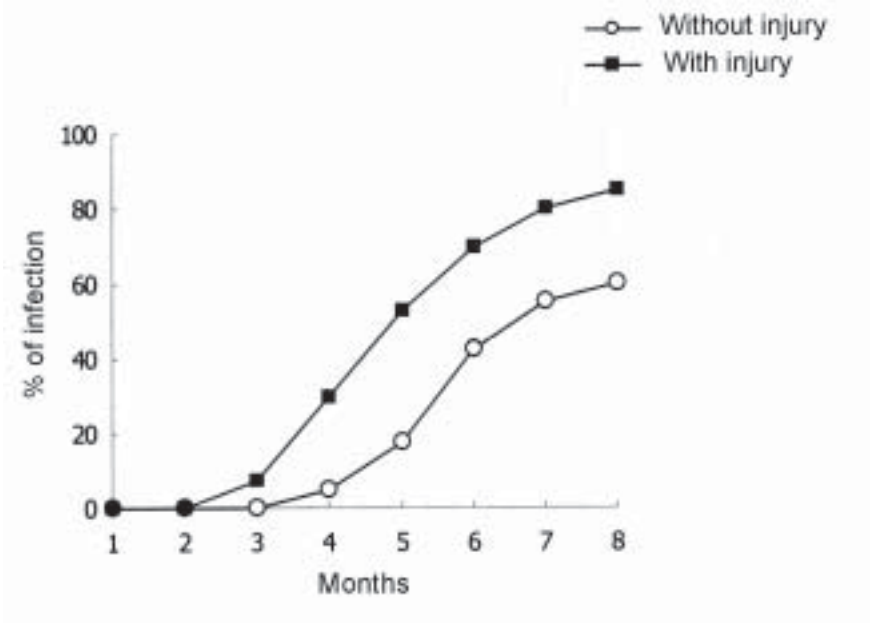

Figure 12. General effect of root injury on the percentage of infected seedlings.

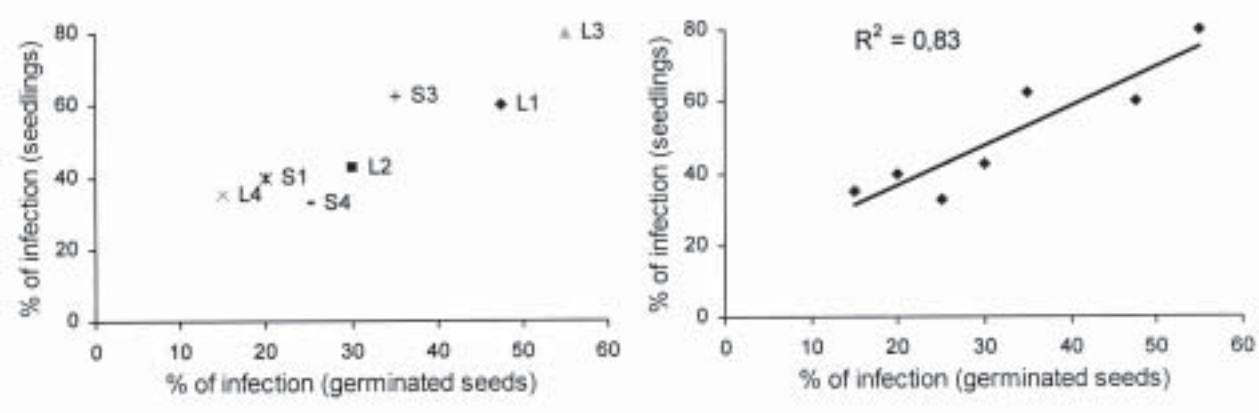

Figure 13. Correlation of the screening of seven progenies with their susceptibility to Ganoderma *at two physiological stages.*

Shade

This is clearly an element that is conducive to the success of the infection process. It was under natural shade that disease kinetics was fastest, whereas no symptoms occurred in the absence of shade. However, natural shade, be it bamboo or the oil palm canopy, did not enable a uniform comparison of a sufficiently large number of crosses. It ought to be possible to consider artificial shade in the long-term, depending on the volume and longevity of any resistance screening, by using suitable greenhouse nets that filters sufficiently.

