THREAT FROM FUSARIUM WILT DISEASE OF OIL PALM TO SOUTH-EAST ASIA AND SUGGESTED CONTROL MEASURES

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ABSTRACT

Fusarium wilt of oil palm caused by Fusarium oxysporum *f. sp* elaeidis (Foe) *has in the past rendered oil palm production uneconomic in some regions of Central and West Africa, where it is endemic. It is an anomaly that the disease has not appeared in South-east Asia, where the palm lines used are susceptible to African* Foe *isolates and the climate should be conducive. Various evidence and speculation are offered here to explain the absence of the disease so far.* Foe *is a soil-borne fungus that infects intact roots, traverses the cortex to the stele to invade the xylem and systemically colonise entire palms. Yield loss and even death result from induced water stress and hormonal imbalance. Disease spread is localised and typical of a soil-borne pathogen. Breeding for resistance over several decades has markedly reduced losses and disease incidence, even though expression of resistance appears to be partial. Resistance is proving durable, probably because* Foe *is monophyletic and resistance is based on multiple genes. Contamination of seed and pollen by* Foe, *has implications for importation of oil palm breeding material from the African centre of diversity. Isolated outbreaks in South America were linked to inter-continental seed movement. Quarantine has long been enforced for imported seed and pollen imported to Malaysia. This laboratory devised a method of fungicide infiltration for eradication of* Foe *from seed and the method is used by some seed companies to their market advantage, and in intermediate quarantine on exported seed batches. Specific DNA, PCR-based probes for rapid detection of the oil palm pathotype has long been required to distinguish* Foe *from the commonly present species* F. oxysporum; *advanced progress based on a unique* Foe *virulence gene is described. This article will consider the biology, spread, impact, detection and control of this aggressive pathogen in order to enhance or maintain awareness of the disease in this region and maintain the status quo of plantations remaining free from Fusarium wilt.*

INTRODUCTION

Malaysia and other major palm oil producing countries have escaped vascular wilt disease caused by *Fusarium oxysporum* f. sp. *elaeidis (Foe)*, endemic to Africa, in spite of long-term importation of considerable amounts of breeding materials, often contaminated with *Foe*, before quarantine measures were enforced. At first sight, this absence is difficult to explain. In previous decades and currently, Malaysian oil palm genotypes have been shown to be highly susceptible to *Foe* (Ho *et al.*, 1985; Durand-Gasselin *et al.,* 2000; Rusli *et al.,* 2014a) (*Figure 1*). Lack of disease in the field may be due to biotic or

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Figure 1. Three Malaysian palm genotypes inoculated with an African isolate of Foe. *Uninoculated seedling is shown on the right (Rusli, 2012).*

abiotic conditions. In particular, it is tenable that natural biological control systems are operating, such as other forms of *Fusarium* which out-compete *Foe* (Cooper, 2009; Flood *et al.*, 1989). Advances in understanding of plant defence systems also suggests that limited infection of palm roots by weak pathogens or mutualistic root colonisers can prime host defences. In this context, Mepsted *et al.* (1988) showed that two avirulent Malaysian isolates of *F. oxysporum* systemically colonised young palms. Vascular colonisation by one isolate was comparable to that by virulent African isolates of *Foe*, so could be considered as an endophyte. Crucially, one non-pathogenic Malaysian strain prevented infection by pathogenic African isolates. Non-pathogenic isolates of *Fusarium oxysporum* have been linked with disease suppression in *Fusarium*-suppressive soils (Olivain *et al.*, 2006). Ongoing investigations are exploring the possibility of suppressive soils by infecting young palms in plantation soils from sites around palms still remaining from the pre-quarantine 1970s plantings, originating from African seed. Disease incidence and severity are much reduced in certain soils (Rusli *et al.*, 2014b).

By default, the climate in South-east Asia should be conducive to Fusarium wilt. However, Turner (1981) suggested that the prolonged dry season in some regions of Africa could have contributed to *Foe* infection. Furthermore, Fusarium wilt is highest in areas where the annual dry season moisture deficit is greatest. These speculations and indirect evidence do not remove the fact that the threat remains, so vigilance and understanding of the disease are crucial to continue to avoid what could be potentially devastating to the oil palm industry.

THE DISEASE

The pathogen is a soil-borne fungus that produces macro- and micro-conidia and long living chlamydospores that survive in soil and debris. *Foe* is a very successful saprotrophic coloniser in plantations, but this property means that, from a pathologist's standpoint, is easy to grow, inoculate and re-isolate, especially when compared to the slow-growing and more recalcitrant *Ganoderma*. *Foe* can infect intact roots (Cooper *et al.,* 1989) (*Figure 2*). Presumably elongating roots contact infected roots, dead roots or debris containing *Foe* chlamydospores, which are then stimulated to germinate by root exudates. After penetration, the pathogen then crosses to the stele and establishes in the xylem vessels from where it colonises palms systemically (Cooper, 2011a).

Fusarium wilt has been reported from plantations in Cameroon, Ghana, Democratic Republic of Congo (DRC), Nigeria, Ivory Coast and other West African regions. Also it has appeared in localised
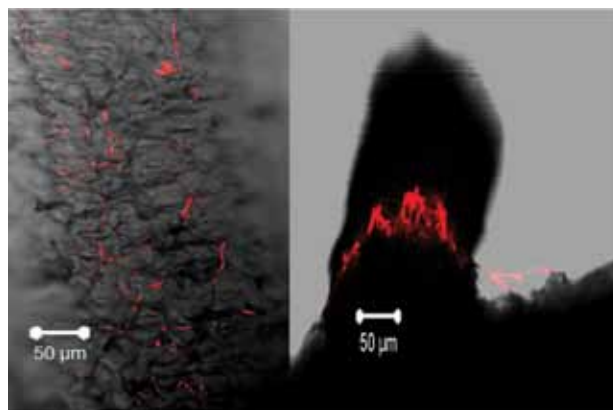

Figure 2. Colonisation of oil palm roots by Foe *expressing the DSRed 2 gene coding for red fluorescent protein. Confocal microscopy showing overall view of a young root (left) and hyphae around the base of a pneumathode (right) (Rusli, 2012).*

plantations in Ecuador and Brazil. The main host is *Elaeis guineensis*, but the South American palm *E. oleifera* can be infected artificially (Renard *et al.*, 1980).

