

A LABORATORY METHOD FOR INDUCING SPOROPHORE FORMATION AND PATHOGENICITY IN *Ganoderma* *zonatum* MURILL

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A technique is reported for the production of sporophores of *Ganoderma zonatum* Murill. in Kilner jars in four weeks, by inoculating stem dust medium (SDM) with 1 mm³ of the fungal mycelium. *Basidiomycetes* agar medium (BAM) was found effective for the isolation of the fungus from sporophores and infected materials. Root inoculations of fifteen-year old palms, using inoculum produced by the technique, resulted in *Ganoderma* disease development within three years. Two out of three seedlings planted 1 m from the base of a palm killed by *Ganoderma* disease were also killed by the disease within two and three years respectively.

INTRODUCTION

The disease called *Ganoderma* trunk rot in Nigeria is known as basal stem rot in South-East Asia: it is one of the most devastating diseases of the oil palm in that region. In Malaysia and North Sumatra, losses of up to 80% have been recorded (Turner and Bull, 1967; Turner, 1976). The disease is widespread in oil palm groves in West Africa, and the common cause of death of wild palms (Robertson *et al.*, 1968). Survey reports of the disease indicate that it occurs mostly among palms that are over 25 years of age but also on palms 10–15 years old.

In Nigeria the disease is widespread throughout the native palmeries, and the incidence is likely to increase with the effort at present going on to convert wild groves to plantations in the Eastern states of Nigeria.

The symptoms of the disease have been fully described by Navaratnam (1961), Turner and Bull (1967), Robertson *et al.* (1968) and Hartley (1988). The disease is caused by several species of *Ganoderma* including *G. lucidum* Leysex, Fr., *G. colossus* Fr., *G. applanatum* Pers ex Wallb, and

G. zonatum Murill. (Anon, 1978). Turner (1981) also listed these species among fifteen known to cause the disease, and stated that a single species is unlikely to be the sole cause of disease in any particular area.

Information on the physiology of the fungus is scanty, probably because of the difficulties encountered in growing it in pure culture. It has been suggested that basidiomycetes, to which group *Ganoderma* belongs, are generally difficult to grow in isolation plates, particularly in competition with fast-growing fungi. However, Navaratnam (1961) was able to grow the fungal mycelium on moist sand containing maize meal, malt extract and marmite. Tamblyn and Da Costa (1958), after testing a number of methods, were able to produce sporophores of many species of basidiomycetes on enriched sawdust medium by allowing the fungi to grow through blocks of decayed wood incubated in diffuse daylight in a humid atmosphere. Both methods were described as cumbersome and not wholly satisfactory (Navaratnam, 1961; Tamblyn and Da Costa, 1958).

Testing the pathogenicity of the fungus against its hosts has never been easy. Venkatarayan (1936) used portions of *Cassia simea* artificially infected with *G. lucidum* to inoculate *Areca* and coconut palms to produce the symptoms. In Papua New Guinea neither inoculation of young palms with infected wood, nor direct inoculation of stem and root, produced any sign of infection after several years (Anon, 1988). In the same country there were no symptoms of *Ganoderma* infection in any of 300 seedlings planted one metre from the bases of felled palms. In his inoculation of a 40-year old palm with *G. lucidum*, Navaratnam (1961) showed that one palm out of twenty inoculated on roots showed foliage symptoms after two years, and three out of twenty stem inoculations were successful. He doubted the suitability of the inoculum and stated that a conclusive method could not be recommended from his work.

This paper describes a simple laboratory method of growing and inducing sporophores in *Ganoderma zonatum*, and reports its pathogenicity and its relationship to the host palm.

EXPERIMENTAL

Isolation of fungus

Among several media tested, Basidiomycetes agar medium (BAM) was found to be the most suitable for isolating the fungus. It contains 10 g D-glucose, 1 g KH_2PO_4 , 1 g $(\text{NH}_4)_2\text{SO}_4$, 0.5 g MgSO_4 , 1.5 g bacteriological peptone, and 15 g of Bacto Agar in 1000 ml of water (Taylor, 1971). This mixture was autoclaved at 15 psi (121° C) for 15 minutes and 15 ml lots were dispensed into petri dishes. Sporophores from palm number 3.1155 in NIFOR Main Station and palm number 501.6840 in Abak Substation were identified by Dr R.L. Steyart of the National Botanic Garden of Belgium as *Ganoderma zonatum* Murill. A sporophore was cut into 5 mm³ pieces which were surface-sterilized with 0.1% mercuric chloride in distilled water and dried with sterile tissue paper (Oruade-Dimaro, 1990). Petri dishes containing 15 ml of BAM were each inoculated with three pieces of sporophore and incubated at laboratory temperature (28° C) for ten days.

