GANODERMA DISEASE OF THE OIL PALM: HYPOTHESIS ON NATURAL INFECTION AND IMPLEMENTATION OF AN EARLY ARTIFICIAL INOCULATION TEST TO SCREEN OIL PALM PROGENIES FOR THEIR LEVEL OF RESISTANCE

F. BRETON*, R. MIRANTI**, Z. LUBIS***, Z. HAYUN***, S. UMI**, A. FLORI*, S.P.C. NELSON**, T. DURAND-GASSELIN*, J.C. JACQUEMARD****, H. DE FRANQUEVILLE*

*Cirad Oil Palm Breeding, TA A 28/01, Avenue Agropolis, Montpellier F34398 Cedex 5, France.

**PT PP London Sumatra Indonesia TBK, Sumatra Bioscience, Jl. Jend. A Yani No. 2, P.O. Box 1154, Medan 20111, Indonesia.

***PT Socfin Indonesia, Jl. K.L. Yos Sudarso No. 106, P.O. Box 1254, Medan 20115, Indonesia.

**** Cirad Oil Palm Breeding, Scientific and Technical Adviser to PT Socfin Indonesia

Keywords: *Elaeis guineensis*, *Ganoderma*, Basal stem rot, Oil palm, early screening test, breeding for resistance, artificial inoculation

SUMMARY

Genetic resistance to basal stem rot (BSR) of oil palm is a major component of an integrated control strategy for BSR disease. Early detection of the level of resistance or susceptibility is of paramount importance for the breeding and sustainability of this crop particularly in Southeast Asia. A screening test of oil palm progenies has been developed and validated by using planting material from two Indonesian private companies - PT PP London Sumatra Indonesia (Lonsum) and PT Socfin Indonesia (Socfindo). This test was performed in the nursery, at the germinated seed stage, using a shade characterized by a light filtration of around 85%. Rubber wood blocks (RWBs) infected by *Ganoderma*

boninense 12 weeks prior to inoculation were used as the inoculum source. The first disease symptoms appeared between 8 to 10 weeks after the nursery bags were inoculated and germinated seed planted. Analyses for each tested cross showed positive correlation between 16 and 28 weeks indicating that early selection is realistic and there is minimal interaction of disease susceptibility with time. These results were consistent and reproducible and no changes in the ranking were observed between external and internal symptoms recordings. Statistical analyses demonstrated significant variation between progenies and consistent ranking of progenies tested in independent trials. The analyses of the F values indicated that the kinetics of the screening test reached an acceptable level of discrimination 20 weeks after inoculation. Nursery results compared with field results, recorded in natural condition of infection, showed that with the test, no highly-susceptible progenies would have been planted. The major aim of this paper is to highlight the efficiency and the reproducibility of this early prenursery inoculation test which allows to screen now around 100 progenies per month. In parallel, the development of a melanised tough mycelium named stromatic-like structure has been observed in the lignified cavity at the base of infected palm; Root-Bol interface. Randomly field observations of the young and mature palm seem to demonstrate that the initial point of *Ganoderma* penetration is localised in this lignified area. No initial *Ganoderma* invasion of young palms by the fungus was observed at the peripheral area of this lignified cavity.

INTRODUCTION

Oil palm cultivation is subject to parasitic threats in each of its main growing areas. In Latin America, “bud-rot type” diseases are prevalent (de Franqueville, 2003) and recent findings on the possible role of *Phytophthora palmivora* are promising in terms of research and disease management (Martinez *et al.*, 2008; Sarria *et al*., 2008). In Africa, the main disease is *Fusarium* wilt (vascular wilt), caused by *Fusarium oxysporum* f.sp. *elaeidis* (de Franqueville and Diabaté, 1995, 2004; Flood, 2006). In Southeast Asia, which is now the main production zone, basal stem rot caused by *Ganoderma boninense* leads to considerable damage in North Sumatra, Indonesia and in many parts of peninsula

Malaysia. BSR is known also in Africa and in Latin America, but with a lower incidence. Based on field observations, an hypothesis on the mode of the natural infection of the palms is proposed in this paper. This is the reason why the paper will be divided in two parts: i) a possible pathway for natural infection and ii) implementation of an early inoculation test to screen progenies for their resistance to BSR.

i) A possible pathway for natural infection

The damages caused by the disease increase when oil palm is replanted after oil palm and these damages are more and more drastic through the successive replanting cycles (Singh, 1991; Ariffin and Idris, 2002).*Ganoderma boninense* is mainly considered as a soil-borne pathogen transmitted through root contacts in the soil. The role of basidiospores in the infection process remains unclear even if the genetic diversity can be linked to this stage of the fungal life cycle. No natural or artificial infection involving only basidiospores as a source of inoculum has been reported so far. Therefore, it seems that the infection pathway through the root plays a major role in the spread of BSR within plantations and there is very little doubt that the fungal progression in these roots leads to the disease. What about the bole colonisation?

