A NON-DESTRUCTIVE QUANTITATIVE METHOD FOR THE ASSESSMENT OF INFECTION OF OIL PALMS BY *Fusarium oxysporum* f. sp *elaeidis*

Keywords: Infection; Oil palm; *Fusarium oxysporum*; Vascular wilt; Root xylem; Pathogen; Trunk rot

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A non-destructive technique, involving removal of cylinders from stems with an auger, is described for the quantification of disease in mature oil palms infected with Fusarium *wilt. Reisolation from cores confirmed that discoloration of vascular tissue is diagnostic for* Fusarium *wilt.*

Good agreement was obtained between disease assessment based upon leaf symptoms and this new technique. Auger sampling was the more sensitive method and in some crosses revealed 50% infection in otherwise symptomless palms. The absence of the pathogen in older 'resistant' palms indicated that they were truly resistant and not tolerant to the disease.

High levels of vascular occlusion occur in diseased palm trunks but not in roots, which suggests that blockage of the trunk xylem system is the critical phase in symptom development.

Auger sampling in Malaysia failed to reveal the presence of the fungus inside palm trunks.

It is suggested that this technique could be useful in breeding programmes to identify resistant palms and reduce the risk of collecting seed from infected but symptomless palms. Other uses for the auger are also described.

INTRODUCTION

Production of palm oil in South-East Asia, notably in Malaysia and Indonesia, has steadily increased in recent years in contrast to the decline observed in much of West Africa. This fall in production is partly due to disease, of which the most serious is a vascular wilt caused by *Fusarium oxysporum* f.sp *elaeidis*. This infection is responsible for widespread losses; for example in Ivory Coast the disease has destroyed 1% of palms per year (Guldentops,

1962) while in Nigeria up to 40% of trees are affected in older plantations (Aberungboye, 1981).

There have also been unconfirmed reports of wilt in South America, from Surinam (Anon., 1951) and Colombia (Sanchez Potes, 1966), but more recently *F. oxysporum* has been isolated and identified in Brazil (van de Lande, 1984) and a Brazilian isolate has been shown to be virulent to clonal oil palm (Flood, Cooper, and Lees, 1989).

The disease has never been reported from South-East Asia despite the importation from West Africa of large quantities of seed and pollen samples, some of which have subsequently been shown to be contaminated with the pathogen (Locke and Colhoun, 1973; Flood, Mepsted and Cooper, 1990). The failure of the disease to become established in South-East Asia may be due to climatic differences (Colhoun, 1981) or to a lack of pathogenic isolates of *F. oxysporum* in that region (Ho, Varghese and Taylor, 1985).

The pathogen is a soil-borne fungus which enters the host via the roots, penetrates the xylem vessels (Locke and Colhoun, 1977) and spreads throughout the plant as propagules in the transpiration stream (Renard, 1970). In response to this invasion the host attempts to seal off the infected vessels, which become characteristically brown and occluded with gels and tyloses (Wardlaw, 1950; Ho, Varghese and Taylor, 1985). Vascular occlusion probably also contributes to water stress of the host and is believed to be the cause of the progressive leaf desiccation observed in infected palms (Wardlaw, 1950; Turner, 1981; Obuckwe and Osagie, 1989). As the symptoms extend upwards through the canopy the resultant leaf necrosis is accompanied by fracturing of fronds at some distance from the base, causing leaves to hang around the trunk in a cluster (Wardlaw, 1950).

In view of the large areas involved, the economics of the crop and the soil-borne nature of the pathogen, chemical control is not feasible and most effort has therefore been directed towards the selection and planting of resistant palms (Renard, 1976). The production of resistant breeding material in Africa depends partly upon a field census which allows for the selection of crosses with a lower than average incidence of wilt. This 'wilt census' is based upon the assessment of visible symptoms in field palms. Unfortunately, as foliar symptoms of *Fusarium* wilt can easily be confused with those caused by other diseases, such as *Ganoderma* and *Armillaria* trunk rot (Wardlaw, 1950), the accuracy of the wilt census is questionable. In contrast to foliar symptoms, the presence of brown vessels in the vascular strands of the trunk is diagnostic for *Fusarium* wilt (Wardlaw, 1950). However in the past the examination of the trunk vessels necessitated the destructive sampling of whole palms, which precludes the continued monitoring of individual trees and is too damaging to be acceptable for more than a few palms.

