Journal of Oil Palm Research
DOI: https://doi.org/10.21894/jopr.2017.0007

DISEASE EPIDEMIOLOGY AND GENETIC DIVERSITY OF *Fusarium oxysporum* f. sp. *elaeidis*, Cause of *Fusarium* wilt OF OIL PALM (*Elaeis guineensis* Jacq.)

M HEFNI RUSLI*; ALAN E WHEALS; SWETA SHARMA**; IDRIS A SEMAN* and RICHARD M COOPER****

ABSTRACT

Vascular wilt disease caused by Fusarium oxysporum *f. sp.* elaeidis (Foe) *has devasted of oil palm in west and central Africa. This study investigates the spatial distribution of* Foe, *whereby non-random, clustered patterns of the disease were recorded in four separate plantations in Ghana; infection from tree to tree via elongating roots therefore plays a more significant role than aerial distribution by conidiospores, with management implications. Control of* Foe *with disease-resistant palm lines can depend on the genetic variability of* Foe *isolates. Twenty-two putative* Foe *isolates from several African countries, including Ghana, were obtained from oil palms in infected areas for phylogenetic analysis along with 19 fungal out-groups, using the TEF-1α gene. The data showed* Foe *isolates have a monophyletic origin, and therefore limited diversity. Palm adapted isolates of* F. oxysporum *appear to have evolved independently, as ff. spp.* elaeidis, albedinis *and* canariensis *were nested into three independent groups. Slowly developing (chronic) and fast, severe (acute)* Fusarium *wilt are both evident in plantations and we provide preliminary evidence that* Foe *isolates' different aggressiveness might contribute to this variation. Sampling for* Foe *infection from xylem in extracted stem cores revealed the deficiency of field surveys based only on visual symptoms.*

Keywords: *Fusarium oxysporum* f. sp. *elaeidis*, oil palm.

Date received: 5 June 2017; **Sent for revision:** 6 June 2017; **Received in final form:** 9 August 2017; **Accepted:** 11 August 2017.

INTRODUCTION

Fusarium vascular wilt is the most important disease of oil palm, endemic in western and central Africa including the Ivory Coast, Ghana, Benin, Nigeria, Cameroon and Congo Democratic Republic (Turner, 1981; Corley and Tinker, 2003). The causal agent is the soil-borne, pathogenic fungus *Fusarium oxysporum* f. sp. *elaeidis* (*Foe*) that invades intact or wounded roots (Cooper, 2011; Cooper and Rusli, 2014). Elongating roots are thought to contact dead or infected roots or debris containing long-lived chlamydospores of *Foe*, which germinate in response to root exudates (Cooper and Rusli, 2014; Flood, 2006). Systemic colonisation then occurs via xylem vessels (Cooper, 2011). *Fusarium oxysporum* strains are classified based on their host specificity into formae speciales (ff. spp.) of which there are more than 150 (Baayen *et al*., 2000).

Diseased palms have been found frequently in pairs, indicating spread of the pathogen between neighbouring palms (Prendergast, 1957). This model of tree to tree spread was supported by a

* Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.
E-mail: mohd.hefni@mpob.gov.my

** Department of Biology and Biochemistry,
University of Bath, Bath BA27AY, United Kingdom.

later report of infected palms in pairs or groups and the greater infection of palms with missing neighbours than those without (Dumortier *et al*., 1992). Although it is generally accepted that vascular wilt is spread this way, *F. oxysporum* can also sporulate on male inflorescences. Significant numbers of *F. oxysporum* conidia were trapped from plantation air with four of these isolates pathogenic to seedlings, suggesting the possibility of aerial transmission (Cooper *et al*., 1989). Aerial dispersal plays a role in *Fusarium* wilt of some other plant species, including date palm (Cooper *et al*., 1989; Cooper, 2011). This study, based on four plantations in Ghana, makes the first attempt to undertake a statistical analysis of disease spread.

Two syndromes are seen in adult field palms, 'acute wilt' in which leaves dehydrate and die while retaining their original erect positions until broken off by wind action, follows rapid disease progression; affected palms die within three months. With 'chronic wilt' the disease can develop for many months and even years, but palms become progressively stunted associated with desiccation of older leaves and fracturing of the rachis near the base. The crown can also reduce in diameter and eventually collapse (Corley and Tinker, 2003; Cooper and Rusli, 2014). These differences might reflect local conditions, pathogen inoculum level, and variation between seedling palms or pathogen isolates' aggressiveness. The latter possibility is considered in this study.

Various cultural practices such as planting new palms at distance from old stumps have been attempted (Corley and Tinker, 2003). However, the only sustainable means of controlling Fusarium wilt is through selection and introduction of disease resistant genotypes (Cooper, 2011; Cooper and Rusli, 2014). Stability of resistance depends on the genetic controls of host resistance and pathogen virulence. Single resistance genes have provided long-term protection to relatively slow-moving, soil-borne *F. oxysporum*, such as host-adapted forms to brassicas and tomato (Lievens *et al*., 2008; Andolfo *et al*., 2014). Nevertheless, virulent pathogen races have evolved (Michielse and Rep, 2009). *Fusarium* resistance in oil palm is claimed to be based on many resistance genes (Meunier *et al*., 1979), but others have implicated only few genes (de Franqueville and de Greef, 1988; Renard *et al*., 1993). If resistance to *Foe* is polygenic, it would increase the chance of resistance remaining durable, especially if *Foe* spreads locally and clonally and has limited genetic diversity. Currently little is known about the genetic variability of *Foe* isolates.

Three separate vegetative compatibility groups (VCG) were associated separately with *Foe* populations in Ghana, Zaire (now known as the Democratic Republic of the Congo) and Ivory Coast/Benin (Dossa *et al*., 1991). Others claimed groups generally corresponded to geographic origin (such as Zaire, Benin, Ivory Coast) based on restriction fragment length polymorphism (RFLP) using one random DNA probe (Mouyna *et al*., 1996). We showed, based on RFLP and VCG, isolates were similar within Zaire and Brazil but distinct between them (Flood *et al*., 1992); however it should be noted that the Brazil isolates originated from West Africa (probably Ivory Coast) (Flood *et al*., 1992; Mouyna *et al*., 1996). Therefore based on these early methods, some diversity of *Foe* populations is apparent.

It was long considered that host plant-specialised forms of *F. oxysporum* would be monophyletic and isolates that shared a host all derived from a single pathogenic genotype (Gordon and Martyn, 1997). However, many ff. spp. are polyphyletic (Laurence *et al*., 2015). For example f. sp. *cubense* (banana) and *F. oxysporum* f. sp. *lycopersici* (tomato) are scattered over several clades of the species complex (O'Donnell *et al*., 1998; Kawabe *et al*., 2005; Fourie *et al*., 2009). Characterisation of the phylogeny of f. sp. *cubense* isolates by compiling sequence data from two ubiquitous genes throughout *F. oxysporum* (small subunit of mitochondrial DNA mtSSU and TEF-1α) revealed five different lineages (O'Donnell *et al*., 1998). It appears that pathogenicity and virulence mechanisms involved in specificity have evolved multiple times, possibly through mutation or transposition or spread to distantly related strains through horizontal gene transfer (Baayen *et al*., 2001) or horizontal chromosome transfer (Ma *et al*., 2010). Horizontal gene transfer is common in the *F. oxysporum* species complex and has recently been suggested as the most likely explanation for non-monophyly of most ff. spp. (Laurence *et al*., 2015).

Previous studies showed that nuclear large subunit (LSU) and/or ITS rDNA sequence data failed to differentiate several species complexes within *Fusarium* (O'Donnell *et al*., 2007). RNA polymerase II subunit (*RPB2*) nucleotide sequences gave poor bootstrap support for several *Fusarium* species complexes, even though the sequence was easily aligned across the entire genus. Genes for protein virulence effectors (*'SIX'* named from proteins secreted in *x*ylem) can contribute to host specificity but do not reveal polyphyly (Laurence *et al*., 2015). The translation elongation factor TEF-1α gene is a highly informative locus at the species level in *Fusarium* (Laurence *et al*., 2015) and non-orthologous copies of the gene have not been detected in the genus. Universal primers of TEF-1α have been designed that work across the phylogenetic breadth of the genus (Geiser *et al*., 2004). Therefore this approach was used here in order to study the phylogenetic diversity of the *Foe* isolates from different backgrounds.

Understanding of pathogen variability and the nature of disease spread is part of our on-going programme to design sustainable and effective disease control.