This is one of the most serious diseases of oil palm in Africa, especially in replantings, inflicting substantial losses (Corley and Tinker, 2003). Dumortier *et al.* (1992) recorded yield loss of >50% from palms with 'acute' wilt in the year preceding death, and from palms with chronic wilt as 30% that of healthy palms. Renard and de Franqueville (1989) showed 16% yield reduction in palms only six years after replanting of which fewer than 5.5% showed external symptoms. They suggested that the losses came from 20%-30% of symptomless palms that were actually infected.

Water stress is a key contributory factor to symptomatology, as is typical of the many microbial diseases involving xylem colonisation. This can result from a variety of causes, including production of microbial polysaccharides, breakdown of vessel walls by cell wall degrading enzymes and host xylem occluding defence responses (Cooper, 2000).

In the *chronic* form of the disease, which can develop for years, symptoms include desiccation of older leaves and fracturing of the rachis near the base causing the fronds to hang around the stem. Subsequently, younger fronds are successively affected, resulting in still green erect leaves becoming reduced in size and they can become chlorotic. The crown reduces in diameter, flattens and eventually collapses (*Figures 3* and *7*). Mature palms can alternatively undergo *acute* wilt, when still erect leaves rapidly dehydrate, then break off from the trunk by wind action. These palms can die within a few months. Intermediate stages can be found and palms from more tolerant crosses may recover. *Fusarium* isolates might differ in aggressiveness perhaps causing these two conditions. It is likely that propensity of individual palms (resulting from seedling variation) or the inoculum level received locally would also contribute to the extent and rate of infection. Influence of region, climate, palm age, plantation history, forest versus savannah soils and nutrition (K and N) are reported by Corley and Tinker (2003).

Microscopic analysis of diseased palms reveals internal symptoms comprising xylem browning, resulting from phenolic infusion into vessel walls and adjacent parenchyma. This is diagnostic and notably distinguishes *Foe* infections from those caused by *Ganoderma boninense* (*Figure 4*). The pathogen is present exclusively in xylem as hyphae (*Figure 4*) and microconidia. These structures facilitate vertical and horizontal spread from vessel to vessel via pits. These are natural points of weakness, where the secondary wall is not deposited onto the primary wall.

Internal symptoms of vascular browning and facile re-isolation of *Foe* onto a *Fusarium*-selective medium (FSM) (Papavizas, 1967) allow critical analysis of infection and evaluation of tolerant or resistant palm genotypes in breeding programmes. Non-destructive sampling involving extraction of stem cylinders with an increment tree borer (*Figure 4*), led Mepsted *et al.* (1991) to determine that many (c. 25%) palms exhibiting no external symptoms but having internal symptoms of *Foe*. Likewise, Buchanan (1999) found considerable disparity between external and internal symptoms: one palm group showed 54% of 'healthy' palms with internal symptoms of *Foe* infection and in contrast, 40% of palms with external symptoms showed no vascular browning. Similar disease symptoms can result from infections by *Ganoderma* and *Cercospora*, and emphasises the need for careful analysis based on in depth understanding of the host-pathogen interaction. Rusli *et al.* (2014c) found similar trends in a survey in Ghana when they detected the presence of *Foe* in c.11% of symptomless palms. This shows, that symptoms alone are not reliable indicators to *Foe* infection, similar to *Ganoderma* where surveys are often visual and in some cases diagnosis may be made in the absence of basidiocarps.

EPIDEMIOLOGY

Fusarium oxysporum sporulates abundantly on male inflorescences and therefore, in theory, *Foe* could also be aerially dispersed. Indeed, 96 and 36 viable spores m^{-3} of *F. oxysporum* respectively were detected from wilt and non-wilt areas by Cooper *et*

Figure 3. Symptoms of Fusarium wilt. Image shows a localised but severe infection of mature palms in Ghana (Rusli et al.*, 2014c).*

Figure 4. Non-destructive sampling of palm trunks for systemic infection by Foe *using an increment borer by the authors in Ghana. The extracted cylinders show uninfected vascular bundles containing xylem (right) and infected xylem discoloured brown (left). The micrograph shows two infected xylem vessels containing hyphae, and intense localised browning as evident in the cylinder core sample.*

al. (1989). Spread of date palm Fusarium wilt and re-colonisation of steamed soil in tomato glasshouses by *F. oxysporum* have been linked with aerial dispersal (Cooper *et al.,* 1989). Of significance to importation of breeding materials, spores of *F. oxsporum* were found in batches of lyophilised pollen and some isolates were shown to be pathogenic (Flood *et al.,* 1990). Transcontinental spread could occur by this means therefore this aspect is considered later in more detail.