Breton *et al.* (2005a), investigating and setting up the best parameters to rapidly induce symptoms by artificial inoculation, showed that rubber wood blocks (RWB) inoculated with *Ganoderma* developed at the surface an extensive fungal mass, highly melanised. This fungal tissue extremely tough forms a stromatic-like structure (SLS), or a pseudo-sclerotium, on the inoculum source. The use of a rhizotron (or glass chamber) method for monitoring *in vivo* the infection process of oil palm seedling, demonstrated that the development of these melanised SLS by *Ganoderma* could be observed only on a solid substrate, such as RWB (Breton *et al.* 2005b). No SLS were produced using pre-infected rubber sawdust and therefore no infection was obtained. Moreover, artificial inoculation of seedlings using inoculated RWB previously cleaned of SLS demonstrated also the necessity of this structure to induce a rapid infection (unpublished data). What can be the hypothesis which links the results of artificial infection (prenursery) involving a major role of the SLS with the observations in natural infection of mature palms? The presence and the localisation of this SLS in natural Infection were explored on oil palm plantations. Irrespective of the continent, the age of a palm or its sanitary status, the presence of

a natural basal star-shape crack or cavity is always observed. Randomly observations of this natural crack were performed in order to determine whether this cavity can play a role in the palm infection.

ii) Implementation of an early inoculation test to screen progenies for their resistance to BSR

Screening for resistance to vascular wilt in Africa, based on artificial inoculations of *Fusarium oxysporum* f.sp. *elaeidis*, started in the 1960s at the nursery stage first, then at the pre-nursery stage in the 1970s (Renard *et al*., 1980) and proved to be highly efficient for controlling the disease (de Franqueville and Renard, 1990). Flood (2006) notes that dramatic reductions in losses from this disease have been reported from some areas following introduction of resistant material. For BSR, Corley and Tinker (2003) consider that the best approach to controlling the disease may, in the longer term, be to develop tolerant material using nursery inoculation for screening, in much the same way as has been done for *Fusarium* wilt.

However, and despite some outdated observations (Akbar *et al.,* 1971) that variations in susceptibility to BSR could be detected from some of the main gene pools, very little attention was paid to the genetic aspects of integrated control until recently. Sources of resistance and susceptibility were found in field trials implemented at Socfindo in North Sumatra (de Franqueville *et al.,* 2001; Durand-Gasselin *et al.*, 2005). Field observations have proven to be consistent, given the genetic and statistical designs on which the trials were based. These results opened up the way for using available genetic resources to improve the level of resistance in planting material proposed to oil palm growers in BSR-risk areas.

The breeding process, of course, takes a very long time to develop if it only relies on field trials. To make it possible to distinguish rapidly between sources of susceptibility and resistance to the disease, it is, therefore, important to develop an early screening test involving artificial inoculation of the pathogen. It was this hypothesis that led to the collaboration of Cirad with two companies, Socfindo and Lonsum, to set up an early screening test to improve resistance to BSR disease.

Due to the nature and variability of the pathogen (Miller *et al.*, 1999; Pilotti *et al.*, 2002), developing such a tool is complex. Nevertheless, Breton *et al.* (2005a, 2006) characterised and standardised

several major parameters governing the success of an early screening test (artificial inoculation). These included the physiological stage of the planting material, nursery shade, temperature, incubation time and size of the inoculum source- most of them confirmed by Rees *et al.* (2007). The method of germinated seed inoculation described by Breton *et al.* (2005a, 2006) led to reproducible disease symptoms only three months after inoculation. It was demonstrated that oil palm progenies can be screened early in prenursery for their resistance level to *Ganoderma* infection. Idris *et al.* (2006) obtained similar results at the same stage which corroborates the usefulness for breeders to adopt this method of screening.

The aim of this second part of the paper is to highlight the efficiency and the reproducibility of this screening test, following the first step of the parameters' characterisation.

MATERIALS AND METHODS

I. Observations of star-shape cracks/cavities

The natural star-shape crack was observed for on old and healthy oil palms excavated before replanting, on healthy mature palms (10-15 years), on healthy 20 months seedlings remaining in nursery and on *Ganoderma* affected mature palms. Before observation, roots were excised and root-bole interface dissected by several longitudinal and transverse sections of the bole.

II. Implementation of an early screening test

Planting material

The planting material at the germinated seed stage came from breeding and genetic programmes of Lonsum's Sumatra Bioscience and Socfindo's Bangun Bandar seed production section.

Each cross was assessed with 5 replications of 20 germinated seeds. Twenty additional seeds per cross were inoculated at the same time for use as replacements when necessary. One month after

inoculation, and before disease expression, ungerminated seeds/seedlings, or those with an abnormal growth, were replaced with an inoculated seedling from the same progeny.

Ten independent trials were implemented, each of them comparing 15 to 20 progenies, and 71 individual crosses were tested at least twice in these trials of these crosses, 22 were tested at least four times.

Inoculum source

Dikaryotic isolate "J" of *Ganoderma boninense* used in this work was obtained from a basidiocarp and was previously characterised as an aggressive isolate by Breton *et al*. (2005a, 2006). The fungal culture was maintained on potato dextrose agar (PDA) in Petri dishes in dark conditions.

The inoculum source was based on artificial inoculation using Rubber wood blocks (RWBs) as a substrate. Fresh RWBs characterized by a volume of 216 cm^3 were boiled for 6 hours and placed in heat-resistant polypropylene bags (2 RWBs/bag). Thirty millilitres of Potato Agar medium (PA) were added and the polypropylene bags were sealed with stoppers of hydrophobic cotton covered with aluminium foil. The bags were autoclaved for 1 hour at 121°C, cooled overnight then inoculated with 6 fragments of mycelium (25 mm^2) from a 15-day old PDA *G. boninense* culture. The polypropylene bags containing inoculated RWBs were incubated in the dark at 27°C at 34% relative humidity. The RWB incubation time used in this work was previously standardised at 12 weeks according to the variability rate of seedlings infection recorded in nursery and for its efficiency to discriminate different progenies (Breton *et al*., 2005a; 2006).