Production of sporophores

Stem dust medium (SDM) was made up with 160 g ground oil palm trunk, sieved through No. 18 mesh (aperture: 0.850 mm B.S.), 10 g of milled corn, 30 ml of 10% malt extract and 250 ml of nutrient broth. An oil palm or rubber stem block 16 cm long and 3.5 cm in diameter was planted firmly in the centre of a 450 ml Kilner jar containing the above medium. The jar was then tightly corked with cotton wool and the whole assembly was sterilized by autoclaving at 15 psi (121° C) for one hour. After cooling to laboratory temperature, the medium was inoculated with 1 mm³ of fungal mycelium at the side of the wood block resting in the medium. The inoculated jars were incubated at laboratory temperature (28° C) for four weeks for the production of sporophores. These formed inocula for studies on pathogenicity.

Stem blocks planted on BAM in Kilner jars were also inoculated with the fungus and incubated in the same way for sporophore production.

Pathogenicity trials

Root inoculation. The contents of a Kilner jar was harvested after four weeks and thoroughly mixed; the sporophores produced were chopped into pieces of peanut size and these formed the inocula for pathogenicity trials. Six 4-month old

seedlings of oil palm in polythene bags were inoculated by spreading the inoculum of *G. zonatum* on the exposed roots, which were then covered up with nursery top soil. The seedlings were watered according to the usual regime for nursery care (Hartley, 1988). These similar seedlings were inoculated with SDM without the fungus and these served as control. In another experiment, 15-year old palms in field 51 in NIFOR Main Station were inoculated as follows:

The inoculum was prepared in a Kilner jar as described above. The roots of the palm on one side were dug to a depth of 15 cm and 6 to 10 actively growing roots were led into polythene bags containing the inoculum which were then buried using plantation soil. Seven palms were inoculated in this way. Two further palms were inoculated in a similar way, using SDM without the fungus as a control. In another treatment, harvested laboratory sporophores of the fungus were bound to exposed roots of three palms which were then covered up with plantation soil.

Stem inoculation. Stem inoculation was carried out by removing 1 cm cubes from the stems at a standard height of one metre from the ground. The hole thus bored was filled with inoculum and then tightly covered up with a tape which was held in place with drawing pins. The area of the stem around the inoculated point was kept moist by spraying water on alternate days for a week (Navaratnam, 1961). Seven palms were inoculated in this way. Two further palms were inoculated with SDM in the same way as control.

In another trial the exudate formed in the Kilner jars during production of sporophores was used to inoculate the stems of three palms in this case: the 1 cm cube compartments on the stems were bevelled so as to hold the exudate. All inoculated palms were separated from one another by digging a trench round the base of each palm 3.6 cm from the base. The trench was 30 cm wide and 30 cm deep. This was done to control any cross infection that might occur between palms through the roots (Bryce, 1925; Bhaskeran *et al.*, 1990).

The toxicity of the exudate was tested with rice, bean and maize grains, by soaking them in the exudate overnight, then planting them out in polythene bags containing nursery soil.

In another investigation, three-month old oil palm seedlings were planted at about 1 m from the base of the stump of a palm killed by *Ganoderma* trunk rot disease, with the sporophores of the fungus well exhibited. The seedlings were observed for symptoms.

Isolations were made from all infected materials using BAM.

RESULTS AND DISCUSSION

Ganoderma zonatum was easily isolated with BAM, producing white compressed mycelia in culture, but the production of sporophores on stem blocks of rubber or oil palm in BAM was slow. Although abundant mycelium was observed on the surface of the medium, sporophores were produced only after four months on rubber stem blocks and seven months on oil palm stem blocks.



Figure 1. Production of Sporophores of *G. zonatum* in Kilner jar.