MATERIALS AND METHODS

Sampling Palm Stems with Increment Borers

Tree increment borers (400 mm length; Mattesson, Sweden) were used to remove cores (Mepsted *et al*., 1991; Cooper, 2011) (5.5 cm diameter) from trunks of diseased and apparently healthy palms in Ghana. Cores were taken perpendicular to the longitudinal stem axis. Brown, discoloured xylem vessels were noted (indicative of *Foe* infection) and distinction was made between these and natural browning of fibres (Cooper, 2011). Isolation of *Foe* was from fragments of stem cores (2.5 cm length), which were surface sterilised in 2% (v/v) sodium hypochlorite for 5 min before rinsing twice in sterile distilled water (SDW). The materials were then plated onto a *Fusarium*-selective medium (FSM) (Papavizas, 1967) and incubated for four days at 28°C.

Locations of Plantations, Palm Genotypes and Sampling

Data for disease epidemiological and phylogenetic studies were obtained from four locations in three separated oil palm plantations in Ghana: Benso Oil Palm Plantation (BOPP) based at the Adum Banso Estate in Takoradi (001°54′821″N 05°06′643″W), NORPALM Ghana Limited Plantation (NPM) (5°03'58.3"N 1°53'50.7"W) also in Takoradi; and Ghana Oil Palm Development Company (GOPDC) in Kade, the eastern region (06°15′22″N 00°56′38″W). Disease symptom types observed in all fields included palms with chronic and acute symptoms. Three hundred sixty oil palms [*Dura* x *Pisifera* progeny (DxP), planted in 1981] including diseased (34 palms) and apparently healthy palms (326 palms) were visually assessed in BOPP plantation for disease. Tissues from diseased (10 palms) and healthy-looking palms (five palms) representative of the affected area were sampled by removing cylinders from trunks with an increment borer from BOPP Plantation and similarly, 15 palms were sampled from NPM Plantation. In NPM Plantation, 520 palms (40 diseased; 480 apparently healthy) were visually recorded. NPM Plantation records showed that the first disease symptoms appeared in 2004 after planting in 1994 with DxP progeny. In GOPDC Plantations (GOPDC1, GOPDC2: different areas of the same plantation) comprising DxP planted in 1979, 20 random stem core samples from palms with symptoms were collected and 317 palms were visually recorded in affected areas. All core samples were isolated onto FSM (Flood *et al*., 1989).

Fungal Isolates and Growth

Twenty-five isolates of *Foe*, other *F. oxysporum* ff. spp. and other *Fusarium* spp. were obtained from the University of Bath Culture Collection, CABI, United Kingdom, Agricultural Research Services, USDA, and nine were isolated from Ghanaian Plantations by the authors (*Table 1*).

Test of Randomness to Establish the Nature of Spread of *Fusarium* wilt

The disease spatial distribution was analysed using a statistical test. This test supposes that N infected palms are observed within the affected area S and perimeter length P, and considers the 'null hypothesis' that the infected palms are 'randomly' (*i.e*., independently and uniformly) distributed over the affected area (Marcus *et al*., 1984).

Let d1 . . . . , dN denote the distances from each infected palms to its nearest (infected) neighbour. Previous report suggested a test of randomness based on the average nearest-neighbour distance đ = (d1 +... + dN)/N. Their test statistic is CE = [đ – E (đ)] / √var (đ) where E(đ) is the expectation of đ and var(đ) is the variance of đ (Clark and Evans, 1954). Note that the observed distances are not independent and may include pairs of identical measurements between some nearest-neighbour pairs. Thus, the following approximations for the expectation and variance of đ:

$$E(đ) = 0.5\,(S/N)^{½} + (0.514 + 0.412/N^{½})P/N$$
$$√var\,(đ) = 0.07S/N2 + 0.037\,PS^{½}/N^{⅔}$$

Using these values, CE follows an approximately standard normal distribution (Ripley, 1979). The null hypothesis of randomness is rejected in favour of non-randomness at level of significance if CE < $Z\alpha$, where $Z\alpha$ is the lower α-quartile of the standard normal distribution.

Polymerase Chain Reaction (PCR) Amplifications of Translation Elongation Factor 1 Alpha (TEF- 1α) Gene

PCR reactions were conducted using a PTC-100™ (MJ Research). A standard PCR protocol was used to amplify the TEF-1α gene region. Forward primer ef1 (5′ – ATGGGTAAGGAR (A/G) GACAAGAC – 3′) and reverse primer ef2 (5′ – GGAR(A/G)GTACCAGTS(G/C)ATCATGTT-3′) based on (O'Donnell *et al*., 1998) were used. The annealing temperature was 53°C. The PCR programme was 90 s at 94°C, followed by 40 cycles of 30 s at 94°C, 90 s at 55°C, and 2 min at 68°C,

TABLE 1. FORMAE SPECIALES OF *Fusarium oxysporum* AND OUT-GROUPS. ISOLATES OBTAINED FROM GHANAIAN OIL PALM PLANTATIONS WERE SAMPLED FROM CHRONIC (C), ACUTE (A) AND SYMPTOMLESS PALMS (H)

F. oxysporum f. sp.	Code	Culture collection	Source of origin	Country of origin
elaeidis	F1 SS1	University of Bath	ex. oil palm, Binga nursery. J. Flood.	Republic Congo (DRC)
elaeidis	F2 ORI		ex. oil palm, Binga field microplot. J. Flood.	Republic Congo (DRC)
elaeidis	F2 SS2		ex. oil palm, Binga field microplot. J. Flood	Republic Congo (DRC)
elaeidis	F1		ex. oil palm, Binga nursery. J. Flood	Republic Congo (DRC)
elaeidis	Y1 ORI		ex. oil palm. Yaligimba. H. Corley.	Republic Congo (DRC)
elaeidis	F3		ex. oil palm, Binga nursery. J. Flood	Republic Congo (DRC)
elaeidis	16F		ex. oil palm. IRHO screening isolate	Ivory Coast
elaeidis	Y1 SS1		ex. diseased oil palm, Yaligimba.	Republic Congo (DRC)
elaeidis	OPC1		ex. oil palm. Infected root.	Nigeria
elaeidis	BRAZIL	CABI	ex. oil palm	Brazil
elaeidis	GHANA		ex. oil palm	Ghana
elaeidis	NRRL 36359	USDA	ex. oil palm, K O'Donnell	Republic Congo (DRC)
elaeidis	BOPP 11H	Isolates from Ghana	BENSO Oil Palm Plantation, Ghana, from symptomless palm.	Ghana
elaeidis	BOPP 12A		BENSO, from palm with 'acute' *Fusarium* wilt.	Ghana
elaeidis	BOPP IC		BENSO, from palm with 'chronic' wilt	Ghana
elaeidis	GOPDC 18C		Ghana Oil Palm Development Centre (GOPDC), from palm with 'chronic' wilt	Ghana
elaeidis	GOPDC 2C		GOPDC, from palm with 'chronic' wilt.	Ghana
elaeidis	GOPDC 42H		GOPDC, from symptomless palm.	Ghana
elaeidis	NPM 3C		NORPALM Plantation, Ghana, from palm with 'chronic' wilt.	Ghana
elaeidis	NPM 4A		NORPALM, from palm with 'acute' wilt.	Ghana
	NPM 1H		NORPALM, from symptomless palm.	Ghana
Other *F. oxysporum* ff. spp.:				
pisi	1307		ex. pea, UK. University of Bath. R. M Cooper.	United Kingdom
lycopersici	32A		ex. tomato, UK. University of Bath. R. M Cooper.	United Kingdom
canariensis	87-Guil2		Tenerife, Canary Islands. D. Fernandez.	Canary Islands
canariensis	95-2269 J4G6		Palm Beach Co., Florida, USA. G. Simone.	United States

followed in turn by one cycle of 5 min at 94°C and 14°C holding temperature (O'Donnell *et al*., 2007).

Nucleotide Sequence Accession Numbers

The sequences obtained from 25 isolates of *Fusarium oxysporum* ff. spp. were deposited in the GenBank database under accession ID5399896 (TEF-1α). Fifteen TEF-1α sequences datasets that had previously been deposited in GenBank (http://www.ncbi.nlm.nih.gov) and Fusarium ID (http://www.fusariumdb.org) were used for other *Fusarium* isolates listed in *Table 2*.