Inoculation method

The inoculation of germinated seeds was performed under artificial shade characterized by a temperature and a relative humidity that, during the daytime, do not inhibit mycelium growth (Nawawi and Ho, 1990) but favour the process of seedling infection in a reproducible way (Breton *et al*., 2005a; 2006; Rees *et al.* 2007). The colonized RWB source was first placed inside the nursery polythene bag (size 20 x 30 cm) containing soil and the distance between the germinated seed and the RWB inoculum was easily and rapidly standardized at 5 cm according to the recommendations of Breton *et*

al. (2005a; 2006). The plants were watered daily and a fertilizer (15/15/6/4 NPK-Mg) was applied according to the local standard schedule of oil palm pre-nursery management.

Symptom recording

At first, disease development based on external symptoms was recorded every four weeks, then every two weeks when the first symptoms appeared, including leaf symptoms and/or fruiting bodies. Twenty-two to 28 weeks after inoculation, depending on the average percentage of the trial, the seedlings were split 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* based on a scale established by Breton *et al*. (2006).

Results analyses

The results, based on percentages of infection, were subjected to an ANOVA after each census. F value for the progeny effect was used to measure the significance of the variation and the discriminating power of the trials at each census. The results were then expressed as an index, similar to that used for interpreting vascular wilt early resistance tests in West Africa (Renard *et al*., 1991; de Franqueville *et al*., 1995). This index corresponds to the ratio between the percentage of BSR expressed by a cross and the mean percentage of the trial in which it figures. Base 100 corresponds to the mean of the percentages observed for the set of progenies. The index of a progeny represents the ratio of the plants in that progeny with BSR to the mean of the percentages of affected plants for all the test progenies considered. An index under 100 indicates a higher resistance than the mean for progenies assessed in the test; an index over 100 indicates higher susceptibility. The index assumption is that the population of progenies tested in each trial is similar for their resistance/susceptibility to BSR. The index enables overall comparisons and makes it possible to rapidly and clearly establish a susceptibility range, provided standard crosses with a wide range of resistance/susceptibility are involved in the trials. Where the distribution of the number of indexes obtained in a set of trials of a progeny falls below 100 and the number of indexes is greater or equal to 100, a genitor or an origin is

generally indicated together with the index. Comparing index results between trials is therefore potentially misleading without the use of standard crosses.

RESULTS

I. Observations of star-shape cracks/cavities

A lignified empty cavity with a star shape is observed at the interface root-bole, whatever the origin or the physiological stage of the healthy mature palm, (Fig. 1a-d). This particular cavity, generally met in the central position of the basal area, can penetrate inside the bole by several centimetres. For some palms, a 30 cm deep cavity was recorded. The origin of this lignified crack is probably a physical response of tissues in relation with the natural growth of the oil palm tree. The development of this basal cavity can be observed at very young stages of the seedlings, which corroborates the hypothesis of a natural physical reaction (Fig. 1e and 1f). The hypothesis of an abiotic or biotic stress origin of this lignified cavity can be discarded. Observation revealed also the presence of several functional roots attached to the surface of this highly lignified cavity (Fig. 2). This cavity which is empty space seems to play a role in the bole colonisation by *Ganoderma*.

Figure 1

Figure 2

For the early stage of infection in mature oil palms, external observation revealed the presence of a tough fungal mass at surface of the basal lignified cavity (Fig. 3). This highly melanised fungal tissue presents a morphological aspect similar with the stromatic-like structure observed at the surface of the infected RWB.

Figure 3

This empty basal cavity can play the role of culture chamber for the development of *Ganoderma* (with production of SLS) which is well-known to be a poor competitor in natural soil.

Observations of transverse and longitudinal sections of boles from random infected palms within plantations seem to reveal a centrifugal and radial invasion of the boles from this cavity by the fungus (Fig. 4). In commercial area, no initial *Ganoderma* invasion of young palms by the fungus was observed beyond the peripheral area of this lignified cavity. These observations do not exclude simultaneous colonisation of this cavity by more than one isolate of G*anoderma*.

Figure 4

II. Implementation of an early screening test

Disease kinetics - Range of susceptibility

Disease development is illustrated with Figure 5. This figure shows the kinetics of the best, median and worst crosses in terms of susceptibility, within four different and independent trials.

Figure 5

The first symptoms generally appeared 8 to 10 weeks after inoculation and differences between progenies began to be noticeable around 14 to 16 weeks. From week 16, the more susceptible progeny could be clearly distinguished from the others. Depending on the trials, significant differences among progenies appeared after 18 to 20 weeks of incubation. After 28 weeks, the most resistant

progenies scored around 20% of infection, the intermediate ones around 40% and the most susceptible ones around 70%. Figure 6 displays the differences that were observed in the pre-nursery.

Figure 6

Index stability

As stated before, the more susceptible progeny could be clearly distinguished from the others after 16 weeks of incubation. Tables 1 and 2 show the evolution of the indexes. As a matter of fact, it was possible at this stage to start to rank the progenies. The correlation (method of Pearsons) was already high when compared with the results obtained after 28 weeks of incubation. Correlation reached 0.95% when the trial mean approached 30% of infection. An infection mean of 30% provided the experiment with useful information on the level of resistance/susceptibility.

