

# Introduction

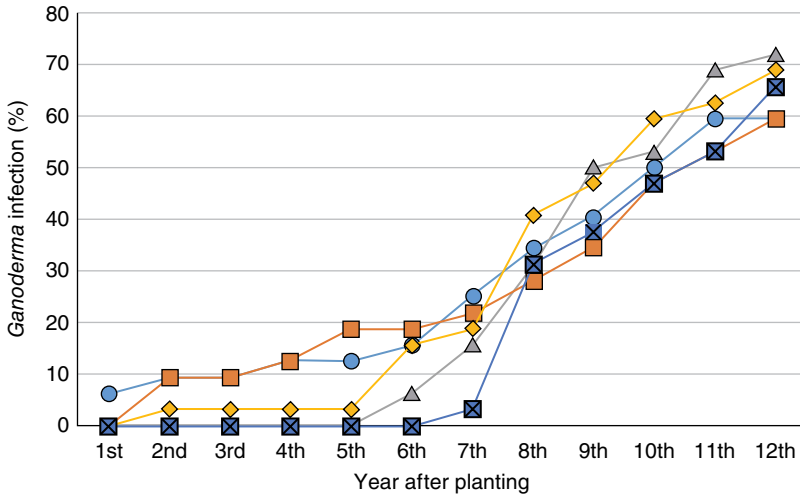
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## Abstract

Oil palm is frequently planted as a continuous monoculture in countries where it is cultivated. This system brings some advantages as it allows standardized agronomic practices in optimizing crop yield per unit of land. However, there are also numerous disadvantages. One of the biggest disadvantages of monoculture is increased susceptibility to pests and diseases. Currently in South-east Asia, oil palm plantations suffer from basal stem rot (BSR) disease caused by the basidiomycete soil-borne fungus, *Ganoderma* spp. The disease is also known in other regions (West Africa and South America), although the incidences are much lower than in South-east Asia (Indonesia and Malaysia). Agronomic approaches have been developed to reduce the impact of the disease. The development of integrated pest management (IPM) practices, by combining chemical, physical and biological controls, has been pursued, but has not shown an overall significant reduction in the incidences of the disease. Breeding to develop *Ganoderma*-resistant material is considered the best additional weapon to overcome the spread of BSR disease. Field selection of mature palms is commonly used, but this can take 10–12 years of observations. Therefore, the development of a quick and early screen is of particular interest. What is described in this manual involves the artificial exposure of seedlings to the disease organism in a nursery screen and recording responses.

## 1.1 *Ganoderma* Disease of Oil Palm

In the past three decades, there have been three diseases particularly that have caused devastation to the oil palm industry: *Ganoderma* disease in South-east Asia, vascular wilt by *Fusarium oxysporum* f. sp. *elaeidis* in Africa and bud rot disease by *Phytophthora palmivora* in South America (Kushairi, 2012; Rajanaidu *et al.*, 2012). These three diseases have caused not only palm stand losses but also significant economic losses. *Ganoderma* disease can cause palm stand losses of up



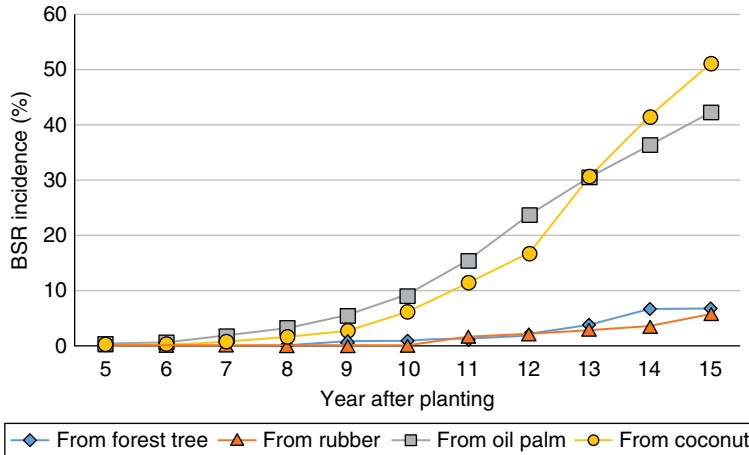
**Fig. 1.1.** The increased *Ganoderma* infection of some progenies in a *Ganoderma* screening trial in Indonesia. (Redrawn from Setiawati *et al.*, 2014).

