Find Review APPEND 2

Electronic nose application in *Ganoderma* detection?

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ABSTRACT

This paper presents a review of several studies on the detection of *Ganoderma* disease in oil palm. The disease known as 'Basal Stem Rot' or BSR is currently a disease of high economic importance as it has incurred high economic losses in terms of revenue to the oil palm industry in South East Asia. The methods engaged in BSR detection are generally lab intensive and time consuming. Arrays of electronic sensors often dubbed as 'electronic noses' have been utilized to differentiate aromas of food and related materials. In addition, e-noses have also been used in medical diagnosis and microbial contamination studies in the environment. This technology is sensitive and accurate and gives quick results. It is fairly new and is still in the development phase, both in respect to hardware and software development. Its application in plant disease detection in agriculture is yet to be exploited and this paper investigates the possibility of e-nose being used as a tool in the early detection of BSR.

Key words: *Ganoderma*; e-nose; oil palm.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) tree is a leading source of edible vegetable oil production in the world having produced more than 32 million tones of oil in 2003. Its cultivation, in much of South-East Asia, is threatened by the 'basal stem rot' caused by *Ganoderma boninense*, where losses can reach 80% after repeated planting cycles. Basal Stem Rot has been causing serious damage to the oil palm plantations in Malaysia for more than 50 years (Shamala *et al.*, 2005) and is currently the most important disease of economical importance causing large amount of losses in revenue. Under severe infestations situations, more than 50% of oil palm stand can be lost to the malady (Golden Hope, 2006). The FELDA Plantations recorded high incidence of the disease in the Peninsular, ranging to about 50%, from 1994 to 2005 (Suhaidi, FELDA personal communication, 2006). The disease has no indication of early infection whereby it progresses through the palm from the base without any symptoms, irremediably ending in palm death. It causes root and stem rots in many tropical perennial crops, including oil palm, coconut, betel palm, rubber, tea, coffee, cocoa and forest trees (Miller *et al.*, 1994). It has been demonstrated that *Ganoderm*a can set in as early as 12 to 24 months after planting but more usually when the oil palm is 4 to 5 years old, particularly in replanted areas (Singh, 1990). The incidence increases rapidly to the extent that by the time the palms were 15 years old, the disease levels can reach between 40 to 50 per cent. In severe cases, up to 85 per cent of the standing palms succumb to BSR by the time the palms are 25 years old (Turner, 1981; Singh, 1990).

PREDISPOSING FACTORS OF BASAL STEM ROT (BSR)

The predisposing factors of the BSR disease in the **tropics** may include: coconut peat soils in the coastal areas of Malaysia (Hasan and Turner, 1994); high soil temperatures in dry coconut areas; irrigation with fertilizer application (Bhaskaran *et al*,1989); soil water stress (Nambiar and Rawther, 1993); and probably poor sanitation in infected fields. Ariffin *et al.* (1996) reported incidence of BSR on oil palm when planted under coconut. It was also observed that infection was very severe when the palm trunks had been buried as a method of disposal, mainly to avoid infestation by rhinoceros beetle. High incidence of BSR was also frequently recorded where the old stand had been felled but the stumps remained in the ground (Noor Hisham, 2005). Personal communication (2006) with Mohd Bazain Yusoh, a FELDA plantation manger, from Ladang Besout, Sungkai Perak and Harmeet Singh, a retired plantation manager from SEDC Johor Plantations in Segamat, revealed that low lying areas with poor soil-drainage system favor the incidence of BSR in oil palm growing areas.

The conditions favoring growth of *Ganoderma* in the cooler **temperate** may differ from that of in the tropics. In the temperate region of Chile (Southern Hemisphere), micro-environmental factors such as low temperature, high humidity, micro-aerobic conditions and very low nitrogen concentrations in Chilean native woods (Barrasa *et al.*, 1992) may constitute to the etiology of rotted log wood samples (palo podrigo) caused by the *Ganoderma* genus (Ferraz *et al.*, 2000a; Ferraz *et al.*, 2001). The selective lignin removal in 'palo podrigo' by the white-rot *Ganoderma* fungi may be due to the occurrence of iron-reducing compounds in the biodelignified rotted wood and an enzymatic process

caused by oxidative enzymes, such as lignin-peroxide and Mn-dependent peroxidase (Kirk and Cullen, 1998).