Table 1 - Index evolution (trial 1)

Table 2 - Index evolution (trial 4)

F-values stability

The discriminating power of the test depends, on the one hand, on the differences of infection among progenies and, on the other hand, reproducibility of the results among replications for a given progeny. The F-value of the progeny effect computed by the ANOVA of infection rate can be used to quantify this discriminating power and to discuss its evolution with time.

As shown in Figure 7, for trials 1 and 4 as described in Tables 1 and 2, F-values of progeny effect reached a maximum before or around the 20th week of incubation. Infection rates at this time were highly contrasted between progenies whose infection started early and progenies whose infection appeared later. After the 24th week, F-values did not vary extensively and relative differences among progenies had stabilized.

External and internal symptoms

Tables 3 and 4 show two sets of data where the indexes were calculated independently: the first one on the basis of visual symptoms, the second on the basis of the internal symptoms. The percentage of diseased seedlings scored with internal symptoms is 7 to 10% higher than the percentage of visual symptoms, but the ranking of the progenies is not significantly modified.

Table 3 - Relationship between external and internal symptoms

(Trial 2)

Table 4 - Relationship between external and internal symptoms

(Trial 5)

Results reproducibility

Table 5 summarises the comportment of the 22 progenies tested at least four times after an early inoculation and indicates also their status in field conditions, when it is known. The indexes were calculated when the trial mean was around 30%.

Table 5 - Field and nursery test status

These data first suggest that no highly-susceptible progeny would have been transferred to the field, if the test results had been available before planting.

Highly-resistant progenies have index values below 100 - that is the case for progenies 4, 10 or 14, while highly-susceptible progenies have index values above 100 - progenies 2 or 8, for example. The consistency of these index results among trials demonstrates the reproducibility of this screening test.

DISCUSSION

Cavity

The understanding of infection process of palm tree by *Ganoderma* involves different steps which is difficult to observe in natural conditions. Therefore, the development of reproducible inoculation methods (in nursery and rhizotron) associated to field observations allows to propose some hypothesis. This study provides further evidence concerning the role of a natural lignified cavity with the shape of star present in mature palms at the interface root-bole. The lignified scar of the future cavity can be observed in the 8 months-old seedling. This cavity seems to result from a physical process induced by the natural growth of palm. In mature palm the cavity was highly lignified and can penetrate the bole of several tens of centimetres. This empty cavity or crack could play a role of culture chamber for *Ganoderma* in this interaction. It is well known that *Ganoderma* is a poor competitor in natural soil, and the presence of competitors in this empty cavity is probably more limited than in the soil. Observations of stromatic-like structure in this cavity demonstrated the presence in this area of adequate conditions for *Ganoderma* development (strong subtract, dark, humidity, low competitors). These tough high melanised mycelia were also described at the surface of infected RWB (Breton *et al.* 2005a; 2005b). With rhizotron method, the authors demonstrated by using infected rubber sawdust and RWB that the development of this structure was necessary for successful infection. No SLS was produced on sawdust only on the tough substrate. The role of the SLS is probably not limited to this pathogenic action but also as a protection from antagonists or host defence reactions…

It is not so easy to link the cavity role in field observations with the nursery inoculation method. In artificial inoculation the infected RWB were used to inoculate young seedlings without cavity. If we compare the two infection process (natural and artificial,) one common point is the development of stromatic-like structures only observed on a tough substrate (RWB for nursery and lignified cavity in mature palm). In nursery, the initial distance between the germinated seed and the upper surface of

infected RWB was standardised at 5 cm. Then, it is possible that even in the absence of cavity in the very young seedlings, the infected RWB can replace this one in nursery inoculation. This could be one hypothesis to explain the necessary presence of SLS to induce *Ganoderma* infection. Some field and nursery trials will be performed to confirm or not this hypothesis, as well as treatment trials located as close as possible to the cavity.

Screening test

Most of the inoculation techniques described to-date (Khairudin *et al.*, 1991; Sariah *et al*., 1994; Idris *et al.*, 2004; Rees *et al*., 2007) have used at least 3-month-old seedlings, with a testing period of several months. This screening test has been reduced to 16 weeks as a result of research on several major parameters involved in the infection process. Among them, the most important to obtain an efficient and rapid seedling infection, are the incubation time of the RWB inoculum and the pre-nursery shade, which maintains a temperature and humidity suitable for the fungus growth (Breton *et al*., 2005a). Inoculations at the germinated seed stage enable the first symptoms to develop 10 weeks later. Breton *et al.* (2005a, 2006) showed a positive correlation between germinated seeds and 6-month-old seedlings in the discrimination between progenies for their susceptibility to *Ganoderma*. This result suggested that the response of progenies to artificial inoculation in the nursery is conserved irrespective of the physiological stage used for the screening test. In parallel, the authors have detected no specific interactions between *Ganoderma* isolates and planting materials tested.

Therefore, obtaining reproducible results in the kinetics of infection and the discriminating power between progenies is highly related to the distance between the germinated seed and the upper surface of the inoculum source.

This distance must be calibrated without damaging the mycelium on the upper surface of the infected RWB. Non-controlled damage of this stromatic-like structure during the calibration of the distance leads to decreased reproducibility of the kinetics of infection between trials (unpublished data).

One of the advantages of inoculating nursery bags at the germinated seed stage is the reduction in time and space. This is crucial when screening hundreds of progenies per year. The screening method described in this paper is rapid and easy to set up in the pre-nursery and limits the source of variability caused by human manipulation. This is the reason why each step of the method was previously studied, calibrated and standardized in order to reach this level of consistency.