Sequencing of DNA and Data Analysis

Sequencing reactions were performed by Fisher Scientific or MyGATC. BLAST searches were performed using the GenBank sequence database to confirm the identity of the sequenced fungal isolates. The output from BLAST algorithms was used to query any unknown sequences against the database of all the fungi in the gene TEF-1α. MEGA5 software (Tamura *et al*., 2007) was used to edit and align the nucleotide data, after which the alignments were improved manually.

Phylogenetic analyses were conducted with MEGA5 on the data set of TEF-1α sequences of all 41 isolates included in the present study. Unweighted parsimony analyses were performed with the heuristic search option and 1000 random addition sequences with the MULPARS function on and with tree bisection reconnection branch swapping. The out-group species, *F. foetens*, selected for rooting the gene trees represents the closest species to *Foe*. Clade stability was assessed by 1000 parsimony bootstrap replications and decay indices were calculated with TREEROOT (Tamura *et al*., 2007).

The evolutionary history was inferred using the Maximum Parsimony method (Eck and Dayhoff, 1966). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap

TABLE 2. TRANSLATION ELONGATION FACTOR 1 ALPHA (TEF-1α) SEQUENCES DEPOSITED IN GENBANK (http://www.ncbi.nlm.nih.gov) AND FUSARIUM ID (http://isolate.fusariumdb.org/) USED AS REFERENCES FOR THE ISOLATES MENTIONED BELOW

Forma specialis	Accession number
elaeidis	FJ985270.1
dianthi	FJ985281.1
canariensis	FJ985388.1
canariensis	AF008485.1
cubense	AF008456.1
cubense	AF008489.1
albedinis	DQ837688.1
albedinis	DQ837686.1
phaseoli	EF056787
gladeoli	EF056787.1
gladeoli	FJ985302
niveum	FJ985410
melonis	FJ985266
batatas	DQ837678
vasinfectum	KP192925
Fusarium sp.	
F. foetens	GU170560.1

test (1000 replicates) was assessed to determine the clade stability. The MP tree was obtained using the Close-Neighbour-Interchange algorithm (Nei and Kumar, 2000) in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option).

Pathogen Isolates, Growth and Inoculum for Pathogenicity Testing

Nine isolates of *Fusarium* spp. (GOPDC 2C, GOPDC 18C, GOPDC 42H, BOPP 11H, BOPP 12A, BOPP IC, NPM 4A, NPM 3C and NPM 1H) were obtained from palms in BOPP, NPM and GOPDC plantations. These isolates were chosen in order to confirm their pathogenicity and aggressiveness as they were sampled from chronic (C) and acute (A) palms. *Fusarium* spp. (identified by TEF-1α sequencing) were sometimes isolated from xylem of healthy-looking palms (H) and these were also tested for pathogenicity. *Foe* 16F from diseased palms in the Ivory Coast was used as a control because the isolate was previously used by Institut de Recherches pour les Huiles et Oléagineux (IRHO) as their pathogenic isolate for *Fusarium* wilt disease resistance screening; we have routinely confirmed its pathogenicity (Flood *et al*., 1993). Isolates were stored at -80°C in 20% glycerol then cultured on Czapek-Dox agar (CDA) and incubated at 25°C for five days.

As described previously (Rusli *et al*., 2015), for inoculum preparation isolates were sub-cultured into 100 ml CD liquid medium in 250 ml conical flasks for three days, shaken at 150 rpm and 25°C then filtered to remove mycelial fragments and centrifuged at 13 000 g for 10 min. The pelleted conidia were re-suspended in 50 ml sterile distilled water and adjusted to 106 spores ml^{-1}. Ten ml of conidial suspension was applied with a sterile syringe onto the soil surface around the base of each palm of age three months. The inoculum was then watered with sterile distilled water for two weeks. Non-inoculated plants served as controls. Each progeny was represented by 10 replicates using randomised complete block design (RCBD).

Plant Materials

Oil palm germinated seeds of a susceptible, commercial progeny DxP (Johor Labis and Yangambi) (Durand-Gasselin *et al*., 2000) were supplied by the Malaysian Palm Oil Board (MPOB). The palms were transferred into black polyethylene bags (15.2 x 125.4 x 25.4 cm) filled with compost (BHGS Ltd Levingtons F2 + sand, BHGS Ltd, Levingtons M2, perlite in ratio 1:1:1) and grown in a greenhouse at 25°C to 32°C at the University of Bath, United Kingdom. Palms were supplied monthly with liquid fertiliser (BHGS Ltd; 1 in 45 dilution, containing N, P, K. in the ratio of 8:3:3 and trace elements); otherwise they were watered from below on alternate days (Rusli *et al*., 2015).

Disease Severity Index

The percentage chlorotic or necrotic leaf index was rated from 0 to 5, 0 = no symptoms, 1 = slight necrosis/chlorosis on 1-2 leaf tips - usually oldest leaves; 2 = necrosis/chlorosis over one-quarter of the leaves and some shortening of the youngest leaves; 3 = severe necrosis/chlorosis over one-half of the leaves. Extensive leaf desiccation and stunting; 4 = severe necrosis/chlorosis over three-quarters of the leaves. Extensive leaf desiccation and stunting; 5 = plant dead, as detailed and imaged in a previous study (Rusli *et al*., 2015). The disease severity index assessment was carried out one month after inoculation then at monthly intervals.

Colonisation of Oil Palm Tissues

Re-isolation of inoculated *Foe* from the plant roots, bulbs (lower stem) and petioles of inoculated and un-inoculated palms was conducted at the end of the experiment in week 40, as previously described (Rusli *et al*., 2015). For qualitative re-isolation, 0.5 cm fragments of plant materials (sections of root, petiole or stem core samples taken with an increment borer, internal diameter 5.5 cm) (Mepsted *et al*., 1994) were surface sterilised in 2% (v/v) sodium hypochlorite for 10 min (5 min for tissue cores) before rinsing

twice in SDW. Samples were then plated onto FSM (Flood *et al*., 1989) and incubated for four days at 28°C.

For quantitative re-isolation, 1 g fresh weight of root, bulb or petiole tissue was surface sterilised as described above. The tissue was ground with 1 cm^3 of sterile sand and 9 ml of SDW. A 10-fold dilution series was prepared and 0.5 ml of the suspension was spread onto triplicate plates of FSM. After incubation at 28°C for four days, colonies of *Fusarium* were counted and the number of colony forming units (CFU) per g fresh weight of palm tissue was calculated. The data collected were subjected to analysis of variance (ANOVA). Means were separated using contrast statements at significance level of $P < 0.05$. Comparison between means of treatment was made using Tukey HSD.

RESULTS AND DISCUSSION

Fusarium wilt Occurrence and Spread within Affected Plantations

For the BOPP Plantation (*Figure 1a*), where 360 plants were recorded, 33 palms appeared visibly infected. Based on the data evaluated from equation $CE = [đ - E(đ)] / \sqrt{var(đ)}$, the hypothesis of randomness is rejected in favour of clustering at a significance level $p < 0.01$ at -10.27. The same conclusion was reached from the data from NPM Plantation (*Figure 1b*) where the disease pattern in the plantation was due to cluster distribution ($p<0.01$). ANOVA performed on the infected palms' distribution in GOPDC 1 Plantation (*Figure 1c*) also showed the CE value significance at $p<0.01$, which reflects cluster distribution instead of random distribution. The same conclusion was reached for the data of the GOPDC 2 Plantation (*Figure 1d*). A cluster distribution was evident in the field and once again, the null hypothesis of randomness is rejected in favour of non-randomness at ($p<0.01$).

Genetic Variation of *Foe* Isolates between and within Countries

Phylogenetic trees from parsimony bootstrap analysis of *Foe* obtained from plantations in Ghana and *Foe* from six different countries were examined based on the *TEF-1α* gene (*Figure 2*). A total of 22 strains of *Fusarium* isolated from oil palm xylem were chosen for the study along with 19 other *Fusarium* strains for comparative purposes. All *Foe* isolates that were isolated from diseased palms from various countries had previously been positively tested for pathogenicity, except *Foe* Brazil, *Foe* F2SS2, *Foe* NRRL 36359 and Foe 38313 (Mepsted *et al*., 1991). The Ghanaian isolates were confirmed as *Foe* by their pathogenicity to oil palm, as part of this study (*Figure 3*). TEF-1α sequences obtained from GenBank and Fusarium ID *(Table 2*) were also included in the analyses.