EPIDEMIOLOGY OF THE DISEASE

In the epidemiology of the disease, BSR is mainly considered as a soil-borne disease, spreading through root-to-root contact or infected debris. However, Sanderson *et al.* (2000) strongly suggest that spores are involved in the spread of the disease. Holiday (1980) reported that *Ganoderma boninense* and other species of *Ganoderma* are saprophytes or weak parasites that can build up massive inocula on debris of woody crops those later affect new crops. The fruiting body (basidiocarp) of *Ganoderma* is flat bracket-shaped and is stalked, varying in size from one to 50 cm in diameter with a thickness of up to more than 10 cm. In the premature button stage, the colour is white but the upper side of the bracket soon becomes shiny and light to dark brown or almost black, and concentrically furrowed. The margin is generally white and so is the underside. For disease initiation, large disease inoculum, a minimum of 750 cubic centimeters, is believed to initiate BSR in perennial crops in the tropics (Hasan and Turner, 1994). Further investigations by Hasan *et al*, (2005)on the causes of upper stem rot (USR) on standing mature oil palms has shown that sufficiently large source of inoculum built up in dead material, probably in frond axils allows invasion of the living tissues. The field trial of Flood *et al*, (2005) concerning the spread of the pathogen from artificially inoculated trunks [used to simulate spread from windrowed trunks at three planting distances for bait seedlings] revealed that the closer the seedling was planted to the source of inoculum the sooner it succumbed to the disease. However, infection only occurred when the trunks were mounded (covered with soil), and seedlings planted around uncovered trunks (at any distance) have showed no symptoms of disease to date.

CLASSIFICATION OF *GANODERMA*

The pathogen causing BSR was originally identified as *G. lucidium* Karts in West Africa in 1920 and also in Malaysia in 1931. In Nigeria, four species of *Ganoderma*, namely, *G. zonatum* Murill, *G. encidium, G. colossus* and *G. applanatum* (Pers.ex.S.F.Gray), have been identified as the causal agents of the disease. In Malaysia four species are associated with oil palm and they are *G. boninense, G. zonatum, G. miniatocinctum,* and *G. tornatum* (Idris *et al.,* 2000). ***Ganoderma boninense*** is the causal agent of basal stem rot in oil palm (Shamala *et al*., 2005).

Basal Stem Rot (BSR) caused by species of *Ganoderma* remains the major disease of oil palm in Southeast Asia. In the nomenclature of the fungus, the genus, ***Ganoderma*** **Karst**., is categorized as a higher fungus, a polyporoid genus, within the *family*: **Ganodermataceae**, *Order:* **Aphyllophorales**, *Subclass*: **Hymenomycetes**, and *Class*: **Basidiomycetes** (Idris *et al*, 2000).

OBJECTIVE OF THE REVIEW

This paper reviews the background of 'basal stem rot' in oil palm and different techniques used in *Ganoderma* detection. In addition the application of e-nose in microbial research is reviewed in order to investigate the efficacy of e-nose in future early plant disease detection in oil palm.

METHODS OF DISEASE DETECTION

BSR has been detected, as reported in literature, by the following methods:

1. **FIELD SYMPTOMS:** The disease has no indication of early infection whereby it progresses through the palm from the base without any symptoms (Shamala *et al.*, 2005). Seedlings planted around diseased stumps (60 cm distances) left in the field became infected within two years. Tissue of the former stand of oil palms acts as the primary inoculum or primary source of infection Yellowing or desiccation of oldest to young fronds and death of supply-palms with or without Ganoderma fructifications are some symptoms that are observed in the field (Idris *et al.*, 2005).

2. **MORPHOLOGICAL CHARACTERISTICS:** Colour of the upper surface of the basidiomata (oval disc-shaped fruiting bodies) collected from the living or dead oil palms in the field were variable. It varied from dark shiny red to dusky red, reddish yellow to reddish red and dark brown to dull white in dead palms (Idris *et al.*, 2000). Basidiomata *in vitro*, propagated on rubber woodblock (3x3x6cm) measured 1.40 to 3.70cm in length, 1.20 to 3.60cm in width, and 0.30 to 1.10cm in thickness. Actively growing margins and the undersurface of the basidiomata were white. The basidiomata formation on the rubber woodblock appeared after 1 – 3 weeks after incubation. The life-span of *Ganoderma* basidiomata on rubber woodblock was 3-4 months as compared with 6-7 months in infected oil palm in the field.