to 80% (Fig. 1.1) and US\$500 million in economic losses per year in Indonesia and Malaysia. In Africa, palm stand reductions of up to 50% and yield reductions of up to 30% were recorded due to vascular wilt disease 10 years after replanting (Flood, 2006; Breton *et al.*, 2010; Hushiarian *et al.*, 2013). Two outbreaks of bud rot in Colombia in 2014 caused losses of US\$250 million (Torres *et al.*, 2016).

In South-east Asia, *Ganoderma* causes both basal stem rot (BSR) and upper stem rot (USR) diseases, although another fungus, *Phellinus noxius*, is suspected as the first disease agent of USR (Flood *et al.*, 2000; Hushiarian *et al.*, 2013; Rakib *et al.*, 2014). Of the two diseases, BSR causes more destruction than USR. USR is generally considered a relatively minor disease and has less frequent manifestations (Rees *et al.*, 2012; Rakib *et al.*, 2014). However, the USR incidence in some plantation estates in North Sumatra is increasing (Rees *et al.*, 2012). It is reported that the ratio of BSR to USR in plantation estates ranges from 10:1 to 1:1 (Hushiarian *et al.*, 2013).

## 1.2 Basal Stem Rot Disease

Although BSR is described as the main disease of oil palm in South-east Asia, the disease was first discovered in Zaire (now the Democratic Republic of the Congo) in 1915. At that time, the disease was not considered a danger to crop production (Turner, 1981). Today, BSR has become the most devastating disease of oil palm, especially in South-east Asia. Although the disease is found in every country that cultivates oil palm on a large scale, Indonesia and Malaysia are the two countries that suffer the most from BSR. Consequently, these countries are active in seeking ways to reduce *Ganoderma* disease (Cooper *et al.*, 2011; Hama-Ali *et al.*, 2014; Rakib *et al.*, 2015).



**Fig. 1.2.** Incidence of BSR disease in oil palm in relation to previous crops. (Redrawn from Singh, 1991).

Continuous monoculture favours disease, and the incidence of BSR has risen sharply in the past two decades, from being very low with little impact on yield to becoming a major devastating disease of oil palm. In the first cycle of oil palm plantations (in which the previous crop is not oil palm), the disease only infects older palms and therefore has no significant impact on the number of dead palms and yield loss. However, after successive replanting from one cycle of oil palm to the next cycle of oil palm (a common practice), the disease occurs increasingly early in younger palms, resulting in severe yield losses (Fig. 1.2) (Singh, 1991; Breton *et al.*, 2010; Purba *et al.*, 2012; Nuranis *et al.*, 2016).

### 1.3 Cause of Basal Stem Rot Disease

Basal stem rot is caused by the basidiomycete soil-borne fungus, *Ganoderma* spp. Infection is thought to be by root contact with infected palms or decaying plant debris. Spores are thought to be the main means of disease spread from plantation to plantation but, once established, one strain predominates (Breton *et al.*, 2006), suggesting spread by mycelia within a plantation. However, the recent findings by Merciere *et al.* (2017) show a high genetic diversity within some areas with a high incidence of *Ganoderma*. The fungus not only causes BSR, but also upper stem rot (USR) disease. The USR incidence supports the epidemiology theory of disease spread by airborne spores. Infected young palms usually die within 1 or 2 years after the appearance of the first symptom (basidiocarps at the base of the tree) (Fig. 1.3a), while mature trees can survive for 3–4 years (Fig. 1.3b) (Hushiarian *et al.*, 2013).