The constructed phylogenetic tree based on the nucleotide sequence alignment of the 680 bp nucleotides, excluding any uninformative characters showed the monophyletic nature of *Foe* (*Figure 2*). The earliest diverging lineage (83% bootstrap support) comprised 17 strains of *Foe* representing clade 1; while other *Foe* isolates BOPP IC, GOPDC 42H, BOPP 11H and GOPDC 2C were in clade 2 with 98% bootstrap support. Strains of *Foe* appear to be closely related with no correlation to geographical origin. Isolates from different plantations and countries are similar to one another. Formae speciales of *F. oxysporum* from other palms, f. sp. *albedinis* and f. sp. *canariensis*, were nested into three independent groups. Representatives of *Foe* isolates obtained from Ghana that appeared within the *Foe* clade were tested for their pathogenicity on oil palm. A single Ghanaian isolate (NPM 1H) that was obtained from a symptomless palm, but fell outside clades 1 and 2, was also subjected to pathogenicity testing in order to compare with the phylogeny results.

Evaluation of Pathogenicity of Ghanaian Isolates from Different Plantations and Based on Chronic, Acute and Symptomless Palms

Foe isolates from Ghana scattered around the *Foe* clade were inoculated onto palm seedlings to evaluate pathogenicity and to confirm identity as f. sp. *elaeidis*. The evaluation of pathogenicity of *Foe* isolates from chronic and acute palms (six isolates, plus aggressive control isolate 16F) and of *F. oxysporum* from symptomless palms (three isolates) also was carried out in order to establish any link between the levels of aggressiveness of the isolates and symptom development and intensity.

All *Foe* isolates tested showed different levels of aggressiveness even though they are from related clades 1 and 2 (*Figure 2*). BOPP 12A resulted in gradually progressing symptoms throughout and gave the highest mean wilt index after 40 weeks, together with the isolate *Foe* 16F, of already established high aggressiveness (Flood *et al*., 1989) (*Figure 3*). Both isolates induced significantly greater vascular necrosis (browning) compared to other isolates (results not shown). The high level of aggressiveness of the *Foe* isolate might reflect that it was isolated from a palm with acute symptoms, yet another *Foe* isolate from an acute symptoms palm (NPM 4A) was much less aggressive, and similar to BOPP IC. Nevertheless, all *Foe* isolates isolated from chronic palms only showed disease progression at or after 24 weeks, which could explain the slow disease development observed in the field.

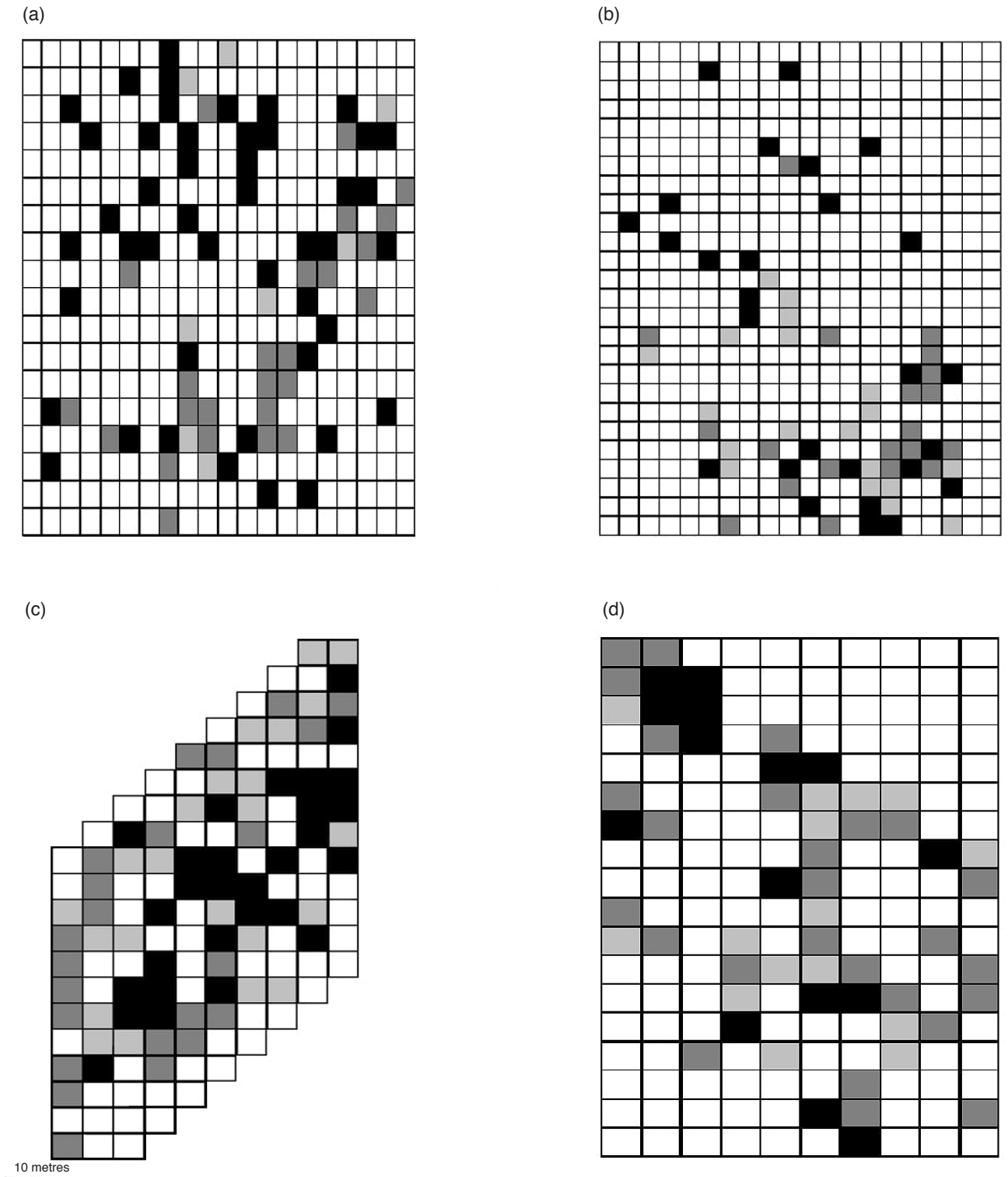

Figure 1. Spatial pattern map of disease severity caused by Foe *in: Benso Oil Palm Plantation (BOPP) (a), NORPALM Ghana Limited Plantation (NPM) (b), Ghana Oil Palm Development Company (GOPDC) 1 (c) and GOPDC 2 (d) affected area. These areas represent hot-spots where* Foe*-affected areas were clearly evident visually. Oil palms were planted at a distance of 10 m from each other; therefore each square represents one palm at 10 m distance from adjacent palms.*