3. **GROWTH CHARACTERISTICS *IN VITRO*:** *Ganoderma*-selective medium (GSM) was developed by Idris and Ariffin in 1992 to facilitate selective isolation of the pathogen, directly from the field, with or without surface sterilization. The medium is prepared in 2 parts: **A** and **B**. Part **A** consists of Bacto-peptone (5g), Agar (20g), $MgSO_4.7H_2O$ (0.25g), K_2HPO_4 (0.50g), distilled water (900ml). The constituents of part **B** are: Streptomycin sulphate (300mg), Chloramphenicol (100mg), PCNB, pure (285mg), Ridomil-25%WP (130mg), Benlate-T20 (150mg), Ethanol, 95% 20ml), Lactic acid, 50% (2ml), Tannic acid (1.5g, Distilled water, pH 5.5 (80ml). Part **A** is stirred in a hot plate set at 100°C until dissolved, then autoclaved for 15 minutes. Part **B** is stirred for 2 hours at room temperature. After this part **B** is added to part **A** when the autoclaved medium has cooled to 45-50°C to get the GSM. The isolates of *Ganoderma* later can be grown or cultured in Potato Dextrose Agar (PDA) or malt extract agar (MEA) in

standard 9cm sterile Petri dishes at 23-28°C (Idris *et al.*, 2000, 2003). Whitish mycelium with slightly sculptured surface would cover the Petri dish 14 days after incubation. The reverse colour in the Petri of the fungal colony is normally dark brown. Colonies diameter in the Petri ranged from 17 – 51mm after 5 days and 34 – 84mm after 10 days The ellipsoid-shaped basidiospores, measuring 7.1 – 13.8mm in length and 4.8 – 8.3mm in width, from the fruiting bodies (basidiomata or basidiocarps) are yellowish brown when observed on slides under light microscope (magnification 40X, Idris *et al.*, 2000).

4. **PATHOGENICITY TEST:** The pathogenicity of 328 isolates of *Ganoderma* was done by Idris *et al* (2000) using an adaptation of the root inoculation technique with 10 months old DxP oil palm seedlings. A small incision was made at the side of the polypropylene bag containing seedling, revealing a part of the root system. One of the primary roots exposed was then pulled through the opening, washed with water to remove the soil, the distal excised and placed into tube containing *Ganoderma* inoculum grown on POPW medium (mixture of paddy, oil palm wood dust and supplemented with sucrose, calcium sulphate and bacto peptone). About 3cm of the root length was inserted into the medium, and the tube then sealed with parafilm and then the whole covered with brown paper to maintain darkness. External symptoms, including the appearance of basidiomata and foliar discoloration such as chlorosis or yellowing of leaves, were recorded 3-monthly until all the seedlings were colonized. *G. boninense* was the most aggressive isolate ($p \leq 0.001$) to infect the seedlings.

   Breton *et al* (2005) developed an early screening test at the nursery stage by artificially inoculating pre-infected rubber wood blocks (that were boiled for 6 hours, autoclaved for 2 h at 120° C in plastic bags containing 100ml of Potato Agar, and left overnight) with four fragments of a 15-day old *G. boninense* culture and left to incubate in the dark at 27° C for a period between 10 to 16 weeks when the fungus penetrated and colonized the tissue. This material was inoculated either by planting germinated seeds in prenursery bags containing the inoculum, or by transplanting 3-month old seedlings in nursery bags containing the inocolum. The distance between the planting material used and the inocolum was standardized to 5 cm, irrespective of bag or inoculum size. The success of this method relied on the aggressiveness of the *G. boninense* isolates, the incubation period of the pre-infected rubber blocks, the volume of the soil for infection, and the quality of the nursery shade. When this set of parameters was optimized, disease symptoms were observed 3 months after the inoculation of germinated seeds. Symptoms were recorded monthly by making two longitudinal cuts in the bole and recording the severity of internal tissue damage by visual estimation for a period between 25 and 30 weeks after inoculation. The estimation was based on the following scale:

   (0) Healthy: no internal rot
   (1) Up to 20% rotting of bole tissue
   (2) From 20to 50% internal rotting
   (3) Over 50% internal rotting

(4) Total rotting of bole tissue along with total desiccation of plant

Development of Breton's method can lead to the selection of isolates to be used in screening for resistance or tolerance to BSR.