Rather than random spread, as would arise from spore dispersal, movement from tree to neighbouring tree and a resulting localised disease pattern would be usual for a soil-borne pathogen. This hypothesis is supported by the statistically greater occurrence of infected palms in pairs or groups and the greater infection of palms with missing neighbours than those without (Dumortier *et al.*, 1992). Rusli *et al.* (2014c) found similar localised spread in three Ghanaian plantations (*Figure 5*). The spatial distribution of infected trees was analysed by a statistical test based on Marcus *et al.* (1984). Similarly Musoli *et al.* (2008) reported coffee wilt disease caused by *Fusarium xylarioides* spread from initial infections to healthy neighbouring trees, resulting in an aggregated pattern with an infected tree able to infect up to three healthy trees away, in any direction.

CONTROL MEASURES

The only practical, sustainable approach for control of Fusarium wilt is breeding for disease resistant lines. Various cultural methods are also practised including: removal of diseased palms and burning; planting new palms at distance from old stumps; changing the cover crop; avoiding use of spent bunch stalks as mulch (Corley and Tinker, 2003). However, all these have limitations thanks to the soil-borne and persistent nature of *Foe* and the per-

ennial habit of the oil palm. Fungicides are not used on the grounds of efficacy, cost, scale and environmental considerations (Turner, 1981).

Screening for disease resistance. Seedling inoculation enables efficient screening of large numbers of progeny. Inoculation is straightforward because *Foe* can be rapidly produced in shaken liquid culture and the resulting spore suspension then poured onto soil and allowed to permeate down to the roots. Symptoms are then assessed after several months and analysed statistically (*Figure 1*). Tolerance to the disease in the nursery by some progenies has been shown to be usually correlated with low incidence of *Foe* in those selected progenies when grown in the plantation (de Franqueville, 1984; Corley and Tinker, 2003; Flood, 2006).

Pathogen isolate(s), need to be representative of an area or country, shown to be pathogenic, created from a single spore, and stored frozen or as resting spores to avoid changes in virulence year to year. The inoculum should be defined in terms of volume and spore concentration and reproduced trial to trial, year to year. In our experience at times we saw that none of these practices was being performed and advised accordingly. The palm seedlings should be shaded to avoid overheating of the soil medium in the tropical sun. We introduced these standardised practices in Zaire (DRC) in the 1990s and this greatly improved infection rates and reduced the extent of required replication. Analogously, shading has highly beneficial effects for seedlings inoculated with *G. boninense*. Soil temperature often exceeded 40°C in full sun, whereas in a plantation the canopy creates shade allowing these soil-borne pathogens to operate near their growth optima of c. 28°C (Rees *et al.*, 2007).

Selection of putative tolerant individuals from field palms in naturally *Foe* infested areas has been reported, but is problematic because the distribution and amount of *Foe* inoculum is likely to vary
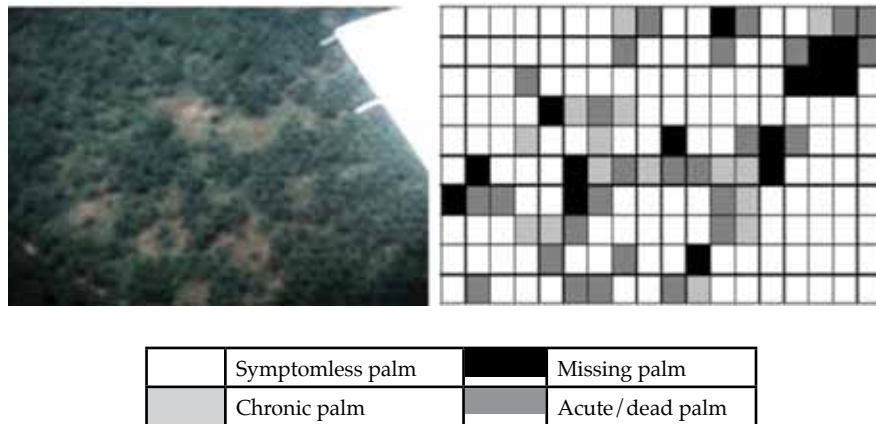
	Symptomless palm		Missing palm
	Chronic palm		Acute/dead palm

Figure 5. Aerial view (left) showing localised outbreaks of Fusarium wilt in Zaire (now Democratic Republic of Congo), reproduced by permission of H C Corley. Survey (right) of a diseased area in an otherwise largely unaffected Ghanaian plantation of individual diseased palms (each square represents one palm) showing distribution was highly localised (Rusli, 2012).

considerably. Symptomless individuals may have merely escaped encounter rather than be expressing resistance or tolerance.

Mepsted *et al.* (1995) described a much more rapid test involving inoculation of 2.5 cm sections taken from the tip of the rachis, by immersion in a *Foe* conidial suspension and infiltration by mild vacuum. It was reported to distinguish susceptible and resistant clones within eight days based on the extensive browning (necrosis) only in susceptible lines. Importantly, the method allows testing of an individual plantation palm, which can be used in the breeding programme. This contrasts with the necessary destruction of palms following a nursery trial. The method has the additional advantage that it can facilitate a study of mechanisms of resistance, which is problematic, because of the non-synchronous and chronic nature of this disease. The method has been field-tested but requires palms to be in good health in order to distinguish susceptible from resistant genotypes (Buchanan, 1999). The method ideally requires further refinement and field evaluation.

Another non-destructive method for pathogen re-isolation and for studying resistance and tolerance involves extraction of core samples from within stems using an increment borer (*Figure 4*). Detection from xylem in these core samples is likely to yield only *Foe*, as other fusaria would not have the adaptation to overcome host physical and chemical barriers and innate immunity.

Genetics and expression of resistance. A sustained breeding programme, using the screening procedures described above, has resulted in losses in the Ivory Coast being reduced from 20%-30% to less than 3% following introduction of 'tolerant' progenies (de Franqeville and Renard, 1990). The disease is becoming increasingly rare to find as a result (Chochard *et al.*, 2005). Our study of the epidemiology of Fusarium wilt in Ghana was by necessity, reliant on few localised areas of infection (Rusli, 2012).