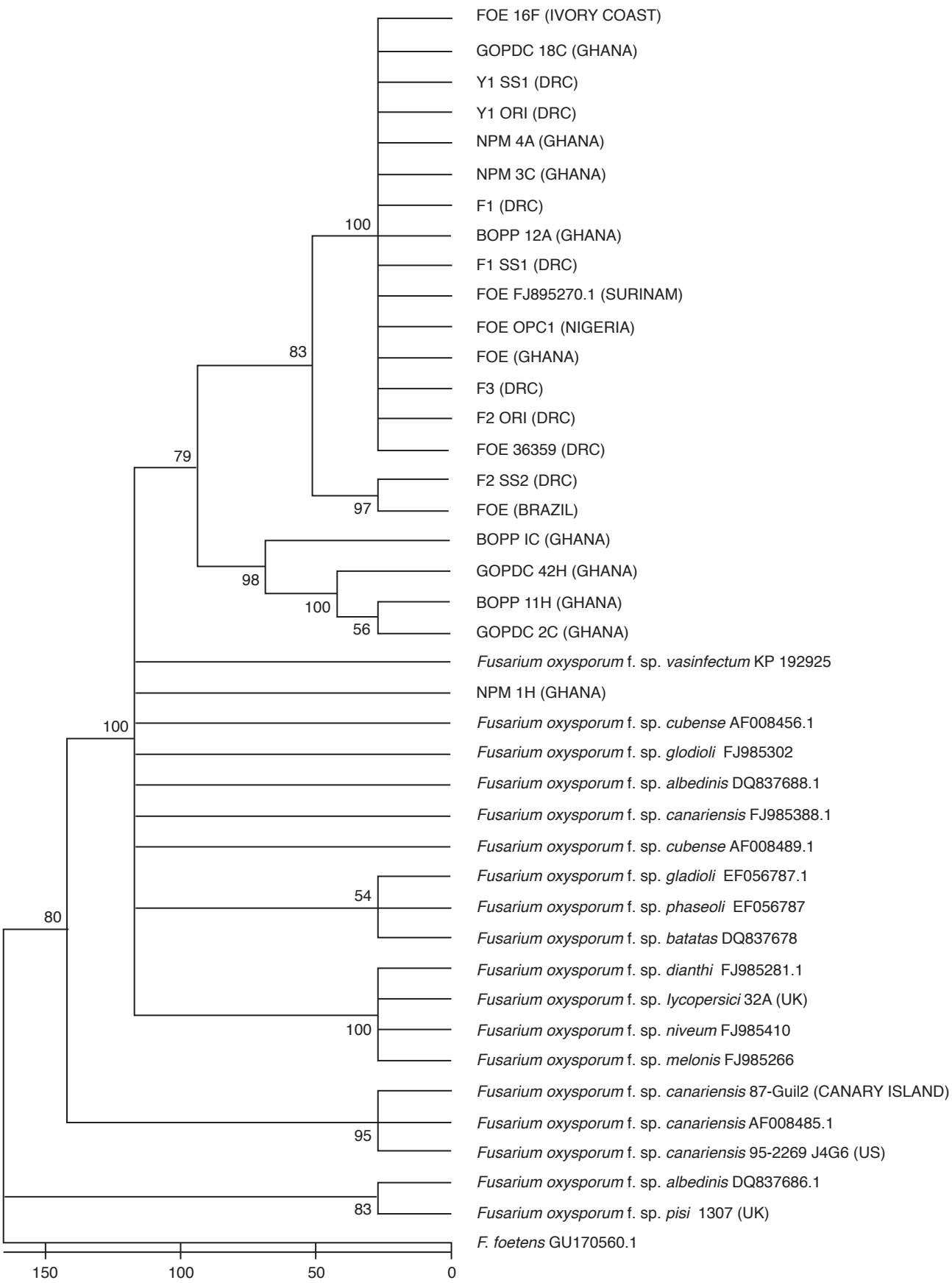

Figure 2. Phylogenetic tree of F. oxysporum *f. sp.* elaeidis *inferred from combined (TEF-1α) sequence data.The phylogenetic tree was constructed using maximum parsimony analysis with 22 strains of* Fusarium *and 19 out groups. The tree is rooted with* F. foetens.

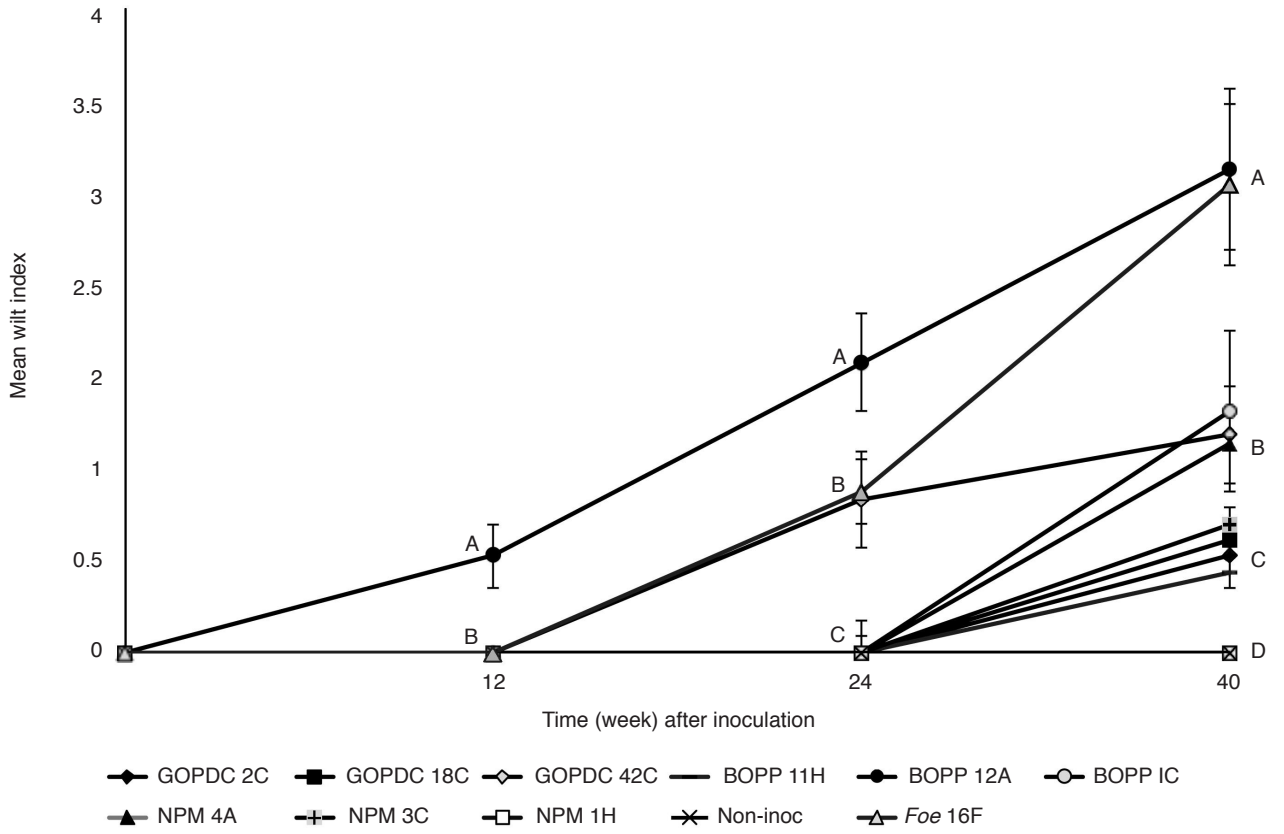

Figure 3. Disease symptoms in oil palms induced by Ghanaian isolates of F. oxysporum*. Values represent n=10 replicates, with each letter denoting significant differences (p≤0.05) between treatments according to week after inoculation; analysed with Tukey (IBM SPSS). Error bars represent standard error (SE) for each treatment. Isolates were from plantations: Ghana Oil Palm Development Company (GOPDC), Benso Oil Palm Plantation (BOPP) or NORPALM Ghana Limited Plantation (NPM), and were from chronic (C), acute (A) or symptomless (H) palms. Isolate 16F is included as a control known pathogenic isolate (see* Table 1 *and methods).*

Qualitative re-isolation showed some of the isolates, such as BOPP 12A and *Foe* 16F colonised root, bulb, 1st, 3rd and 7th leaves of the inoculated oil palms, while others were more limited in their extent of colonisation (data not shown). Based on quantitative re-isolation, *Foe* 16F intensively colonised the root tissue and bulb at ca. 10^4 CFU g^{-1} fresh weight followed by BOPP 12A at 10^3 CFU g^{-1}. (*Figure 4*). These isolates were also present in greater amounts in petioles of the 1st, 3rd and 7th leaves compared to others. Therefore high colonisation density of BOPP 12A and *Foe* 16F CFU was linked with rapid and early disease development (*Figure 3*). Slower and milder disease development by other isolates usually reflected lower colonisation density by these isolates. GOPDC 2C is a notable exception with equivalent colonisation of root and bulb tissues to BOPP 12A and 16F.

Some isolates from symptomless palms (GOPDC 42H and BOPP 11H) also showed substantial colonisation of all tissues and induced eventual symptoms (*Figure 3*), whereas GOPDC 18C colonised systemically but palms remained symptomless; NPM 1H induced neither symptoms nor significant colonisation. Other possibly endophytic *Fusarium* spp., were also recovered from control palms at ca. 10^1 CFU g^{-1} in root and bulb tissues but not in petioles.

Presence of *Foe* and Other *Fusarium* spp. in Symptomless Palms

Forty-five core samples were taken from trunks of 45 standing oil palms in the GOPDC area where eight of the sampled palms exhibited external *Fusarium* wilt symptoms. According to re-isolation onto FSM and identification based on isolates' similarity to *Foe* TEF-1α sequences in GenBank, 13 (29%) palms contained *Foe*, including five palms classed as healthy by external appearance.

Isolates of other *Fusarium* spp. were occasionally re-isolated from symptomless palms (as above) and revealed by TEF-1α sequencing as *F. solani*, *F. equiseti* and *F. oxysporum*.