5. **PCR (Polymerase Chain Reaction) TECHNIQUE (Idris *et al.*, 2003):** is an alternative approach to drilling technique used for symptom less *Ganoderma* detection. It engages molecular techniques to provide a more specific and sensitive detection of the target organism in the host tissue. Repetitive DNA polymorphism analysis, oligonucleotide hybridization to amplified ribosomal DNA (rDNA), and polymerase chain reaction (PCR) amplification of ribosomal DNA and internal transcribed spacer (ITS) regions have been used to detect and identify the pathogen. PCR-based identification is rapid and less time consuming when compared with other molecular and traditional isolation methods. The amplification of *Ganoderma* DNA by the PCR method is reliable and convenient way to detect and identify different isolates of this pathogen to oil palm. In the sensitivity of detection, DNA of *G. boninense* was extracted from the mycelium and was quantified using a spectrophotometer. The DNA was diluted to 1, 5, 10, 50, 100, 500, and 1000pg/ul. One microlitre of each dilution was used for each PCR reactions. The targeted DNA of *G. bininense* was amplified using PER44-123 and LR2 primers at concentrations of template DNA as low as 10pg/ul. When primer PER44-123 and LR1primer were used with total nucleic acid extracted from *G. boninense* basidiospores and diseased stem and frond based tissues, a product of 580 bp. was produced. DNA template of the fungus as little as 0.2ng of template of DNA (per g fresh weight) was adequate for detection of *G. boninense.* No amplification product was observed with DNA extract from healthy tissues.

6. **PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphisms) molecular method (Utomo *et al*, 2005):**
Utomo *et a*l, 2005, used a molecular method for the identification of *Ganoderma* in oil palm. They compared internally transcribed spacers (ITS) of ribosomal DNA from *Ganoderma* associated with BSR with other *Ganoderma* species by selecting two specific primer pairs that provided a specific DNA amplification of pathogenic *Ganoderma* in oil palm. Each primer pair produced a single PCR (polymerase chain reaction) product of about 450 bp (for primer pair IT1-IT2) and 334 bp (for IT-IT3) when oil palm *Ganoderma* DNA was used. No PCR amplification product was observed when other *Ganoderma* species DNA was used in PCR amplification with these primer pairs. Three specific restriction enzyme sites were identified in the ITS and intergenic spacer (IGSI) regions. The restriction enzymes *Mlu*I, *Sac*I, and *Hinf*I digested the ITS-PCR product and *Tfi*I, *Sca*I, and *Hinc*II were used to digest the IGSI-PCR product. *Mlu*I specifically digested the ITS regions and *Tfi*I digested the IGSI region of oil palm *Ganoderma.* Analysis of the published ITS nucleotide sequences of 31 *Ganoderma* species showed that the *Mlu*I restriction site was not present in other *Ganoderma* species. The study of Utomo *et al* (2005) used specific primers and PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length

Polymorphisms) analyses of the rDNA of *Ganoderma* to indicate that the strains associated with BSR disease in oil palms belonged to a single species.

E-NOSE IN MICROBIAL RESEARCH

In the current research, at the School of Bioprocess Engineering, KUKUM, the use of e-nose in early plant disease detection of *Ganoderma* in oil palm is to be attempted. Hence, a review of the application of e-nose in microbial research is given below:

What is an electrochemical nose (E-nose)?

The electronic nose mimics the human olfaction, and comprises three essential elements: (a) a sampling conditioning unit, which delivers the odour volatiles from the headspace of the sample; (b) a test chamber in which a sensor array is based; and (c) a processing unit, which analyses the sensor responses for pattern analyses (Canhoto *et al.*, 2004).