The mycelial growth was faster and more prolific on SDM, and the fungus was visible ramifying through the entire medium within seven days of inoculation. During the same period, a yellowish-brown exudate was seen on the surface of the medium. Well-defined sporophores were produced within four weeks on oil palm and rubber stem blocks (*Figure 1*). The sporophores were sessile, measuring 4–8 cm in diameter and 2.5 cm thick at the base. The upper surface of the sporophores was purplish to brown, and shiny. The spores surface or under-side showed an irregular outline along the margin and was whitish in colour, with tiny pores through which spores could be discharged. The sporophores produced abundant yellowish spores which were ellipsoidal in shape.

Palms inoculated on the stem did not show any disease symptoms even after five years. In the case of root inoculation there was development of disease symptoms three years later, continuing into the fourth year. Four palms out of the seven inoculated in this way developed symptoms of *Ganoderma* trunk rot. The outer leaves of infected palms showed wilting, and within six months of the first signs of the disease, leaves broke at their bases and hung down on the stem, as described by Turner and Bull (1967). Two of the affected palms showed the appearance of sporophores six months before the appearance of crown symptoms. The palms lost their tops, appearing decapitated, after which the stem crumbled. A fifth palm also showed rot at the point of inoculation. The newly-produced leaves of the palm were successively smaller and had a yellowish-brown appearance.

None of the stem-inoculated palms, nor those inoculated with SDM, nor those in which sporophores were bound to the roots showed any disease symptoms. The exudate formed in the laboratory production of sporophores did not induce the disease, and was not toxic to rice, bean or maize grains, as these germinated after being soaked in the exudate over-night.

One of the three palm seedlings planted 1 m from the base of an infected palm showed *Ganoderma* disease symptoms about two years later: these were manifested by desiccation of the outer leaves, followed by death of the central spear leaf. The condition of the palm continued to deteriorate until it collapsed completely six months after the onset of

the disease. A second palm succumbed to the disease three years after planting in a similar way.

In all cases, *G. zonatum* was isolated from the chocolate core of stems of affected palms. No exudate was observed on inoculated palms.

The present laboratory method for the production of *Ganoderma* sporophores has several advantages. It is quick and reliable, and fungal cultures with spores can be easily raised for inoculation. The materials making up the stem dust medium (SDM) are readily available, and no special experimental conditions are required. Navaratnam (1961) experienced difficulty in raising inoculum of *G. lucidum* and attributed the poor results of his work on pathogenicity to this problem. The technique reported here is an improvement.

The fact that the successful inoculation of five palms resulted in actual disease three years later indicates that there is a long time lag between infection with the pathogen and the appearance of symptoms. In his investigations, Turner (1965) considered that there must be a long subclinical period between infection and visual evidence of *Ganoderma* disease of the oil palm, but the duration of this period has not yet been determined. No palm inoculated on the stem showed any sign of the disease in the present investigation, in agreement with the experience of Venkatarayan (1936), Turner (1965), and Turner and Poon (1968). In natural infection exudates are sometimes produced at the site of infection, which form gums (Hartley, 1988). There was no production of exudate in our inoculations, but a lot of it was formed during laboratory production of sporophores and within ten days in culture plates. The exudate appeared not to be toxic to plant materials since seeds and grains soaked in it overnight germinated.

Two of the three palms planted equidistantly from the base of a palm killed by *Ganoderma*, developed the disease two to three years after planting. This indicated that replanted fields are in danger of infection, since *Ganoderma* in most cases quickly colonizes the stumps of felled palms. Old palm tissues left to rot away in the field appeared to act as infection foci for young palms according to Turner (1965) and our observations suggest that palms below ten years of age may succumb to the disease. Our experiment is however being repeated with more palms. Determination of the length of the

period between infection and the appearance of *Ganoderma* disease symptoms in the oil palm requires careful study, and this aspect is being examined in a follow-up experiment.

Our inoculation results and the identification of *Ganoderma zonatum* from two distant sites in Nigeria tend to show that the disease is caused by this known species. This is contrary to the suggestion of Turner (1981), that a single species is unlikely to be the sole cause of the disease in a particular area. Attempts to identify species of the pathogen at NIFOR and throughout the entire oil palm belt in Nigeria are another aspect of our investigations.

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