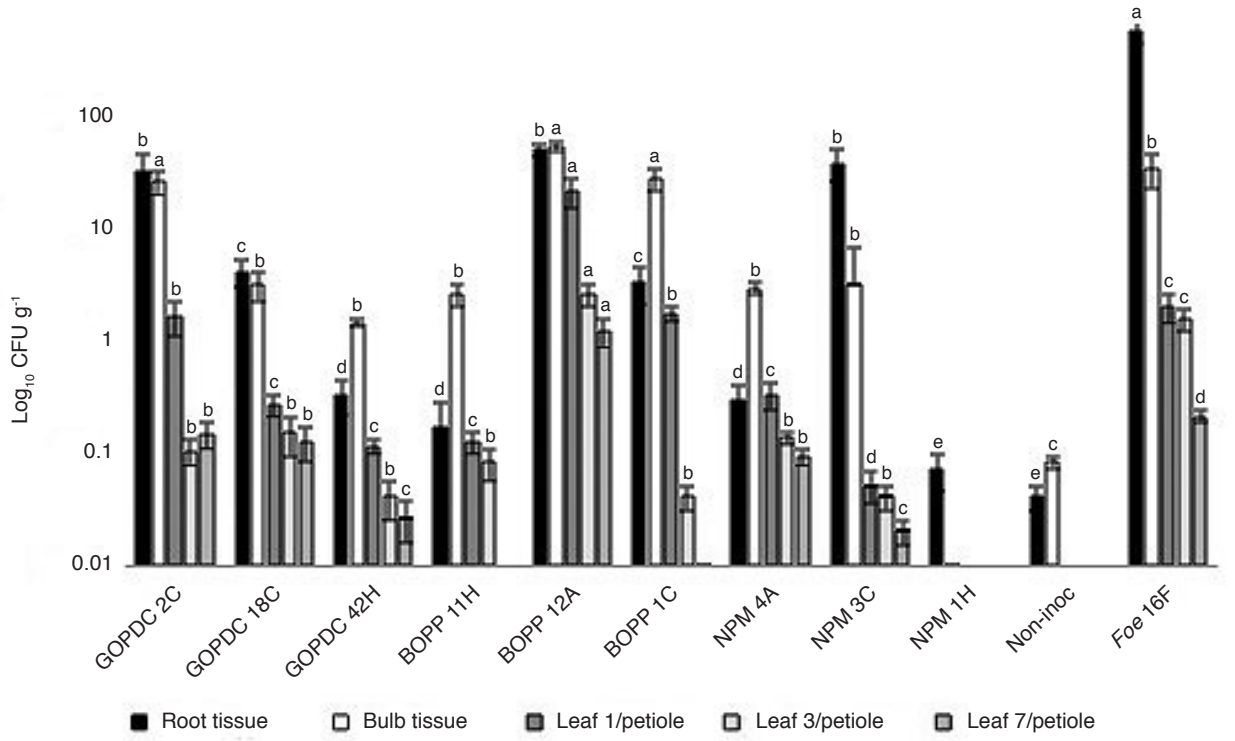

Figure 4. Quantitative re-isolation of Ghanaian isolates 40 weeks post inoculation. Each treatment consisted of 10 replicates of oil palm seedlings. Error bars represent standard error (SE) for each treatment and each letter denotes significant differences (p≤0.05) between colony forming units(CFU) of each plant part (root, bulb, leaf 1, leaf 3 and leaf 7);analysed with Tukey (IBM SPSS). GOPDC, BOPP, NPM are isolates from Ghanaian plantations from chronic (C), acute (A) or symptomless (H) palms. Non-inoc., non-inoculated control. Isolate 16F is included as a control known pathogenic isolate (see Table 1 *and methods).*

CONCLUSION

Spatial distribution analysis of disease incidence indicated non-random patterns of disease were evident in four Ghanaian plantations. These results strongly suggest that palm to palm infection via roots plays the key role in establishment of *Foe* infection, even though the potential for aerial spread by conidia has been suggested (Cooper *et al*., 1989). *F. oxysporum* f. sp. radicis-lycopersici spreads from infected to neighbouring plants via root-to-root dissemination (Rekah *et al*., 2000) and for coffee wilt caused by *Fusarium xylarioides*, one infected tree can infect up to three healthy trees in any direction, resulting in an aggregated pattern (Musoli *et al*., 2008).

Palms killed by *Foe* become a source of nutrients as well as inoculum for extending roots of adjacent palms, which can then form clusters of diseased and dead palms (Flood, 2006; Cooper, 2011). On a larger scale, in an area where the main cause of palm death was *Fusarium* wilt, it was reported that of 1600 palms without missing neighbours 17% had wilt, compared with 24% infection of 1000 palms with one or more neighbours missing (Dossa *et al*., 1991). Clearly, information on the role of diseased palms in extent and rate of disease spread is important for designing effective control management strategies. The secondary roots of oil palm can grow as far as 25 m horizontally (Jourdan *et al*., 2000) thus, eradication of infected palms at the earliest opportunity, before root elongation, should minimise disease spread and inoculum potential.

We detected the presence of *Foe* in ca. 11% of symptomless palms in GOPDC Plantation and also occasionally found in the other Ghanaian plantations, palms with apparent *Fusarium* wilt symptoms that were not infected by *Foe* (Cooper, 2011). This reveals that visual disease surveys may not show the true picture of the level of infection and we recommend that they are supported by internal inspection with increment borers (Mepsted *et al*., 1991; Cooper, 2011). *Fusarium solani*, *F. equiseti* and *F. oxysporum* spp. isolates were also found in symptomless palms, as confirmed through molecular identification. These three *Fusarium* species and *F. verticillioides* and *F. incarnatum* were identified on contaminated oil palm pollen (Flood *et al*., 1990) and *F. equiseti* has been associated with oil palm seedling root rot, spear rot and bud rot (Turner, 1981). We cannot know at this stage if they are pathogens, contaminants or endophytes. On the other hand, *F. oxysporum* isolates from within palms are more likely to be endophytic, in view of the many forms of this

species adapted for vascular (xylem) colonisation; systemic but symptomless invasion of oil palms was found for some Malaysian strains of *F. oxysporum* (Flood *et al*., 1989). We also isolated *F. oxysporum* as one of several putative endophytes from within roots and lower stems of uninoculated oil palm seedlings grown in plantation soils from Malaysia under United Kingdom conditions (unpublished data). Notably, the putative endophytic *F. oxysporum* isolates inhibited to various extents *Foe* isolates on dual culture plates (unpublished data) so could conceivably influence pathogen colonisation.

Based on the sequence information from TEF-1α, this study demonstrates that *Foe* has a monophyletic origin. Seventeen *Foe* isolates from six different countries resolved into one clade comprising two independent lineages with a support value of 83%, suggesting a moderate level of genetic diversification. Four other isolates BOPP IC, GOPDC 42H, BOPP 11H and GOPDC 2C also grouped together but are located in clade 2 with a support value of 98%. They possibly evolved from different local ancestral strains, as argued for f. sp. *vasinfectum* on cotton, which exists as two VCG in Australia (Wang *et al*., 2006). The low level of diversity in *Foe* reported here might explain the reported long-term stability of lines bred in Africa for *Fusarium* wilt resistance (Cochard *et al*., 2005; Rusli *et al*., 2015).

However, there is a risk with a potentially variable pathogen such as *Foe*, that palms selected for resistance in one area might be susceptible to infection elsewhere. In general, this seems unlikely based on findings where inoculation of 14 palm clones with three *Foe* isolates from different parts of Africa showed variation in aggressiveness, but clone-isolate interactions were not significantly different (Mepsted *et al*., 1994). Nevertheless, sometimes the ranking of isolates by clones varied considerably, leading to a possible explanation why a few apparently resistant crosses have proved susceptible when planted in areas remote from where their resistance was assessed (Flood *et al*., 1993; Mepsted *et al*. 1994). We also recently found differential interactions between some *Foe* isolates and palm lines (Rusli *et al*., 2015).