Thus, a non-destructive method for the detection of brown xylem elements within palm trunks was required and a tree auger, designed to remove cores from the inside of the trunk, was evaluated on a heavily infected plantation at Binga in Zaire as an alternative approach to disease assessment.

EXPERIMENTAL

The tree auger consists of a threaded hollow tube which is screwed into the trunk to the required depth (*Figure 1*). A trunk core is then removed from the tube with an extractor (*Figure 2*). In this investigation two 400mm augers were used which extracted cores 5.15mm in diameter. These were manufactured by Haglof of Sweden and supplied by Michael Richmond of Surrey, U.K.

Field palms planted 6, 12 and 16 years previously were selected for investigation and these palms were classified as 'resistant' or 'susceptible' (these terms are relative: see

Figure 1. Insertion of tree auger into a palm trunk.

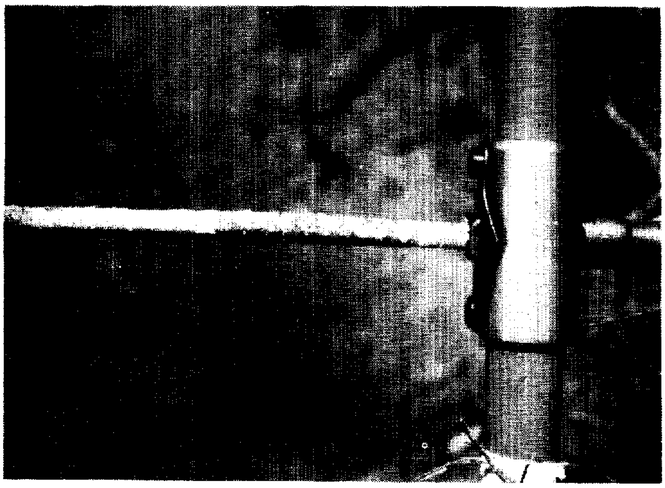

Figure 2. Removal of a trunk core from the end of an auger; dark spots in the core indicate vascular browning.

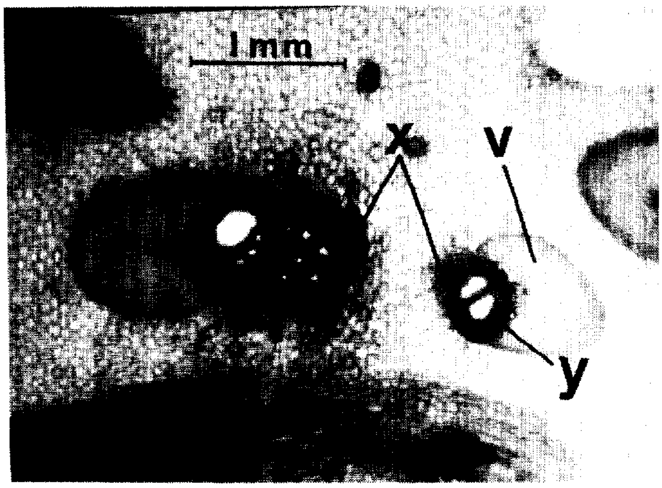

Figure 4. Micrograph of trunk tissue showing browning of xylem parenchyma (X) and gel deposition within xylem vessels (Y); note the absence of browning of vascular fibres (V).

footnote of *Table 2* for details) depending on the percentage of palms of that cross or clone with disease symptoms. Four trunk cores were removed from the base of each 6-yr old palm (*c*. 500mm above the soil) and three cores were taken from the base, middle and top of the trunk of the older palms. The cores were cut longitudinally, at right angles to vascular bundles, and examined with a hand lens (*Figure 3*) and a microscope (*Figure 4*); any brown vessels were noted and the percentage of vessels so affected was calculated. Distinction was made between browning of xylem elements induced by *Fusarium* wilt, and that of vascular bundle

Figure 3. Longitudinal section of trunk core, at right angles to vascular bundles, from (A) an infected palm with brown vascular bundles (arrowed) and from (B) an uninfected palm.

fibres, which can be due to *Ganoderma* infection (Wardlaw, 1950). The pathogen was reisolated from surface-sterilized (1% available chlorine for 5 min) core samples on *Fusarium*-selective media (Papavizas, 1967) and the results were compared with the wilt census, which had been conducted within the previous month.