Another key point with the germinated seed stage is the fact that it avoids root injury which occurs when seedlings are transferred to main nursery bags. Significant root injuries can interfere with the biological mechanisms of resistance to *Fusarium* wilt and then with the relationship between early tests and field results (de Franqueville H., private communication, 2002). As such, the different sources of partial resistance should be taken into account. An effect of root injury has also been detected in the pathosystem *Ganoderma boninense*/oil palm (Breton *et al.,* 2006). The authors demonstrated by using 3 months-old seedlings, that a root injury increased significantly the velocity of disease symptoms appearance.

Kinetics of disease development remains comparable among the various trials and the values of F stabilise after a peak linked with the detection of the most susceptible progenies. It is, therefore, possible to conclude a test after a period of 22 weeks after inoculation, similar to the *Fusarium* wilt test in Africa. This is an important component in the practical realisation and the planning of the successive screening tests involving several hundred crosses per year.

Progeny ranking for susceptibility is detected early during the test and remains stable. It also remains stable when internal and established external symptoms are analysed independently.

As in the case of *Fusarium* wilt results, data are expressed with a mean index (Id) together with the distribution of the number of indexes below 100 and the number of indexes greater or equal to 100 obtained in the set of trials. Progressively, the mean index and this distribution give a clear indication of the level of resistance of the tested material. These are the cumulative results of a seed parent, for example, or those of an origin which clearly indicate whether they transmit resistance/susceptibility. Screening for resistance to *Ganoderma* probably follows the same rule.

In the screening tests for *Fusarium* resistance, a set of standard resistant and susceptible progenies is used to ensure that comparisons among different tests are unbiased. Progenies tested in the trials

treated in this paper and in the following trials, coming from a diversified gene pool, will become, at least for some of them, standard crosses. A set of standard crosses is being developed by both Socfindo and Lonsum to provide an improved comparison of results from different trials and using different *Ganoderma* isolates.

Data presented in this paper indicate that this early screening test performs well. Thus, potential testing capacities are being developed in both companies to allow the screening of several hundred crosses per year, making it possible to integrate this tool in their respective breeding programmes.

In the meantime, continuous improvement of the techniques must be investigated, as has been done for *Fusarium* wilt in Africa. For example, in the *Ganoderma* screening tests, the size of RWBs is important because the quantity that can be processed and sterilised has an impact on the screening capacities.

To conclude, it should be clearly emphasised that breeding for resistance can, in the near future, be a major component of integrated BSR management, though not the only one. There will be different levels of partial resistance, but certainly not total resistance, which would prove to be not durable with this kind of pathogen. As a practical consequence, plantation managers must not reduce the efforts on cultural practices and sanitation. This integrated management, with all its components, will allow progressive decrease of the disease and the replanting of third or fourth planting cycles in as optimal conditions as possible.

ACKNOWLEDGEMENTS

The work described in this paper was carried out under a scientific collaboration agreement between *Centre de Coopération Internationale en Recherche Agronomique pour le Développement* (CIRAD), PT SOCFIN INDONESIA (SOCFINDO) and PT PP LONDON SUMATRA INDONESIA TBK (LONSUM). The authors wish to thank the management of SOCFINDO and SUMATRA BIOSCIENCE (LONSUM) for permission to publish this paper.

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Fig. 1 – Macroscopic observations of the lignified cavity present at the base of the bole of the healthy oil palms from Indupalma, Colombia (a); Palmeras de los Andes (b); Aek Loba Socfindo, Indonesia (c); Tanah Gambus Socfindo, Indonesia (d). Photos e and f show respectively the first steps of the development of this ubiquist cavity in 8 and 18 months-old seedlings.