However, resistance of oil palm crosses to *Foe* appears to be partial. Immunity to *Foe*, as conferred by major resistance genes in other crops to other formae speciales of *F. oxysporum*, such as the tomato pathotype, is not evident in oil palm, other than the high resistance of pure Dumpy Deli *dura* (Rosenquist *et al.,* 1990). Indeed, it remains unclear as to whether resistance is polygenically determined and additive (Meunier *et al.,* 1979) or based on only two genes with simple segregation, according to the inheritance data of Renard *et al.* (1993) and de Franqueville and de Greef (1988). This point is considered further by Corley and Tinker (2003) and Mepsted *et al.* (1994).

Putative genes for resistance, resistance gene analogues (RGA), can be identified by homology to the many cloned resistance genes from other species, as there is much consensus between them (Jones and Dangl, 2006). Attempts were made at Bath by Buchanan (1999) to identify RGA in oil palm. Consensus primers from the NBS region of *R* genes were used to amplify elements from oil palm DNA. Regions were found entirely consistent with *R* genes but three of these did not map to Fusarium wilt resistance. Transfer of major genes for resistance to *Foe* from other species, such as 'I' genes from tomato, so far appears unlikely because of so-called restricted taxonomic functionality.

Single resistance genes of major effect are often overcome, even by slow moving soil-borne pathogens like *F. oxysporum*, leading to the creation of new pathogen races (Huang and Lidhout, 1997). The likely polygenic nature of resistance to *Foe* creates a desirable situation because this type of resistance is usually durable, although breeding and evaluation are much more complex to perform. The relatively resistant or tolerant lines have proved to be durable, since they have been used for more than 40 years.

The chances of resistance being overcome also depend on the inherent genetic variability of *Foe*. Based on the DNA sequence information from three independent loci representing, respectively, the nuclear TEF1α gene, the internal transcribed spacer (ITS) region of the ribosomal DNA and the second largest subunit of RNA polymerase (RPB2), Rusli (2012) demonstrated that *Foe* has a monophyletic origin. Twenty-six *Foe* isolates from six different countries generated one *Foe* clade, whereby four independent lineages of *Foe* appear to be contained within the *Foe* clade suggesting a moderate level of genetic diversification. This analysis concurs with earlier studies of Mepsted *et al*. (1994), where inoculation of 14 clones with three *Foe* isolates from different parts of Africa showed variation in aggressiveness, but clone-isolate interactions were not evident. De Franqueville (1991) came to similar conclusions using three *Foe* strains and 66 palm progenies. However, it must always be borne in mind that there remains a risk with a potentially variable pathogen such as *F. oxysporum* that palm material developed in one area might succumb to infection elsewhere.

Local *Foe* populations appear to have evolved to be similar. Thus, Flood *et al*. (1992) found that all pathogenic isolates from Zaire (DRC) were in the same vegetative compatibility group (VCG) and separate from isolates from Brazil. In contrast, non-pathogenic *F. oxysporum* isolates from soil and roots of healthy palms in Zaire (DRC) and Malaysia had high VCG diversity as supported by RFLP analysis (Flood *et al.,* 1992).

The terms *resistant* and *tolerant* are often inter-changed, when describing reduced susceptibility to *Foe* and to *Ganoderma*. These descriptors need to be distinguished, because *tolerance* is universally accepted as a definition in plant pathology, describing that the pathogen still invades in high amounts (and therefore poses a threat through the continued spread of the inoculum). This is in contrast to virtual exclusion of a pathogen in truly resistant host genotypes.

The nature of expression of oil palm defences to *Foe* is largely unknown. Other than initial root invasion, infection is entirely in the xylem and, as in other vascular infections, defence appear to rely in part on occlusion of infected vessels by gels and tyloses. These structures may in some cases be accompanied by concurrent formation of anti-microbial phytoalexins (Cooper *et al*., 1996). Mepsted *et al*. (1995) using *Foe*-infiltrated petioles, detected but did not identify preformed and induced anti-fungal compounds in oil palm against *Foe*. Rusli (2012) found that one Malaysian progeny, PK 5463, whilst susceptible to one African isolate (16F) expressed some resistance to another (F3), and this was associated with an early up-regulation of the defence-related gene, chitinase.

Biological control by suppressive soils in Malaysia. As mentioned earlier, it is feasible that the non-appearance of the disease in Malaysia is a result of natural suppression by microbial components of soils, either acting directly or indirectly via the host defence system. This study is ongoing and *Figure 6* shows indications of this possibility.

Quarantine: seed treatment and **Foe** *detection*. It is an axiom that prevention of accidental introduction of *Foe* into unaffected areas is the most effective control measure. The continued exploitation of genetic diversity from the West and Central African centre of diversity is essential to improve the performance of oil palms. Zulkifli *et al*. (2012) describe Malaysia's collection as the largest in the world and this is based on germplasms from 11 African countries.

In light of this continued movement of breeding materials, *Foe* contaminated seed and pollen, from West Africa, clearly poses a potential threat to the oil palm industries worldwide. *Foe* can contaminate the surface of seeds (Locke and Colhoun, 1973) and can be found within seeds on the kernel surface (Flood *et al.*, 1990). The level of contamination between batches and between individual seeds varies greatly. About 50% of these seeds from Zaire (DRC) were contaminated and levels up to 5×10^3 propagules or cfu (colony forming units) per seed were detected; contamination of kernels in 30% of these samples was up to 100 cfu. The treatment of fruit bunches after harvest and subsequent retting to remove the pericarp, might be the stage when *Foe* proliferates, then presumably entering through the germ pores (Cooper *et al.*, 1989). In nurseries in Ivory Coast where millions of seeds have been germinated for screening trials, wilt symptoms have never been observed in uninoculated seedlings according to J. L. Renard (pers. com.). However, Flood *et al*. (1994b) reported that seed artificially infested with *Foe* planted in a glasshouse study at the University of Bath resulted in c. 3% palms with Fusarium wilt, underlining that transmission via seed can occur. Also the limited outbreaks in South America act as a warning.