**Fig. 1.3.** (a) Fallen palm infected by *Ganoderma*; (b) standing palm infected by *Ganoderma*.

## 1.4 *Ganoderma* Diversity

*Ganoderma* species that cause BSR vary in different regions. Although the common species in West Africa is generally reported to be *Ganoderma lucidum* Karts, the predominant disease species in Nigeria, which is also part of West Africa, are *Ganoderma encidum*, *Ganoderma colossus* and *Ganoderma applanatum*. In South-east Asia (Indonesia and Malaysia), where the disease is the most devastating, the primary causative species is *Ganoderma boninense* (Susanto, 2002; Hushiarian *et al.*, 2013). The origin of *G. boninense* is still not known – whether the fungus came from Africa or originally was in South-east Asia (Merciere *et al.*, 2017). Moreover, studies by Wong *et al.* (2012) and Rakib *et al.* (2014) found that other *Ganoderma* species, *Ganoderma zonatum*, *Ganoderma miniatocinctum* and *Ganoderma tornatum*, were also present in diseased oil palms in Malaysia.

The first identified *Ganoderma* species was *G. lucidum* in 1881, identified by a Finnish mycologist, Peter Adolf Karsten, although its origin was not mentioned (Seo and Kirk, 2000; Susanto, 2002). The identification was based on host specificity, geographic distribution and the macromorphology of the bracket fruiting body (basidiocarp). The next identification was based on the morphology of spores, hyphal and basidiocarp characteristics. Currently, there are more than 300 *Ganoderma* species identified (Susanto, 2002). Genetic markers such as internal transcribed spacers (ITSs) of the nuclear rDNA can be used to distinguish between and within species of *Ganoderma*. Based on this method, *Ganoderma* is divided into three large groups and some subgroups (Table 1.1). *G. boninense* is in Group 2, Subgroup 2.1 (Moncalvo, 2000). However, it is interesting that *G. miniatocinctum* is not described clearly in this table, although it has been associated with disease. Another category described by Steyaert (1967) distinguishes *Ganoderma* based on the existence of laccate. *Ganoderma* with laccate (varnished or polished) can be recognized by their shiny, reddish-brown and shelf-like basidiocarp (Lyod *et al.*, 2017). *G. miniatocinctum* is placed in the laccate *Ganoderma* category (Steyaert, 1967).

A recent study based on mating compatibility reveals that *G. boninense* is 75% compatible with *G. miniatocinctum*. This percentage could represent different strains of the same species. However, there was not enough evidence to conclude that *G. boninense* and *G. miniatocinctum* represented different species or strains, but morphological studies of both fungi suggested that they might be regarded as synonymous (Jing *et al.*, 2015). The biodiversity of *Ganoderma* is now being studied and characterized at the DNA level. This allows the identification of different species and strains within a species (Zaremski *et al.*, 2013).

**Table 1.1.** Taxonomic groupings of *Ganoderma* based on DNA analysis of internal transcribed spacer region. (From Moncalvo, 2000.)

Group	Geographic categories	Hosts
Group 1		
1.1 <i>Ganoderma lucidum</i> complex <i>sensu stricto</i>		
– <i>G. lucidum</i>	Europe	Woody dicots, Conifers
– <i>Ganoderma valesiacum</i>	Europe	Conifers
– <i>Ganoderma carnosum</i> (= <i>G. atkinsonii</i> )	Europe	Conifers
– <i>Ganoderma ahmadii</i>	India, Pakistan	Woody dicots
– <i>Ganoderma tsugae</i>	China, Korea, North America	Conifers
– <i>Ganoderma oregonense</i>	North America	Conifers
– <i>Ganoderma praelongum</i> , <i>Ganoderma oerstedii</i>	South America	Woody dicots
1.2 <i>Ganoderma resinaceum</i> complex <i>sensu lato</i>		
<i>G. resinaceum</i> complex <i>sensu stricto</i> :		
– <i>G. resinaceum</i> (' <i>Ganoderma pfeifferi</i> ')	South Africa, Europe	Woody dicots
– <i>G. cf. resinaceum</i> ( ' <i>Ganoderma lucidum</i> ' )	Florida, North America	Woody dicots
– <i>G. cf. resinaceum</i> ( <i>Ganoderma sessile</i> , <i>Ganoderma platense</i> )	South America	Woody dicots
– <i>Ganoderma weberianum</i> complex:		
– <i>G. weberianum</i> (= <i>Ganoderma microsporium</i> )	Taiwan, South-east Asia, Australia	Woody dicots
– <i>Ganoderma cf. capense</i>	China, Korea, South-east Asia	Woody dicots
– <i>Ganoderma</i> sp.	South-east Asia	Woody dicots
– <i>Ganoderma</i> sp. (' <i>Ganoderma subamboinense</i> ')	South America, Neotropics	Woody dicots
– <i>Ganoderma trengganuense</i>	South-east Asia	Woody dicots
1.3 <i>Ganoderma curtisii</i> complex:		
– <i>G. curtisii</i> (= <i>Ganoderma meredithae</i> )	Neotropics, Florida, North America	Woody dicots, Conifers
– <i>G. curtisii</i> ( <i>Ganoderma fulvellum</i> , ' <i>Ganoderma tsugae</i> ')	China, Korea, Japan, Taiwan, South-east Asia	Woody dicots
1.4 <i>Ganoderma tropicum</i> complex <i>sensu lato</i> :		
– <i>Ganoderma</i> sp. 'clade A'	South-east Asia, Neotropics	Woody dicots
– <i>Ganoderma</i> sp. 'clade B' ( ' <i>Ganoderma lucidum</i> ' )	India, Pakistan, Taiwan, South-east Asia	Woody dicots