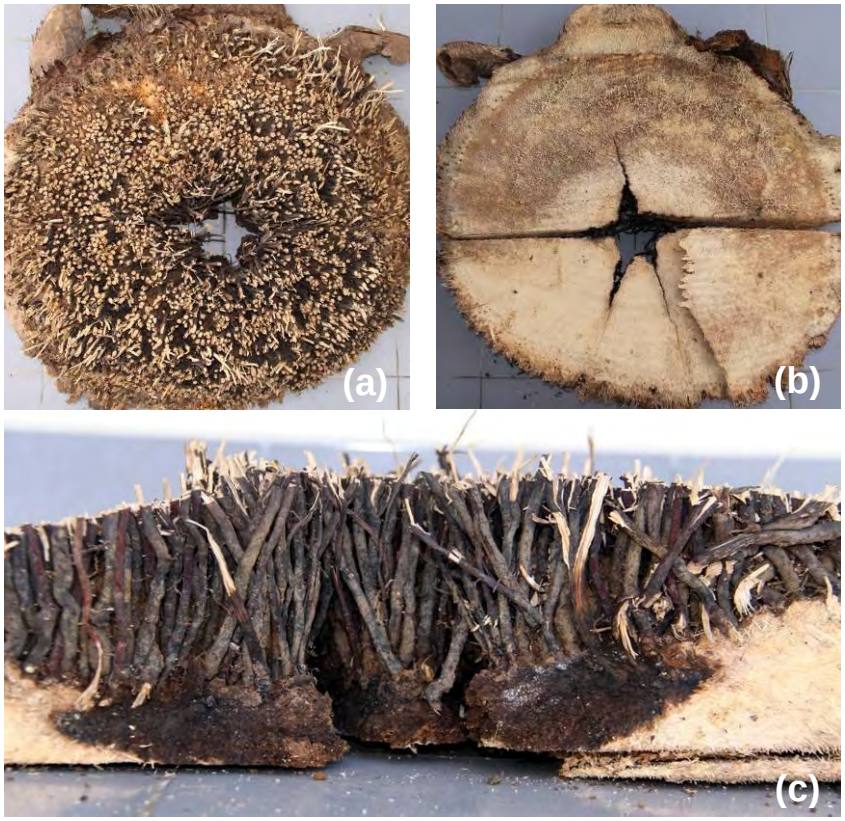

Fig. 2 – Transversal and longitudinal section of the lignified cavity localised at the base of a healthy bole from a 12 years-old palm. The photo (a) and (b) show respectively the external interface root-bole and the lignified cavity penetrating inside the bole. Transverse view (c) reveals the attachment of functional roots inside the cavity.

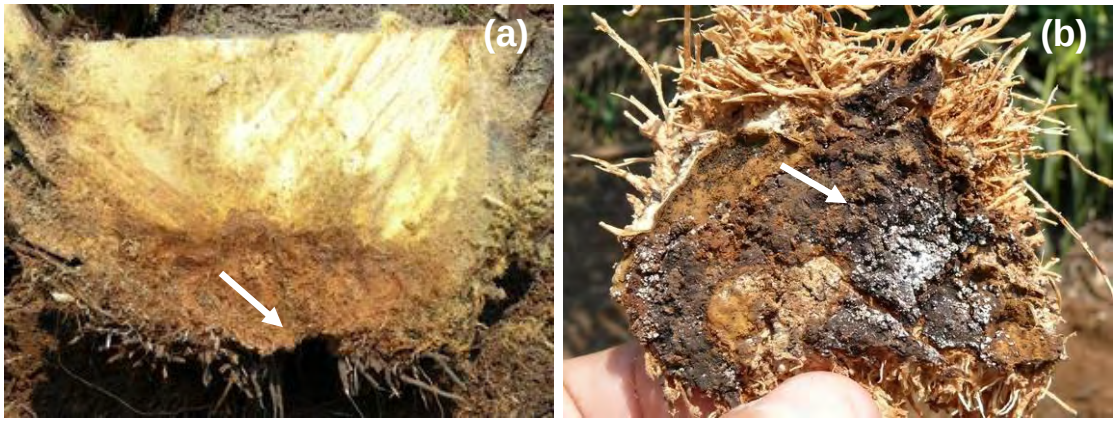

Fig. 3 – Dissection of bole from an infected 10 years-old palm (a). A fragment of a lignified cavity was collected (b) and the stromatic-like structures covering the entire external surface are indicated by arrow.

Fig. 4 – Observation of bole-root interfaces from infected mature palms within a plantation in north Sumatra (Indonesia). The progression of the rot (arrows) seems to indicate a centrifugal colonisation of the bole by the fungus from the basal lignified cavity.