Some of the other reported examples of seed contamination with *F. oxysporum* formae speciales are: *pisi, lycopersici, phaseoli, asparagi, betae, callistephi, elaedis, cumini, ciceri, carthami, lactucae, lini, vasinfectum, mathioli, perniciosa, niveum* and *spinacia*. Seed contamination is thought to have contributed much to the worldwide spread of the tomato pathotype f. sp. *lycopersici*. Race 2 was first found in Ohio in 1945 then spread throughout the USA, West Asia, North Africa and Latin America (Cooper, 2011b).

Trans-continental spread of *Foe* has already occurred. Localised, single plantation outbreaks in Brazil (Van de Lande, 1984) (*Figure 7*) and Ecuador (Renard and de Franqueville, 1989) resulted from

Figure 6. Foe *inoculated palms in a Malaysian soil: in pre-sterilised soil, the disease has established (left). In untreated soil, there is a markedly reduced infection and disease severity, even in the presence of a high inoculum density (centre). Endophytes were often detected from soil-grown palms such as other fusaria, antagonistic* Trichoderma *spp. (right).*

Foe isolate(s) with identical RFLP patterns and vegetative compatibility with isolates from the Ivory Coast (Flood *et al.*, 1992; Mouyna *et al.*, 1994). This suggests exported, contaminated seed was responsible, although contaminating seed or debris has been suggested as an alternative source of *Foe* (Flood, 2006). Clearly, movement of breeding materials constitutes a major threat to regions without *Foe*.

It was therefore necessary to develop a method to eradicate *Foe* from seed. Heat treatment of seeds from some other plant species can be effective in removing other *F. oxysporum* form species, such as f. sp. *lycopersici* and f. sp. *lagenariae* (Cooper, 2011b). However, the standard dormancy-breaking heat treatment at 40ºC to induce seed germination of oil palm substantially reduces but does not eradicate *Foe* (Flood *et al.*, 1994a). A suitable fungicide (Sportak Alpha: prochloraz and carbendazim) was identified with sufficient toxicity to *Foe*, but seed soaking did not remove *Foe* from within seeds. Consequently, this laboratory developed a method of vacuum infiltrating the fungicide, which eradicated *Foe* from the seed coat and from the seed kernel (Flood *et al.*, 1994a). This method is used by the quarantine facility at CABI Bioscience, United Kingdom in collaboration with MPOB for all materials entering Malaysia. Two seed companies in Africa also employ the method to their market advantage.

Pollen is less frequently imported but can also be contaminated with *Foe*, so requires screening at quarantine. Unfortunately, a decontamination method has yet to be achieved for pollen (*Figure 8*). The company ASD in Costa Rica use 95% ethanol treatment for pollen; however, *Foe* is not endemic there and the efficacy of this treatment against *Foe* is unknown to date.

Detecting *Foe* from seed samples involves placing fragments from cracked seeds (or placing pollen directly) onto FSM, then checking morphology after growth. If growth and identification are positive, cultures are then subjected to DNA-based PCR detection for *F. oxysporum*. Currently, only

Figure 7. Fusarium wilt in Para, Brazil, introduced by seed shipments from West Africa.

the species *F. oxysporum* can be diagnosed. DNA primers are designed on the translation elongation factor (TEF) gene (Geiser *et al.*, 2004). A related probe developed at the University of Bath is more discerning than previous ones because it has been shown to exclude the fungi most closely related to *F. oxysporum* (Rusli *et al.*, 2014d). It is sensitive enough to detect only one to two conidia of *Foe*. However, actual confirmation of the oil palm pathotype *Foe* is required because seed batches shown to contain *F. oxysporum*, a common contaminant, must be treated or discarded. Confirmation of *Foe* would otherwise require lengthy artificial inoculation of young oil palms, but at >six months for disease development, this procedure is not practicable. As part of ongoing research sponsored by MPOB, the Bath laboratory has developed a DNA-based probe to distinguish *Foe* from other *F. oxysporum* formae speciales. This proved to be very challenging, because in some cases, host specificity in *F. oxysporum* appears to have evolved multiple times; for example formae speciales *cubense* (banana), *gladioli* (gladiolus) and *lycopersici* are polyphyletic (Lievens *et al.*, 2008). A wide range of strategies were employed in an attempt to find a specific probe (Geiser *et al.*, 2004), including

Figure 8. Pollen dispersed onto Fusarium-selective medium reveals contamination by Fusarium, including F. oxysporum, *as confirmed using PCR primers (Rusli, 2012). Right image shows Fusarium sporulating on male inflorescences in the field.*

RAPD and SCAR and housekeeping genes such as TEF, IGS, RPB2, but none discriminated sufficiently between *Foe* and other host specific formae speciales. Therefore, we exploited the genomic information from the related *F. oxysporum* f. sp. *lycopersici* (Ma *et al*., 2010) and from knowledge of its virulence effectors, which are secreted in xylem and thus abbreviated to 'SIX' (Lievens *et al*., 2008). Virulence effectors are produced by most if not all microbial pathogens, targeting host defence components such as pathogen receptors and defence signalling (Aslam *et al*, 2008). This led to identification of a gene unique to *Foe*. A patent application is in process and so details cannot be given here. The primer pair designed, based on a secreted effector, is able to amplify a unique DNA fragment of all *Foe* isolates from different geographic backgrounds, but did not amplify 45 *Fusarium* spp., *Trichoderma* sp., *Aspergillus* sp. and *Sclerotinia sclerotiorum* tested as out groups (*Figure 9*).