However, *in vitro* mycelium growth measurements taken *in situ* showed that it slowed down under artificial shade and especially when there was no shade. This observation tallies with data obtained in the laboratory by Nawawi and Ho (1990) who reported that mycelium growth slowed down over 30°C-35°C.

It is always risky to extrapolate this type of result to what really happens under natural conditions, but it is nonetheless interesting to note that in replanting palms that are about the ages of six or seven years, *i.e.* when the entire soil of the plantation is covered by the oil palm canopy, that BSR development reaches significant proportions.

Inoculum Preparation

Several studies have emphasized the importance of the volume of inoculum in the success of the infection process, be it under artificial conditions (Khairudin *et al.*, 1991) or under natural conditions (Turner, 1981).

In a trial described by Khairudin (1993), the earliest symptoms were obtained after six months with an inoculum of 432 cm^3 (11.8% infection) and the same percentage was only achieved after nine months with an inoculum of 216 cm^3; 5.9% infection was obtained at 11 months with inocula of 54 and

108 cm^3. Furthermore, Khairudin *et al.* (1991) pointed out that Navaratnam and Chee (1965) established that 734 cm^3 was the threshold beyond which infection could occur. The single root inoculation technique described by Idris *et al.* (2004) gave positive results, but little information was given about incubation times prior to symptom expression.

The inoculations carried out in the study described in this paper mostly involved RWBs measuring 90 or 216 cm^3, and ratios of around 5% to 7%, inducing symptoms three months later.

It is therefore not very clear whether it is the volume of inoculum that matters or whether it is the ratio between that volume and the volume of soil to be contaminated, or even the area of contact between the root and the inoculum. The RWBs need to be sufficiently colonized internally by *Ganoderma boninense*. Under our experimental conditions, it appeared that a minimum incubation time of 12 weeks was required to obtain sufficient uniform symptoms. However, it needs to be borne in mind that the incubation time depended not only on the RWB preparation and sterilization process, the size of the inoculum, but also on the nature of the isolate and its ability to colonize the substrate used. The incubation time is a parameter that it is very important to standardize as it will have a direct effect on screening results in terms of the resistance/ susceptibility of the progenies (results not shown).

Good colonization is reflected in the presence of stromatic-like structures on the surface of the RWBs and by the presence of mycelium inside the substrate. In the case of *Ganoderma*, the formation of such structures seems to be an important and essential parameter for seedling infection via the roots (Breton *et al.*, 2005b). Therefore, the role of the stromatic-like structure in the infection process requires further and specific investigations.

Root Injury

Root wounds speeded up the expression of symptoms, but were not essential for the success of the infection process, which tallied with the results obtained in Malaysia on older plants (Ariffin and Idris, 2002).

This is an important point insofar as, for vascular wilt, excessive root injuries can modify a resistance range that is otherwise known and confirmed (de Franqueville, pers. comm.). When the time comes, it will be necessary to check whether that is the case with *Ganoderma boninense*.

Isolate Aggressiveness

Classification of the aggressiveness of a range of *Ganoderma boninense* isolates was not modified depending on whether inoculation was carried out at the germinated seed stage or when the three-leafed seedlings were transferred to nursery bags. Of course, two different trials were involved, but they were conducted under the same kind of shade. Disease kinetics was generally faster at the germinated seed stage for moderately aggressive to very aggressive isolates, slower for slightly aggressive isolates. In addition, statistical analyses seemed to indicate that the discrimination between isolates was greater at the germinated seed stage than at the time of transfer to large polybags.

For the rest of this work, it is essential to compare the aggressiveness of the different isolates, given that it is not advisable to work with non-discriminatory isolates, either because they are too aggressive, or because they are not aggressive enough. In the results presented isolates such as GI4 or 9805033 would be candidates for future studies. However, this result needs to be fine-tuned in further experiments.

Inoculation Stage

The work described in this paper constitutes the preliminary development of a test protocol for the early detection of sources of resistance in oil palm to *Ganoderma* infection. This approach takes its inspiration from that used for vascular wilt in West Africa (de Franqueville and Diabaté, 1995) and is considered justified by Corley and Tinker (2003).