An 'electronic or artificial nose' is an instrument , which consists a sampling system, an array of gas sensors with differing selectivity, and a computer capable of qualitative or quantitative analysis of simple or complex gases, vapors, or odors. An 'electronic tongue' uses an array of liquid sensors. The artificial 'chemical' senses include taste and olfaction. The entire genus of electronic noses include those with conductive polymer, polymer composite, quartz microbalance, surface acoustic wave, calorimetric, and other classes of sensors. Modern E-noses are constructed with more than one class of sensors in them. Multi-parameter chemical data that include infrared spectrometers, gas chromatographs, and mass spectrometers have been used to identify odors and therefore called E-noses (Stetter & Penrose, 2001). According to Staples (2000), an electronic nose is a vapor analyzer which provides a recognizable image of specific vapor mixtures (fragrances) containing possibly hundred of different chemical species. The zNose™ is a combination of fast chromatography, an integrating surface acoustic wave sensor, and a programmable gate array (PGA) microprocessor. It is distinguishable from other sensor arrays called eNoses.

Electronic noses has been used as an environmental monitoring tool to detect microbial and chemical contamination of portable water (Bourgeois *et al.*, 2001); in sewage treatment works to discriminate between different residues in water samples (Baby *et al.*, 2000); in medical diagnostics (Gardener *et al.*, 2000); in detection and identification of bacterial cultures (Bachinger & Mandenius, 2000); detection of fungal contamination in library paper (Canhoto *et al.*, 2004); detection of microbial volatile organic compounds (MVOCs) produced by moulds on various materials (Fiedler *et al.*, 2001);

Application of E-nose in microbial research

Early detection of fungal species producing mycotoxins is very important to prevent human and animal risk deriving from the entry of volatile mycotoxins (headspace), such as alfatoxins from *Aspergillus flavus*, sesquiterpenes from *Fusarium sambucinum*, into the food chain. Electronic nose for headspace investigation could represent a valid

method of choice for its rapidity, simplicity and low cost besides overcoming the potential health hazard to the human tasters and the low predictive ability of odor classification system for mycotoxins contaminations. Falasconi *et al.*, 2005, used electronic olfactory system EOS835 (Sacmi Imolascarl, Italy), based on thin film semiconductor metal oxide (SMO) sensors, to detect fuminisins produced by *Fusarium verticillioides* in corn, that were later verified by means of immunological screening (CD-ELISA test). Toxigenic and non-toxigenic strains of *Fusarium* spp. cultured on wheat agar medium were differentiated by Keshri and Magan (2000) using an electronic nose based on conductive polymers (BH114, Bloodhound Sensors Ltd.).

Growth of bacteria and fungi on organic matter generates a broad range of volatile organic compounds and fixed gases. The presence of certain volatile compounds can be used as an indicator of the presence and identity of microorganisms. Electronic nose can enable differentiation between species in bacteria (Gibson et al., 1997), yeasts (Magan *et al.*, 2001) and fungi (Keshri *et al.*, 2003; Schiffman *et al.*, 2000).

Canhoto and Magan (2005) used two e-nose systems for comparisons of early detection bacterial species, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and fungal spores of *Aspergillus fumigatus*, *Fusarium culmorum* and *Penicillium* sp.

CONCLUSION

Electronic nose technology is still in its development phase, both in respect to hardware and software development. The instruments contain an array of from one to 32 sensors, using a variety of different sensor technologies – from organic polymers to metal oxides to micro-balances. Electronic noses are being used as a quality control instrument (Chair, 2006); microbial volatile organic compounds as indicators of fungi (Kuske *et al.*,2005); early detection of 'undesirable off-odours and microbial contaminants' in diary and bakery products (Anonymous, 2006). Strengths of e-noses include high sensitivity and correlation to human sensory panels in detection of microbial contamination in food industry and indoor environments many applications. They do have a high sensitivity (ppt to ppm) and are often more sensitive than the human nose. However, the limitations may include loss of sensitivity in the presence of water vapor or high concentrations of a single component like alcohol; sensor drift and the inability to provide absolute calibration; relatively short life of some sensors; necessity to do considerable method development work for each specific application and lack of being able to obtain quantitative data for aroma differences (Anon. 2006).

In the introduction of any new technology there is always bound to be some initial teething problems; but with the onset of time and experience, e-nose technology has a great potential in the early detection of diseases in plants, animals and humans. With the correct hardware, software and development work, e-nose has great potential to be used as a tool for the early detection of BSR in oil palm.

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