Likewise the gene SIX6 has enabled clear distinction (using sequence differences between SIX6 homologues) of Australian isolates of the cotton pathogen *F. oxysporum* f. sp. *vasinfectum* from non-Australian isolates, and allows a specific diagnosis (Chakrabarty *et al*., 2010). A *Foe*-specific probe will not only have great value for quarantine of seed and pollen but it will aid tracking of *Foe* in plantations and provide a more rapid and specific assessment of infection in individual palms in breeding programmes.

CONCLUSION

It is perhaps remarkable that *Foe* has not established in South-east Asia in view of the inevitable importation of contaminated breeding materials and the robust nature of the pathogen, with its long-lived resting spores and strong saprotrophic ability. At this stage, we can remain optimistic that this region will remain free from *Foe*, because of its non-appearance to date and the enforcement of quarantine. Never-theless, continued vigilance by companies, government bodies, consultants and researchers is essential. The placement of the second author (M Hefni Rusli) in the United Kingdom laboratory of R M Cooper in order to acquire expertise with Fusarium wilt, shows the commitment of the Malaysian Palm Oil Board (MPOB) to keep the disease at bay. Never-theless, global trade sometimes results in accidental introductions in spite of phytosanitary checks, as we are only too well aware, with several, serious, non-endemic tree diseases recently affecting the United Kingdom. We need to continue to improve detection methods, such as by adopting the *Foe*-specific PCR primer kit described above. Analysis of *Fusarium*-suppressive soils might reveal competing or antagonistic soil microorganisms, or ideally endophytes, that could be introduced into palms to provide or induce resistance. These natural phenomena are not just of academic interest but are there to be exploited.

REFERENCES

ASLAM, S; NEWMAN, M-A; ERBS, G; MORRISSEY, K L; CHINCHILLA, D; BOLLER, T; TANDRUP JENSEN, T; DE CASTRO, C; IERANO, T; MOLINARO, A; JACKSON, R W; KNIGHT, M C
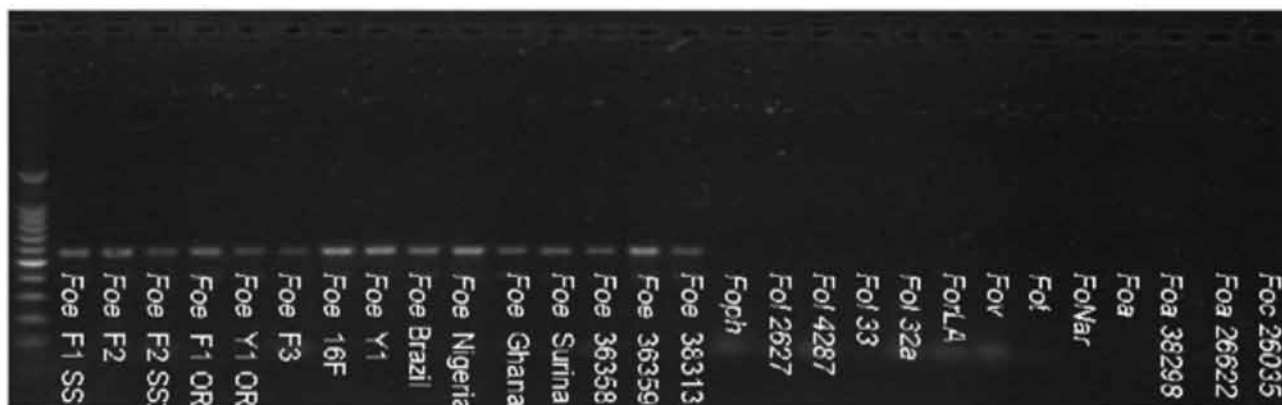
Figure 9. Agarose gel electrophoresis of PCR-amplified products using the Foe *specific primers ORF-F1 and ORF-R1. Lane1, 100-bp DNA ladder marker. Similar results were obtained in two replicates. Abbreviations other than* Foe, *refer to other form species such as Fol (f. sp.* lycopersici), *Fov (f. sp.* vasinfectum), *Foa (f. sp.* albedinis). *This gel is representative as many more isolates were tested.*

and COOPER, R M (2008). Bacterial polysaccharides suppress induced innate immunity by calcium chelation. *Current Biology, 18*: 1078-1083.

BUCHANAN, A (1999). *Molecular Genetic Analysis of* Fusarium *wilt Resistance in Oil Palm*. Ph.D thesis, University of Bath, Bath, UK.

CHAKRABARTI, A; REP; M; WANG, B; ASHTON, A; DODDS, P and ELLIS, J (2010). Variation in potential effector genes distinguishing Australian and non-Australian isolates of the cotton wilt pathogen *Fusarium oxysporum* f.sp. *vasinfectum. Plant Pathology, 60*: 232-243.

CHOCHARD, B; AMBLARD, P and DURAND-GASSELIN, T (2005). Oil palm genetics and sustainable development. *Oleagineux Corps Gras Lipids, 12*: 141-147.

COOPER, R M (2000). *Advances in Verticillium* (E C Tjamos *et al*., eds.). *Verticillium*-host interactions: past achievements and future molecular prospects. APS Press. p. 144-150.

COOPER, R M (2009). Vascular wilt - biology, economic importance, epidemiology, detection, control and future. *Proc. of the PIPOC 2009 International Palm Oil Congress – Agriculture Conference*. Kuala Lumpur.