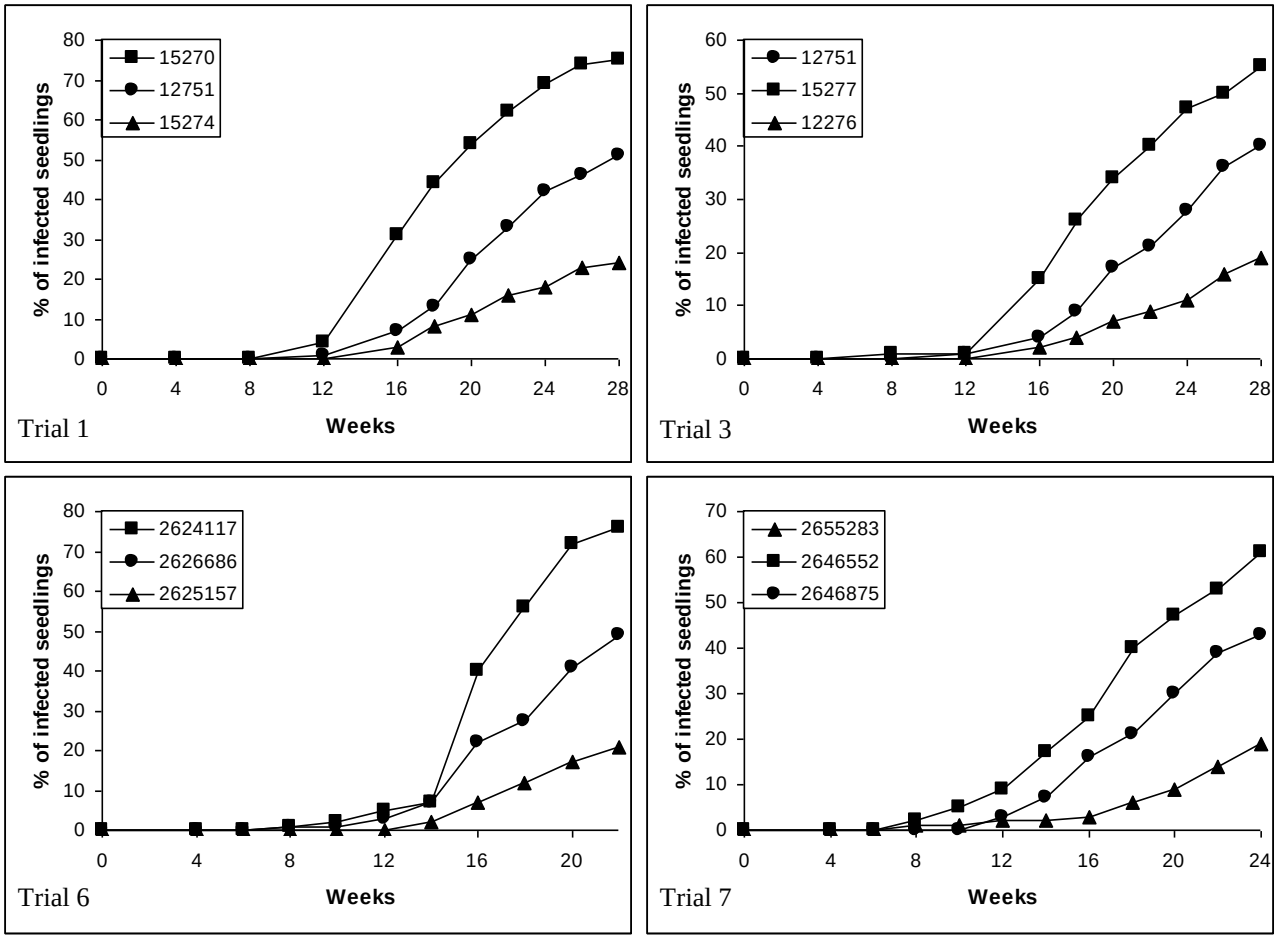

Fig. 5 - Kinetics of BSR development following artificial contamination of oil palm germinated seeds by *Ganoderma boninense*. For each trial 1, 3, 6 and 7, 3 independent progenies characterized by different levels of susceptibility to the BSR were selected among the progenies tested in these trials. Each data point represents the average of a total of 5 replicates of 20 seedlings.

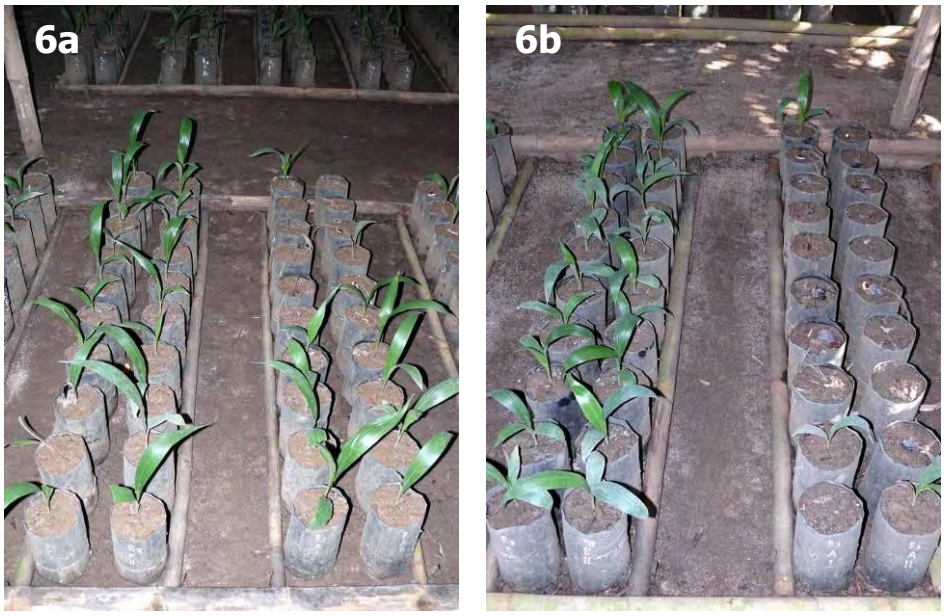

Fig. 6 - Disease expression observed 20 weeks after artificial inoculation of germinated seeds by *Ganoderma boninense*. **6a**: resistant progeny on the left and intermediate progeny on the right. **6b**: resistant progeny on the left and highly-susceptible progeny on the right

Table 1 - Index evolution (trial 1)

Progenies code tested in Trial 1	Index (based on external symptoms)						
	16W	18W	20W	22W	24W	26W	28W
12675	164	152	135	127	121	117	116
12700	78	95	82	81	78	89	88
15270	267	210	178	167	158	154	143
12751	60	62	82	89	96	96	97
12752	78	86	96	97	89	94	95
15271	86	95	109	108	117	121	113
12773	103	119	119	116	119	114	118
12276	52	57	76	78	78	81	82
15272	138	119	112	110	114	112	115
15273	112	100	102	110	112	104	103
11672	95	105	86	89	85	83	88
12444	60	57	76	75	80	77	84
11143	52	67	79	83	89	96	97
15274	26	38	36	43	41	48	46
15275	129	138	132	127	123	114	115
Trial mean (% infection)	11.6	21.0	30.3	37.1	43.7	48.1	52.4
Correlation with index 28W	0.83	0.86	0.95	0.96	0.98	0.98	1.00

Table 2 - Index evolution (trial 4)