Continued

Table 1.1. Continued.

Group	Geographic categories	Hosts
– <i>Ganoderma</i> sp. ‘clade C’	Indo, PNG	Woody dicots
– <i>Ganoderma</i> sp.	South Africa	Woody dicots
– <i>Ganoderma</i> sp.	Neotropics	Woody dicots
– <i>G. tropicum</i> complex <i>sensu stricto</i> ( <i>Ganoderma fornicatum</i> )	Taiwan, South-east Asia	Woody dicots, palms
Group 2		
2.1 ‘palm clade’:		
– <i>Ganoderma zonatum</i> - <i>boninense</i> group:		
– <i>G. zonatum</i>	Florida	Palms
– <i>Ganoderma</i> sp.	South Africa, India, Pakistan	Palms
– <i>Ganoderma boninense</i>	Japan, South-east Asia, Indo, PNG, Australia	Palms
– <i>Ganoderma</i> sp.	South-east Asia, Australia	Woody dicots, palms
2.2 <i>Ganoderma</i> species:		
– <i>Ganoderma</i> sp.	South Africa	Woody dicots
– <i>Ganoderma</i> sp.	Neotropics	Woody dicots
– <i>Ganoderma</i> sp. (‘ <i>Ganoderma</i> cf. <i>tornatum</i> ’)	South-east Asia	Woody dicots
2.3 <i>Ganoderma</i> cf. <i>balabacense</i>	South Africa, South-east Asia	Woody dicots
2.4 <i>Ganoderma</i> sp.	South-east Asia	Woody dicots
2.5 <i>Ganoderma sinense</i> (= <i>Ganoderma formosanum</i> = ? <i>Ganoderma neojaponicum</i> )	China, Korea, Taiwan	Woody dicots
Group 3		
<i>Ganoderma austral-applanatum</i> complex <i>sensu lato</i> :		
<i>Ganoderma applanatum</i> A ( <i>Ganoderma lobatum</i> , <i>Ganoderma adspersum</i> ):	Europe, Japan, North America	Woody dicots
– <i>Ganoderma cupreolaccatum</i> (= <i>Ganoderma pfeifferi</i> )	Europe	Woody dicots
– <i>Ganoderma australe</i> complex <i>sensu stricto</i> :		
• <i>Ganoderma australe</i> complex A:		
▪ ‘Clade A.1’	China, Korea, Taiwan, South- east Asia, Indo, PNG	Woody dicots, palms
▪ ‘Clade A.3’	Neotropics, Florida	Woody dicots, palms
▪ ‘Clade A.2’	South-east Asia	Woody dicots, palms
• <i>G. australe</i> complex B	South Africa, Australia, New Zealand, South America	