COOPER, R M (2011a). Fusarium wilt of oil palm: a continuing threat to the Malaysian oil palm industry. *The Planter, 87*: 409-418.

COOPER, R M (2011b). *Fusarium oxysporum* wilt of oil palm: seed contamination, intercontinental spread and the development of eradication and rapid detection for seed quarantine. *Management of Plant Diseases - Technological Innovations in Seed Health*. Brasília-DF: Sociedade Brasileira de Fitopatologia. p. 29-46.

COOPER, R M; RESENDE, M L V; FLOOD, J; ROWAN, M G; BEALE, M H and POTTER, U (1996). Detection and cellular localization of elemental sulphur in disease resistant genotypes of *Theobroma cacao. Nature, 379*: 159-162.

COOPER, R M; FLOOD, J and MEPSTED, R (1989). *Vascular Wilt Diseases of Plants* (E C Tjamos and C H Beckman, eds.). Fusarium wilt of oil palm: transmission, isolate variation, resistance. NATO ASI Series. Springer-Verlag. p. 247-258.

CORLEY, H and TINKER, B (2003). *The Oil Palm*. Blackwell Scientific Press, Oxford.

DE FRANQUEVILLE, H (1984). Vascular wilt of the oil palm: relationship between nursery and field resistance. *Oleagineux, 39*: 513-518.

DE FRANQUEVILLE, H (1991). Former savannah or former forest: effect of pathogen isolates on the performance of oil palm families with respect to Fusarium wilt. *Oleagineux, 46*: 179-186.

DE FRANQEVILE, H and DE GREEF, W (1988). Hereditary transmission of resistance to vascular wilt of the oil palm: facts and hypotheses. *Proc. of the 1987 International Oil Palm Conference: Progress and Prospects* (A Halim Hasan *et al.,* eds.). PORIM, Bangi. p. 118-129.

DE FRANQUEVILLE, H and RENARD, J L (1990). Improvement of palm vascular wilt tolerance. Results and development of the disease at the R. Michaux plantation. *Oleagineux, 45*: 399-405.

DUMORTIER, F; VAN AMSTEL, H and CORLEY, R H V (1992). Oil palm breeding at Binga, Zaire. 1970-1990, Unilever Plantations, London. Cited in Corley, H and Tinker, B (2003). p. 404.

DURAND-GASSELIN, T; DIABAT, S; DE FRANQUEVILLE, H; COCHARD, B and ADON, B (2000). Assessing and utilizing sources of resistance to Fusarium wilt in oil palm (*Elaeis guineensis* Jacq.). *Proc. of the International Symposium on Oil Palm Genetic Resources and their Utilization.* Kuala Lumpur, Malaysia. p. 446-470.

FLOOD, J (2006). A review of Fusarium wilt of oil palm caused by *Fusarium oxysporum* f. sp. *elaedis. Phytopathology, 9*: 660-662.

FLOOD, J; COOPER, R M and LEES, P E (1989). An investigation of pathogenicity of four isolates of *Fusarium oxysporum* from South America, Africa and Malaysia to clonal oil palm. *J. Phytopathology, 124*: 80-88.

FLOOD, J; MEPSTED, R and COOPER, R M (1990). Contamination of oil palm pollen and seeds by *Fusarium* spp. *Mycological Research, 94*: 708-709.

FLOOD, J, MEPSTED, R and COOPER, R M (1994a). Population dynamics of *Fusarium* species on oil palm seeds following chemical and heat treatments. *Plant Pathology, 43*: 177-182.

FLOOD, J; MEPSTED, R; TURNER, S and COOPER, R M (1994b). Eradication of Fusarium from oil palm by seed treatments. *Seed Treatment: Progress and Prospects. BCPC Monograph, 57*: 201-205.

FLOOD, J; WHITEHEAD, D S and COOPER, R M (1992). Vegetative compatability and DNA polymorphisms in *Fusarium oxysporum* f.sp. *elaeidis* and their relationship to isolate virulence and origin. *Physiological and Molecular Plant Pathology, 41*: 201-215.

GEISER, D M; JIMINEZGASCO, M; KANG, S; MAKALOWSKI, I; VEERARAGHAVAN, N; WARD, T J; ZHANG, N; KULDAU, G A and O'DONNELL, K (2004). FUSARIUM-ID v. 1.0: a DNA sequence data base for identifying Fusarium. *European Journal of Plant Pathology, 119*: 473-479.

HO, Y W; VARGHESE, G and TAYLOR, G S (1985). Pathogenicity of *Fusarium oxysporum* isolates from Malaysia and *Fusarium oxysporum* f. sp. *elaeidis* from Africa to seedlings of oil palm. *Phytopathologische Zeitschrift, 114*: 193-202.

HUANG, C C and LINDHOUT, P (1997). Screening for resistance in wild Lycopersicon species to *Fusarium oxysporum* f. sp. *lycopersici* race 1 and race 2. *Euphytica, 93*: 145-153.

JONES, J D G and DANGL, J L (2006). The plant immune system. *Nature, 444*: 323-329.

LIEVENS, B; REP, M and THOMMA, B P H J (2008). Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum. Pest Management Science, 64*: 781-788.

LOCKE, T and COLHOUN, J (1973). *Fusarium oxysporum* f. sp *elaeidis* as a seed borne pathogen. *Transactions of the British Mycological Society, 60*: 594-595.

MA, L J; VAN DER DOES, H C; BORKOVICH, K A; COLEMAN, J; DABOUSSI, M J; DI PIETRO, A; DUFRESNE, M; FREITAG, M; GRABHERR, M and HENRISSAT, B *et al.* (2010). Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. *Nature, 464*: 367-373.