It is tenable that *F. oxysporum* ff. spp. that have adapted to different palm hosts might share similarities because they will have had to overcome related anatomical and biochemical barriers. However, we found *Foe*, f. sp. *albedinis* (date palm) and f. sp. *canariensis* (Canary Islands date palm) were nested into independent lineages. Formae speciales *canariensis*, f. sp. *albedinis*, f. sp. *palmarum* (*Syagrus romanzoffiana* and *Washingtonia robusta* hosts in Florida) and *Foe* have independent evolutionary origins (Phyler *et al*., 2000). Previous work also shown that f. sp. *palmarum* is more closely related to f. sp. *albedinis* than to *Foe* or to f. sp. *canariensis* (Elliot *et al*., 2010). It appears that evolution of palm pathogenic forms were probably driven by the geographical separation of their host plants. Notably, Australian isolates of f. sp. *canariensis* are diverse and show an independent evolutionary origin from an extensive international population, which showed little genetic diversity (most isolates belonged to a single VCG and with only four RFLP haplotypes) (Phyler *et al*., 2000; Gunn and Summerell, 2002; Laurence *et al*., 2015).

The different forms of *Fusarium* wilt expression in the field, expressed as chronic or acute, might reflect variation in individual palms, which are not clonal but derive from seed; or they might result from different levels of aggressiveness in *Foe* isolates; or could reflect the timing of infection and inoculum load of an individual palm. All isolates from both types of syndrome were pathogenic, but varied in aggressiveness. The most aggressive isolate came from an acute infection and as well as inducing extreme symptoms, it was present in highest amounts in palm roots, stems and petioles. Some isolates from chronic infections were also effective colonisers but resulted in later and milder symptoms. Some *F. oxysporum* isolates (coded 'H') from symptomless palms, colonised systemically but showed low or no aggressiveness, as reported previously in Malaysia with soil isolates of *F. oxysporum* (Flood *et al*., 1989).

This wide range of responses may well reflect the field situation of this chronic disease, where the presence within a palm of a *Foe* isolate might be a recent colonisation event or an infection that has been accumulating for months or even years. Even under controlled conditions with inoculated seedlings, disease expression takes several months (Cooper, 2011; Rusli *et al*., 2015). Greater numbers need to be tested to establish any firm correlation between isolate aggressiveness and chronic or acute wilt, but there was limited availability of palm seedlings in our United Kingdom-based facility to provide sufficient replication in light of palm seedling variation. Future work should ideally involve clonal palms for both natural infection in the field and for pathogenicity testing in the laboratory.

Knowledge of the phylogeny and epidemiology of *Foe* is important in developing control strategies based on use of resistant genotypes and cultural control measures. This is the first report of either phenomenon for this major disease.

ACKNOWLEDGEMENT

This research was supported by MPOB with a studentship to the first author. We thank colleagues in MPOB for providing the oil palm seedlings for this study. This work was conducted at the University of

Bath, United Kingdom under the FERA Plant Health Licence PHL 188A/6475. We gratefully acknowledge the cooperation of plantations in Ghana to allow access, evaluations and sampling: Benso Oil Palm Plantation (BOPP) Takoradi, NORPALM Ghana Limited Plantation (NPM), Takoradi and Ghana Oil Palm Development Company (GOPDC) in Kade. We are indebted to the Oil Palm Research Institute, Kusi, Kade, Ghana for organising these field surveys in particular S Tagoe and K Afrim. We acknowledge K O'Donnell's (USDA) generous provision of many *Fusarium* isolates. The authors wish to thank the Director-General of MPOB for permission to publish this study.

REFERENCES

ANDOLFO, G F; FERRIELLO, F; TARDELLA, L; FERRARINI, A; SIGILLO, L; FRUSCIANTE, L and ERCOLANO, M R (2014). Tomato genome-wide transcriptional responses to *Fusarium* wilt and tomato mosaic virus. *PLoS ONE, 9*: e94963. http://dx.doi.org/10.1371/journal.pone.0094963

BAAYEN, R P; O'DONNELL, K; BONANTS, P J, CIGELNIK, E; KROON, L P; ROEBROECK, E J and WAALWIJK, C (2000). Gene genealogies and aFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. *Phytopathology, 90*: 891-900. https://doi.org/10.1094/PHYTO.2000.90.8.891

BAAYEN, R P; O'DONNELL, K; BONANTS, P J M; CIGELNIK, E; KROON, L P N M; ROEBROECK, E J A and WAALWIJK, C (2011). Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic formae speciales causing wilt and rot disease. *Phytopathology, 90*: 891-900. DOI: 10.1094/PHYTO.2000.90.8.891.

CLARK, P J and EVANS, F C (1954). Distance to nearest neighbour as a measure of spatial pattern in biological populations. *Ecology, 35*: 445-453. http://links.jstor.org/sici=0012-9658%28195410%2935%3A4%3C445%3ADTNNAA%3E2.0.CO%3B2-J

COCHARD, B; AMBLARD, P; and DURAND-GASSELIN, T (2005). Oil palm genetics and sustainable development. *Oleagineux Corps Gras Lipids, 12*: 141-147. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.917.8451&rep=rep1&type=pdf

COOPER, R M; FLOOD, J and MEPSTED, R (1989). *Fusarium* wilt of oil palm: transmission, isolate variation, resistance. *Vascular Wilt Diseases of Plants* (Tjamos, E C and Beckman, C H eds.). p. 247-250. NATO ASI Series. Springer-Verlag. DOI: 10.1007/978-3-642-73166-2_18.

COOPER, R M (2011). *Fusarium* wilt of oil palm: a continuing threat to Southeast Asian plantations. *The Planter, 87*: 409-418. https://www.cabdirect.org/cabdirect/abstract/20113317744

COOPER, R M and RUSLI, M H (2014). Threat from *Fusarium* wilt disease of oil palm to South-east Asia and suggested control measures. *J. Oil Palm Res. Vol. 26*: 109-119. http://jopr.mpob.gov.my/threat-from-fusarium-wilt-disease-of-oil-palm-to-south-east-asia-and-suggested-control-measures/?v=true

CORLEY, R H V and TINKER, P B (2003). *The Oil Palm*. 4th Edition. Blackwell Science Ltd, United Kingdom. http://onlinelibrary.wiley.com/doi/10.1002/9780470750971.fmatter/pdf

DE FRANQUEVILLE, 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.

DOSSA, C; PANDO-BAHUON, A; RENARD, J L and BOISSON, C (1991). Determination of vegetative compatability groups in African *Fusarium oxysporum* strains isolated from vascular wilt-infected oil palms. *Oleagineux, 46*: 145-147. http://agritrop.cirad.fr/427856/

DUMORTIER, F; VAN AMSTEL, H and CORLEY, R H V (1992). *Oil Palm Breeding at Binga, Zaire. 1970-1990*. Unilever Plantations, London. http://www.cabi.org/isc/datasheet/24639

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.* p. 446-470. http://agritrop.cirad.fr/525609/

ECK, R V and DAYHOFF, M O (1966). *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Springs, MD. http://www.worldcat.org/title/atlas-of-protein-sequence-and-structure-1966/oclc/499052378

ELLIOTT, M L; DES JARDIN, E A; O'DONNELL, K; GEISER, D M; HARRISON, N A and BROSCHAT, T K (2010). *Fusarium oxysporum* f. sp. *palmarum*, a novel forma specialis causing a lethal disease of *Syagrus*

romanzoffiana and *Washingtonia robusta* in Florida. *Plant Dis., 94*: 31-38. DOI:10.1094 / PDIS-94-1-0031.

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. Phytopathol., 124*: 80-88. DOI: 10.1111/j.1439-0434.1989.tb04898.x.

FLOOD, J; MEPSTED, R and COOPER, R M (1990). Potential spread of Fusarium wilt of oil palm on contaminated seed and pollen. *Mycol. Res., 94*: 708-709. http://agris.fao.org/agris-search/search.do?recordID=MY19940025666

FLOOD, J; WHITEHEAD, D S and COOPER, R M (1992). Vegetative compatibility and DNA polymorphisms in *Fusarium oxysporum* f. sp. *elaeidis* and their relationship to isolate virulence and origin. *Physiol. Mol. Plant. Pathol., 41*: 201-215. DOI: 10.1016/0885-5765 (92)90011-J.

FLOOD, J; MEPSTED, R; VELEZ, A; PAUL, T and COOPER, R M (1993). Comparison of virulence of isolates of *Fusarium oxysporum* f. sp. *elaeidis* from Africa and South America. *Plant Pathol., 42*: 168 - 171. DOI: 10.1111/j.1365-3059.1993.tb01487.x.

FLOOD, J (2006). A review of *Fusarium* wilt of oil palm caused by *Fusarium oxysporum* f. sp. elaeidis. *Phytopathology, 96*: 660-662. DOI: 10.1094/PHYTO-96-0660.