The inoculation techniques described to date (Khairudin *et al.*, 1991) have generally been used on three-month-old seedlings at the moment they are transferred to the main nursery, with symptom incubation generally lasting several months. These techniques are frequently used to study the pathogenicity of an isolate (Khairudin *et al.*, 1991), the effect of fertilization (Mohd Tayeb *et al.*, 2003) or that of a biological control agent (Abdullah *et al.*, 2003; Sundram *et al.*, 2003). So far, few attempts have been made to develop them for screening purposes, apart from those described by Idris *et al.* (2004), where inoculation was carried out on single roots of 12-month-old oil palms, and observations were continued for 12 months after inoculation.

Challenge inoculations at the germinated seed stage, enables the first symptoms to develop three months later. Among the advantages, we would of course point out the time saved and the smaller area required, with a technique that minimizes root injury at the time the seedlings are transferred to polybags, injury that might interfere or negate the biological resistance mechanisms of the intact root. Nevertheless, it needs to be borne in mind that working at the germinated seed stage is not risk-free, notably because we have yet to master the uniform emergence of seedlings after inoculation.

Inoculation at this early stage of development (germinated seeds) gave some encouraging results, rendering differential differences between crosses in response to the inoculation method. A positive

correlation was also obtained between germinated seeds and six-month-old seedlings in terms of the discrimination between progenies for their susceptibility to *Ganoderma*. This preliminary result suggests that the response of progenies to artificial inoculation in the nursery is conserved irrespective of the physiological stage used for the screening test. Other experiments should confirm or refute this result.

Nevertheless, caution is called for because the preliminary work described here indicates that the infection process, and especially its ability to distinguish the object being studied (*e.g.* cross or biofungicide), depends on a delicate balance between the different components of the pathosystem (plant, pathogen, environment). This study is the first to describe and define the importance of a certain number of parameters involved in uniform and reproducible induction of disease symptoms through artificial inoculation of germinated seeds or seedlings. Due to the close relationship between these different parameters, standardization of this screening method would be a lengthy process, but the results to date are encouraging and the success in developing such a selection tool is promising.

This study first identifies and defines the basics prerequisite parameters for a reliable early screening method for tolerance / resistance in oil palm seedlings against *Ganoderma* BSR. The protocol needs to be improved, standardized and validated by a significant correlation with observations carried out under natural infection conditions. Specific mating designs are being implemented that will soon show whether it is possible to reach this correlation between artificial and natural contamination.

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REFERENCES

ABDULLAH, F; IZATTI, M Z N A; ILIAS, G N M; NELSON, M and UMI KALSOM, M Y (2003). The efficacy of *Trichoderma harzanium* as a biocontrol agent of basal stem rot of oil palm. *Proc. of the PIPOC 2003 International Palm Oil Congress – Agriculture Conference*. MPOB, Bangi. p. 1056-1061.

AKBAR, U; KUSNADY, M and OLLAGNIER, M (1971). Influence de la nature du matériel végétal et de la nutrition minérale sur la pourriture sèche du tronc due à *Ganoderma*. *Oléagineux, 26 (8-9)*: 527-534.

ARIFFIN, D and IDRIS, A S (2002). Progress and research on *Ganoderma* basal stem rot of oil palm. *Proc. of the Seminar on Elevating the National Oil Palm Productivity and Recent Progress in the Management of Peat and* Ganoderma. MPOB, Bangi. 5-6 May 2002. p. 167-205.

BRETON, F; HASAN, Y; HARIADI; LUBIS, Z and de FRANQUEVILLE, H (2005a). Preliminary research of pathogenic components from *Ganoderma* isolates, in relation to their aggressiveness after artificial inoculation of oil palm seedlings. *Proc. of the PIPOC 2005 International Palm Oil Congress – Agriculture Conference*. MPOB, Bangi. p. 962.

BRETON, F; HASAN, Y; HARIADI; LUBIS, Z and de FRANQUEVILLE, H (2005b). Rhizotron: a demonstrative tool for monitoring *in vivo* the infection process of oil palm seedlings by *Ganoderma boninense*. *Proc. of the PIPOC 2005 International Palm Oil Congress – Agriculture Conference*. MPOB, Bangi. p. 971.