Progenies code tested in Trial 4	Index (based on external symptoms)						
	16W	18W	20W	22W	24W	26W	28W
12675	236	231	199	171	153	140	135
12700	41	30	50	68	78	78	77
12752	82	82	99	100	104	103	104
15271	216	187	163	157	147	142	128
12271	103	97	81	86	89	91	90
12778	164	149	127	114	104	106	108
13790	144	134	163	157	141	132	128
15278	62	60	81	82	95	98	97
15279	21	45	50	57	63	62	77
15280	82	90	72	86	95	109	113
15281	92	119	104	107	101	93	101
15282	10	22	36	54	55	62	59
15283	92	97	122	104	112	114	110
15284	41	52	68	68	69	75	79
15285	113	104	86	89	95	96	95
Trial mean (% infection)	9.7	13.4	22.1	27.9	34.7	38.7	44.4
Correlation with index 28W	0.88	0.88	0.92	0.93	0.96	0.97	1.00

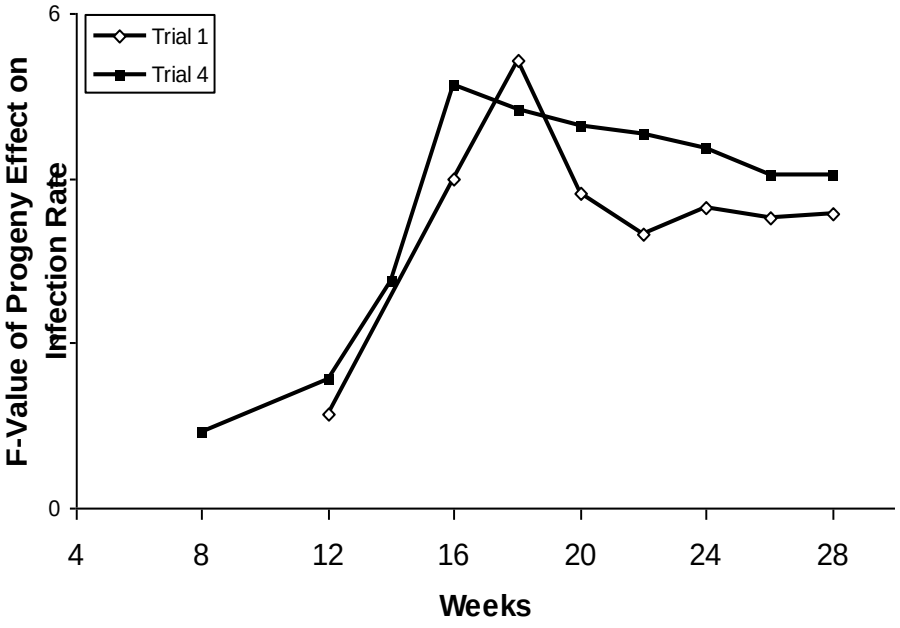

Fig 7 - Evolution of F-values during the trials 1 and 4

Table 3 - Relationship between external and internal symptoms (Trial 2)

Progenies code tested in Trial 2	Index (28 weeks after inoculation)	
	Ext. Sympt.	Int. Sympt.
12675	117	116
15270	125	117
12751	91	94
12752	85	85
12761	147	132
15271	126	123
15276	78	76
12773	108	105
12276	39	62
15273	78	84
11672	119	110
12444	106	108
11143	102	107
15274	59	68
15275	121	114
Correlation Indexes extern. / intern.	0.98	
Trial mean (% infection)	46.3	56.2

Table 4 - Relationship between external and internal symptoms (Trial 5)

Progenies code tested in Trial 5	Index (28 weeks after inoculation)	
	Ext. Sympt.	Int. Sympt.
2605855	91	101
2615580	114	117
2615930	73	81
2623333	91	101
2625369	97	99
2632316	107	103
2618415	114	104
2619450	116	111
2631689	103	94
2649170	99	106
2623332	69	79
2626686	103	103
2641311	83	86
2646554	101	99
2622233	114	108
2623005	105	109
2622745	95	89
2623074	110	104
2622009	99	93
2625157	118	112
Correlation Indexes extern. / intern.	0.88	
Trial mean (% infection)	50.7	60.5

Table 5 - Field and nursery test status

Number	Progeny code	Field status [1]	Number of trials [2]	Index average	Index distribution		Test status
					Id [3]<100	Id>100	
1	12658	Intermediate	4	86	3	1	Resistant to intermediate
2	12675	Susceptible	8	126	2	6	Susceptible
3	15278	-	4	79	3	1	Resistant
4	12700	Resistant	5	75	5	0	Resistant
5	15284	-	4	75	3	1	Resistant
6	12751	Intermediate	4	97	2	2	Intermediate
7	12752	Intermediate	6	109	2	4	Intermediate to susceptible
8	15271	-	8	141	0	8	Susceptible
9	12773	Intermediate	4	101	2	2	Intermediate
10	12276	Resistant	5	46	5	0	Resistant
11	12778	Intermediate	5	91	4	1	Resistant to intermediate
12	2615580	Exp. susceptible	7	113	2	5	Susceptible
13	2648858	Exp. susceptible	8	100	4	4	Intermediate
14	2654198	-	5	74	4	1	Resistant
15	2655003	Susceptible	5	105	2	3	Intermediate to susceptible
16	2648147	-	6	127	2	4	Susceptible
17	2653769	-	5	76	5	0	Resistant
18	2641311	Exp. resistant	4	65	4	0	Resistant
19	2660428	-	4	112	1	3	Susceptible
20	2641666	Exp. resistant	5	77	5	0	Resistant
21	2632316	-	4	95	2	2	Intermediate
22	2649172	Exp. resistant	6	70	6	0	Resistant

[1] : provisional field status, subject to evolution
[2] : number of nursery trials in which the progeny have been tested
[3] : index