Continued

**Table 1.1.** Continued.

Group	Geographic categories	Hosts
• <i>G. austral</i> complex C	India, Pakistan, Taiwan, South-east Asia, New Zealand, South America	Woody dicots
Unclassified		
<i>G. applanatum</i> B	Europe, North America	Woody dicots
<i>Ganoderma</i> sp.	South Africa, South-east Asia	Woody dicots
<i>Ganoderma</i> sp.	Neotropics	Woody dicots
<i>Ganoderma tsunodae</i> ( <i>Trachyderma</i> )	Japan	Woody dicots
<i>Ganoderma colossus</i>	South Africa, South-east Asia, Neotropics, Florida	Woody dicots

Notes: Names in parentheses are commonly miss-applied names (in 'quotes'), synonyms (=) or possible alternative names. Geographic categories and samplings are as follows: 'South Africa' includes collections from South Africa and Zimbabwe; 'Europe' includes collections from the UK, Norway, France, the Netherlands, Belgium, Austria and Germany; 'China' includes collections from mainland China with the exclusion of subtropical and tropical collections from Yunnan; 'South-east Asia' includes subtropical and tropical collections from Yunnan, Thailand, Vietnam, the Philippines, Peninsular Malaysia, Sabah and Singapore; 'Indo, PNG' includes collections from Bali, Maluku and Papua New Guinea; 'South America' includes collections from Argentina and Chile; 'Neotropics' includes collections from Costa Rica, Puerto Rico, Ecuador and French Guyana.

## 1.5 *Ganoderma* Pathogenicity

*Ganoderma* spp. produces enzymes that degrade the cell wall components of plants, lignin, cellulose and hemicellulose. The enzymes consist of polyphenol oxidases, laccases and tyrosinases that are able to degrade lignin. The fungus also produces some cellulose-degrading enzymes such as  $\beta$ -glucosidases (exo and endo) and hemicellulose-degrading enzymes ( $\alpha$ -D-galactosidase, endo- $\beta$ -D-mannanase, exo- $\beta$ -D-mannanase and exo- $\beta$ -D-mannan cellobiohydrolase). In addition, *Ganoderma* also produces amylases, extra-cellular oxidases, invertases, coagulase, protease, pectinases and cellulases (Susanto, 2002).

*Ganoderma* becomes infectious when the inoculum size reaches a certain level. *Ganoderma* pathogenicity can be demonstrated by artificial exposure of oil palm seedling roots to a massive inoculum source (Idris *et al.*, 2006; Breton *et al.*, 2010).

## 1.6 Management Control of Basal Stem Rot

Some protocols have been developed and practised to reduce the spread of *Ganoderma* disease in oil palm plantations. The control techniques are grouped into chemical, biological, agronomic and breeding controls.



## Chemical control

Fungicides are used for living oil palm stands. The effectiveness of this approach is little studied but may be developed. Many fungicides have been shown to be effective in suppressing the development of *Ganoderma* in laboratory conditions. However, in the field, the effectiveness of fungicides is poor (Susanto, 2002; Hushiarian *et al.*, 2013). Methods of field application may require improvement.

## Biological control

Many *Trichoderma* spp. are soil-borne fungi with known antagonism to many pathogens, one of which is *Ganoderma*. The effectiveness of *Trichoderma* has been demonstrated not only in the laboratory but also in nursery and field conditions. *Trichoderma* application to the soil has been implemented by some plantation companies. In Indonesia, the application of *Trichoderma virens* to planting holes has been conducted routinely in oil palm replanting schemes since 2000 (Priwiratama and Susanto, 2014). Soil applications of *Trichoderma* are expected to reduce the incidence of *Ganoderma*.

## Agronomic practice

There are some agronomic techniques that suppress *Ganoderma* disease, such as: soil mounding; surgery; sanitation; removal of diseased material; ploughing; harrowing; fallowing; planting legume cover crops; and fertilizer application. However, with the exception of fallow periods, the effectiveness of these practices is questionable and under review (Hushiarian *et al.*, 2013). Fallow periods do appear to produce a significant reduction in later *Ganoderma* incidence of the disease, but the length of time of a fallow (1 year or more) and its effectiveness are still under investigation.