CORLEY, R H V C and TINKER, P B (2003). *The Oil Palm*. Fourth edition. Blackwell publishing. 562 pp.

de FRANQUEVILLE, H and DIABATÉ, S (1995). La fusariose du palmier à huile en Afrique de l'Ouest – Plantations. *Recherche, Développement, 2(4)*: 5-13.

de FRANQUEVILLE, H; ASMADY, H ; JACQUEMARD, J C ; HAYUN, Z and DURAND-GASSELIN, T (2001). Indications on sources of oil palm (*Elaeis guineensis* Jacq.) genetic resistance and susceptibility to *Ganoderma* sp., the cause of basal stem rot. *Proc. of the 2001 PIPOC International Palm Oil Congress - Agriculture Conference*. MPOB, Bangi. p. 420-431.

DURAND-GASSELIN, T; ASMADY, H; FLORI, A; JACQUEMARD, J C; HAYUN, Z; BRETON, F and de FRANQUEVILLE, H (2005). Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninense* – prospects for future breeding. *Mycopathologia, 159*: 93-100.

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

IDRIS, A S; ARIFFIN, D; SWINBURNE, T R and WATT, T A (2000). The identity of *Ganoderma* species responsible for BSR disease of oil palm in Malaysia. Morphological characteristics. *MPOB Information Series No. 102*. 4 pp.

IDRIS, A S; KUSHAIRI, A; ISMAIL, S and ARIFFIN, D (2004). Selection for partial resistance in oil palm to *Ganoderma* basal stem rot. *J. Oil Palm Research Vol. 16 No. 2*: 12-18.

KHAIRUDIN, H (1993). Basal stem rot of oil palm caused by *Ganoderma boninense*: an update. *Proc. of the 1993 PORIM International Palm Oil Congress - Agriculture Conference*. PORIM, Bangi. p. 739-749.

KHAIRUDIN, H; LING, T K and RAZAK, A R A (1991). Pathogenicity of *Ganoderma boninese* Pat. on oil palm seedlings. *Proc. of the 1991 PORIM International Palm Oil Congress – Agriculture Conference*. PORIM, Bangi. p. 418-423.

MILLER, R N G; HOLDERNESS, M; BRIDGE, P D; CHUNG, G F and ZAKARIA, M H (1999). Genetic diversity of *Ganoderma* in oil palm plantings. *Plant Pathology, 48*: 595-603.

MOHD TAYEB, D; IDRIS, A S and MOHD HANIFF, H (2003). Reduction of *Ganoderma* infection oil palm through balanced fertilization in peat. *Proc. of the PIPOC 2003 International Palm Oil Congress – Agriculture Conference*. MPOB, Bangi. p. 193-219.

NAWAWI, A and HO, Y W (1990). Effect of temperature and pH on growth pattern of *Ganoderma boninense* from oil palm in Peninsular Malaysia. *Pertanika*, *13*: 303-307.

PILOTTI, C A; SANDERSON, F R and AITKEN, E A B (2002). Sexuality and interactions of monokaryotic and dikaryotic mycelia of *Ganoderma boninense*. *Mycological Research, 106 (11)*: 1315-1322.

SANDERSON, F R; PILOTTI, C A and BRIDGE, P D (2000). Basidiospores: their influence on our thinking regarding a control strategy for basal stem rot of oil palm. Ganoderma *Diseases of Perennial Crops* (Flood, J; Bridge, P D and Holderness, M eds). Cabi Publishing. p. 113-119.

SINGH, G (1990). *Ganoderma* - the scourge of the oil palm in the coastal areas. *Proc. of the* Ganoderma *Workshop*. PORIM, Bangi. p. 113-131.

SUNDRAM, S; ABDULLAH, F; ZAINAL ABIDIN, M A and UMI KALSOM, M Y (2003). The use of single and mixed strains of *Trichoderma harzanium* as biological control agents on oil palm seedlings. *Proc. of the PIPOC 2003 International Palm Oil Congress – Agriculture Conference*. MPOB, Bangi. p. 1029-1033.

TURNER, P D (1981). *Oil Palm Diseases and Disorders*. Oxford University Press. 280 pp.