FOURIE, G; STEENKAMP, E T; GORDON, T R and VILJOEN, A (2009). Evolutionary relationships among the *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups. *Appl. Environ. Microbiol., 75*: 4770-4781. DOI: 10.1128/AEM.00370-09.

GEISER, D M; JIMENEZ-GASCO, M; DEL MAR KANG, S; MAKALOWSKA, I; VEERARAGHAVAN, N; WARD, T J; ZANG, N; KULDAU, G A and O'DONNELL, K (2004). FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium. Eur. J. Plant. Pathol., 110*: 473-479. DOI:10.1023/B:EJPP.0000032386.75915.a0.

GORDON, T R and MARTYN, R D (1997). The evolutionary biology of *Fusarium oxysporum*. *Annu. Rev. Phytopathol., 35*: 111-128. DOI: 10.1146/annurev.phyto.35.1.111.

GUNN, L V and SUMMERELL, B A (2002). Differentiation of *Fusarium oxysporum* isolates from *Phoenix canariensis* (Canary Island date palm) by vegetative compatibility grouping and molecular analysis. *Australas. Plant Pathol., 31*: 351-358. DOI:10.1071/AP02039.

JOURDAN, C; MICHAUX-FERRIERE, N and PERBAL, G (2000). Root system architecture and gravitropism in the oil palm. *Ann. Bot., 85*: 861-868. DOI: https://doi.org/10.1006/anbo.2000.1148.

KAWABE, M; KOBAYASHI, Y; OKADA, G; YAMAGUCHI, I; TERAOKA, T and ARIE, T (2005) Three evolutionary lineages of tomato wilt pathogen, *Fusarium* f.sp. *lycopersici*, based on sequences of IGS, AT1 and pg1 are each composed of isolates of a single mating type and a single or closely related vegetative compatibility group. *J Gen. Plant Pathol., 71*: 263-272. DOI:10.1007/s10327-005-0203-6.

LAURENCE, M H; SUMMERELL, B A and LIEW, E C Y (2015). *Fusarium oxysporum* f. sp. *canariensis*: evidence for horizontal gene transfer of putative pathogenicity genes. *Plant Pathol., 64*: 1068-1075. DOI: 10.1111/ppa.12350.

LIEVENS, B; REP, M and THOMMA, P H J (2008). Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Manag. Sci., 64*: 781-788. DOI: 10.1002/ps.1564.

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; HENRISSAT, B; HOUTERMAN, P M; KANG, S; SHIM, W B; WOLOSHUK, C; XIE, X; XU, J R; ANTONIW,J; BAKER, S E; BLUHMN, B H; BREAKSPEARL, A; BROWN, D W; BUTCHKO, R A E; CHAPMAN, S; COULSON, R; COUTINHO, P M; DANCHIN, E G J; DIENER, A; GALE, L R; GARDINER, D M; GOFF, S; HAMMOND-KOSACK, K E; HILBURN,K; HUA-VAN, A; JONKERS, W; KAZAN, K; KODIRA, C D; KOEHRSEN, M; KUMAR, L; LEE, Y H; LI, L; MANNERS, J M; MIRANDA-SAAVEDRA, D; MUKHERJEE, M; PARK, G; PARK, J; PARK, S Y S; PROCTOR, R H; REGEV, A; RUIZ-ROLDAN, M C; SAIN,D; SAKTHIKUMAR,S; SYKES, S; SCHWARTZ, D C; TURGEON, B G; WAPINSKI, I; YODER, O; YOUNG, S; ZENG, Q; ZHAOU, S; GALAGAN, J; CUOMOL, C A; KISTLER, K C and REP, M (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature, 464*: 367-373. DOI:10.1038/nature08850.

MARCUS, R; FISHMAN, S; TALPAZ, H; SALAMON, R and BAR-JOSEPH, M (1984). On the spatial distribution of *citrus tristeza* virus disease. *Phytoparasitica, 12*: 45-52. DOI:10.1007/BF02980797.

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*. *Int.*

J. Oil Palm Res., 3: 329-335. http://www.cabi.org/isc/datasheet/24639

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. http://agris.fao.org/agris-search/search.do?recordID=FR19810608425.

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. http://agris.fao.org/agris-search/search.do?recordID=FR19810608425

MICHIELSE, C B and REP, M (2009). Pathogen profile update: *Fusarium oxysporum*. Mol. *Plant. Pathol., 10*: 311-324. DOI:10.1111/j.1364-3703.2009.00538.x.

MOUYNA, I; RENARD J L and BRYGO, Y (1996). DNA polymorphism among *Fusarium oxysporum* f. sp. *elaeidis* populations from oil palm, using a repeated and dispersed sequence 'Palm'. *Curr. Genet, 30*: 174-180. DOI:10.1007/s002940050117.

MUSOLI, C P; PINARD, F; CHARRIER, A; KANGIRE, A; HOOPE, G M; KABOLE, C; OGWANG, J; BIEYSSE, D and CILAS, C (2008). Spatial and temporal analysis of coffee wilt disease caused by *Fusarium xylarioides* in *Coffea canephora. Eur. J. Plant. Pathol., 122*: 451-460. DOI:10.1007/s10658-008-9310-5.

NEI, M and KUMAR, S (2000). *Molecular Evolution and Phylogenetics*. Oxford University Press, New York. https://books.google.com.my/books?isbn=0199881227

O'DONNELL, K; KISTLER, H C; CILGENIK, E and PLOETZ, R C (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Nat. Acad. Sci. USA, 95*: 2044-2049. http://www.pnas.org/content/95/5/2044.full

O'DONNELL, K; SARVER, B A J; BRANDT, M; CHANG, D C; WANG, J N; PARK, B J; SUTTON, D A; BENJAMIN, L; LINDSLEY, M; PADHYE, A; GEISER, D M and WARD, T J (2007). Phylogenetic diversity and microsphere array-based genotyping of human pathogenic Fusaria, including isolates from multistate contact lens-associated US keratitis outbreaks of 2005 and 2006. *J. Microbiol., 45*: 2235-2248. DOI:10.1128/JCM.00533-07.

PAPAVIZAS, G (1967). Evaluation of various media and antimicrobial agents for isolation of Fusaria from soil. *J. Phytopathol., 57*: 848-852. https://www.cabdirect.org/cabdirect/abstract/19671103688

PRENDERGAST, A G (1957). Observations on the epidemiology of vascular wilt disease of the oil palm (*Elaeis guineensis* Jacq.). *J. West Afr. Inst .Oil Palm Res., 2*: 148-175. https://www.cabdirect.org/cabdirect/abstract/19581100178

PYLER, T R; SIMONE, G W; FERNANDEZ, D and KISTLER, H C (2000). Genetic diversity among isolates of *Fusarium oxysporum* f. sp. *canariensis*. *Plant Pathol., 49*: 155-164. DOI: 10.1046/j.1365-3059.2000.00427.x

REKAH, Y; SHTIENBERG, D and KATAN, J (1999). Spatial distribution and temporal development of *Fusarium* crown and root rot of tomato and pathogen dissemination in field soil. *Phytopathology, 89*: 831-839. http://eprints.icrisat.ac.in/2784/1/Phytopathology_89_9_831-839_1999.pdf

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. http://agritrop.cirad.fr/387880/

RIPLEY, B D (1979). Tests of randomness for spatial point patterns. *J. Royal. Stat. Soc. Ser., B 41*: 368-374. DOI : 10.1051/ps/2012027.

RUSLI, M H; IDRIS, A S and COOPER, R M (2015). Evaluation of Malaysian oil palm progenies for susceptibility, resistance or tolerance to *Fusarium oxysporum* f. sp. *elaeidis* and defence-related gene expression in roots. *Plant Pathol., 64*: 988-995. DOI: 10.1111/ppa.12301.

TAMURA, K; DUDLEY, J; NEI, M and KUMAR, S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol., 24*: 1596-1599. DOI: 10.1093/molbev/msm092.

TURNER, P D (1981). *Oil Palm Diseases and Disorders*. Oxford University Press, Kuala Lumpur. DOI: 10.1111/j.1744-7348.1968.tb03860.x.

WANG, B; BRUBAKER, C L; TATE, W; WOODS, M J; MATHESON, B A and BURDON, J J (2006). Genetic variation and population structure of *Fusarium oxysporum* f. sp. *vasinfectum*. *Australas. Plant Pathol., 55*: 746-755. DOI: 10.1111/j.1365-3059.2006.01445.x.