MARCUS, R; FISHMAN, S; TALPAZ, H; SALAMON, R and BARJOSEPH, M (1984). On the spatial distribution of citrus tristeza virus disease. *Phytoparasitica, 12*: 45 -52.

MEPSTED, R; FLOOD, J and COOPER, R M (1988). Biocontrol of Fusarium wilt by an avirulent isolate. *Proc. of the British Society for Plant Pathology - Novel and Unusual Methods of Disease Control.*

MEPSTED, R; FLOOD, J and COOPER, R M (1994). Fusarium wilt of oil palm; 'susceptibility' of some palms of a 'resistant' clone. *Oleagineux, 49*: 205-208.

MEPSTED, R; FLOOD, J; PAUL, T and COOPER, R M (1994). Virulence and aggressiveness in *Fusarium oxysporum* f.sp. *elaeidis*; implications for screening for disease resistance. *Oleagineux, 49*: 209-212.

MEPSTED, R; FLOOD, J; PAUL, T; AIREDE, C and COOPER, R M (1995). A model system for rapid selection for resistance and investigation of resistance mechanisms in Fusarium wilt of oil palm. *Plant Pathology, 44*: 749-755.

MEPSTED, R; NYANDUSA, C; FLOOD, J and COOPER, R M (1991). A non-destructive, quantitative method for the assessment of infection of oil palm by *Fusarium oxysporum* f.sp. *elaeidis. J. Oil Palm Res. Vol. 3*: 329-335.

MEUNIER, J; RENARD, J L and QUILLIC, G (1979). Heredity of resistance to Fusarium wilt in the oil palm *Elaeis guineensis* Jacq. *Oleagineux, 34*: 555-561

MOUYNA, L; RENARD, J L and BRYGOO, Y

(1994). Characterization and DNA polymorphism in *Fusarium oxysporum* f. sp. *elaeidis* isolated from oil palm. *Final Report. Defence Reaction Mechanism in Oil Palm with Respect to* F. oxysporum *f. sp.* elaeidis. Cytological studies and biochemical analysis. Application to the strengthening of selection criteria (J L Renard, ed.). CIRADCP, Paris. p. 1-11.

MUSOLI, C P; PINARD, F; CHARRIER, A; KANGIRE, A; KABOLE, C and OGWANG, J (2008). Spatial and temporal analysis of coffee wilt disease caused by *Fusarium xylarioides* in *Coffea canephora*. *European Journal of Plant Pathology, 122*: 451-460.

OLIVAIN, C; HUMBERT, C; NAHALKOVA, J; FATEHI, J; L'HARIDON, F and ALABOUVETTE, C (2006). Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into soil. *Applied and Environmental Microbiology, 72*: 1523-1531.

PAPAVIZAS, G (1967). Evaluation of various media and antimicrobial agents for isolation of Fusarium from soil. *Phytopathology, 57*: 848-852.

REES, R W; FLOOD, J, HASAN, Y and COOPER, R M (2007). Effect of inoculum potential, shading and soil temperature on root infection of oil palm seedlings by the basal stem rot pathogen *Ganoderma boninense*. *Plant Pathology, 56*: 862-870.

RENARD, J L and DE FRANQUEVILLE, H (1989). Oil palm vascular wilt. *Oleagineux, 44*: 342-347.

RENARD, J L; DE FRANQUEVILLE, H; DIABATE, S and OUVRIER, M (1993). Study on the impact of vascular wilt on FFB production in oil palm. *Oleagineux, 48*: 495-504.

RENARD, J L, NOIRET, J M and MEUNIER, J (1980). Sources and ranges of resistance to Fusarium wilt in the oil palms *Elaeis guineensis* and *Elaeis melanococca*. *Oleagineux, 35*: 387-393.

ROSENQUIST, E A; CORLEY, R H V and DE GREEF, W (1990). Improvement of *tenera* populations using germplasm from breeding programmes in Cameroon and Zaire. *Proc. of the Workshop Progress of Oil Palm Breeding Populations*. PORIM, Bangi. p. 37-69.

RUSLI, M H (2012). *Detection, Control and Resistance Expression in Oil Palm Caused by* Fusarium oxysporum f. sp. elaeidis. Ph. D thesis, University of Bath, Bath, UK.

RUSLI, M H; IDRIS, A S and COOPER, R M (2014a). Evaluation of Malaysian oil palm progenies for susceptibility, resistance or tolerance to *Fusarium oxysporum* f. sp. *elaeidis* and defence-related gene expression in roots. Accepted in *Plant Pathology*.

RUSLI, M H; IDRIS, A S and COOPER, R M (2014b). The possible role of suppressive soils in Fusarium vascular infection of oil palm (in preparation).

RUSLI, M H; IDRIS, A S; WHEALS, A E and COOPER, R M (2014c). Disease epidemiology and evolutionary relationship of *Fusarium oxysporum* f.sp *elaeidis* (in preparation).

RUSLI, M H; IDRIS, A S; WHEALS, A E and COOPER, R M (2014d). Specific detection of *Fusarium oxysporum* f. sp. *elaeidis* of oil palm based on a unique virulence effector (in preparation).

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

VAN DE LANDE, H L (1984). Vascular wilt disease of oil palm (*Elaeis guineensis* Jacq.) in Para, Brazil. *Oil Palm News, 28*: 6-10.

ZULKIFLI, Y; MAIZURA I and RAJINDER, S (2012). Evaluation of MPOB oil palm germplasm (*Elaeis guineensis*) populations using ESTSSR. *J. Oil Palm Res. Vol. 24*: 1368-1377.