## Breeding

The three previous approaches aim to reduce the incidence of disease. However, it is believed that the disease incidence can be reduced significantly and infection appearance retarded if new resistant oil palm varieties are bred. Breeding programmes for *Ganoderma* resistance have been set up in Indonesia and Malaysia. Success would be a major advance in improving the integrated pest management to BSR disease (Susanto, 2002; Breton *et al.*, 2010; Purba *et al.*, 2012), but as yet little is known about the genes involved and potent resistant sources for breeding. Some seed producers in Indonesia and Malaysia claim to have planting material showing some degree of resistance (PT Socfin Indonesia (SOCFINDO), Indonesia Oil Palm Research Institute (IOPRI) and Felda Agricultural Services Sdn Bhd (FASSB)) (Amri, 2014; Anon., 2016; PPKS, 2016).

## 1.7 Genetic Potency of *Ganoderma* Partial Resistance

The first report of potential genetic partial resistance to *Ganoderma* in oil palm was by Akbar *et al.* (1971). From a field census of two estate plantations in Indonesia, it was reported that Deli material was more susceptible to BSR disease than the material of more African origin. A more recent census by Purba *et al.* (2012) also demonstrated that African Dura germplasm was more resistant than Deli Dura. Based on these results, it was suggested that African material had retained a wider gene pool than Deli material. This is not surprising as north-west Africa is the centre of diversity of the species, and Deli Duras are highly selected. The related oil palm species, *Elaeis oleifera*, from South America, also shows good resistance to *Ganoderma*. However, there are no reports of any oil palm material having zero incidence of BSR disease.

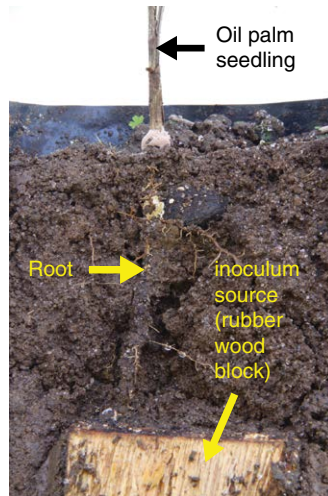
Breton *et al.* (2010) reported that some resistant progenies from La Me populations (originating from the Ivory Coast, Africa) transmitted resistance to their offspring. Likewise, susceptible parents tend to produce more susceptible progenies. It is suggested that resistance is a multi-genic character, and so suitable breeding strategies for *Ganoderma* partial resistance will need to be considered in developing resistant populations. Mutation breeding has recently been initiated with *Ganoderma* resistance as one target (Nur *et al.*, 2018).

## 1.8 Screening for *Ganoderma* Partial Resistance

The initial screening for *Ganoderma* resistance was done by conducting a census of diseased palms in the field (Akbar *et al.*, 1971; Purba *et al.*, 2012). However, these field surveys were not properly based on random locations. Therefore, the census results might have been due to a bias from the locations and not the materials. Field screening with sound statistical designs were conducted in Indonesia by Sumatra Bioscience in 2000 and by the IOPRI in 2008. Although it takes several years to collect all the results from field screening, the screening can determine the correlation between *Ganoderma* resistance, yield and yield components. Purba *et al.* (2012) reported that both *Ganoderma* resistance and yield were independent. Therefore, resistant material could be either high- or low-yielding.

## 1.9 Early Screening for *Ganoderma* Partial Resistance/ Susceptibility in the Nursery

It has been noted in the previous section that field screening for *Ganoderma* resistance takes several years. Therefore, a quicker method is needed, especially for breeding purposes. Breton *et al.* (2006) and Idris *et al.* (2006) first reported the possibility of screening seedlings in the nursery. Massive inoculum sources are needed to infect the roots of oil palm seedlings (Fig. 1.4).



**Fig. 1.4.** Root artificial inoculation of *Ganoderma* to infect oil palm seedling.

Both papers demonstrated the use of rubber wood blocks, inoculated with a *Ganoderma* isolate, as a source of infection for seedlings grown in polybags. CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), London Sumatra, IOPRI and MPOB (Malaysian Palm Oil Board) were among the first companies developing this method (Rajanaidu *et al.*, 2012). This approach not only reduces the time for selection but also saves on land resources, as well as allowing the selected lines to be advanced for trialling and breeding. These nursery trials take 5–7 months to conclude after germinated seeds are inoculated.

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