Since the pathogen initially invades roots, an attempt was also made to quantify the level of root xylem occlusion. Thus, ten samples from primary roots were taken from the base of 6-yr old clonal palms and after sectioning and microscopic examination, as previously described for auger samples, the percentage of infected vessels was calculated. These results were then compared with estimates of trunk xylem occlusion obtained by examination of auger cores from the same palms.

Following evaluation in Zaire, a similar auger survey was conducted on three plantations in Johore and Perak, Malaysia, in which healthy palms and some that had symptoms similar to vascular wilt were examined; sampling and re-isolation methods were as previously described for 6-yr old palms in Zaire.

RESULTS AND DISCUSSION

The auger effectively removed samples from palm trunks, and xylem browning in cores was easily observed with a hand lens (*Figure 3*). Cores removed from the trunk base and middle contained more brown vessels than those removed from the top of the trunk (*Table 1*). When reisolation was attempted, *F. oxysporum* was found in over 88% of samples containing brown vessels and therefore vascular discoloration in auger cores was confirmed as being diagnostic for infection by *F. oxysporum* f. sp. *elaeidis*.

TABLE 1.
PERCENTAGE OF XYLEM VESSELS OCCLUDED IN TRUNK CORES FROM DISEASED PALMS AT THREE DIFFERENT HEIGHTS

Position of cores in trunk[a]	Percentage of xylem vessels affected[b]
Top	12.8
Middle	27.3
Base	32.4

KEY

Values represent the mean of eight 12-yr old palms and are not significantly different ($p > 0.05$, STP test based on U statistic, Sokal and Rohlf, 1981).

[a] At each height, three cores were extracted from each palm.
[b] Affected vessels contained gels, tyloses and hyphae, and surrounding parenchyma cells were brown.

The comparison between the wilt census and the auger survey (*Table 2*) demonstrates close agreement between the two techniques for palms regarded as wilted in the census, where 26 of the 29 palms contained brown xylem vessels in the trunk. Also, among trees not considered wilted in the census, good agreement was found for 12- and 16-yr old palms that were regarded as disease-resistant and 6-yr old randomly selected palms; no brown vessels were found in the trunk of any of the 20 palms in this group. However, of the remaining symptomless palms, comprising 6- and 12-yr old trees of susceptible crosses and 16-yr old palms of unknown resistance, 11 out of 21 trees contained brown vessels in the trunk.

The auger survey also demonstrated a significant correlation between the extent of external symptoms and the degree of vascular blockage in the trunk (Mepsted, unpublished) and for susceptible cross 109 and clone UF4 the percentage of vessel browning in symptomless but infected trees was significantly less than in obviously wilted palms (means 16.6% and 45.1% respectively: $p=<0.01$, T-Test).

The absence of any brown vessels in the trunks of older 'resistant' palms suggests that these palms are truly resistant rather than tolerant to this disease. However, in some susceptible and older palms the auger revealed slight infection in over 50% of palms with no external symptoms. Further work would be required to demonstrate how often such low levels of infection occur in plantations and if it has any effect on yield. In a large (>750 palms) survey of the effects of agronomic practices on *Fusarium* wilt, Renard and de Franqueville (1989) reported that 8% of 6-yr old symptomless trees contained brown fibres in the trunk and a 3% to 15% yield reduction was noted for these palms. The authors also reported brown fibres in the trunks of 50% of older palms that had no obvious wilt symptoms. Unfortunately no details of sampling technique were given for these experiments. Nevertheless their observations and the results presented here support the theory that symptomless but infected palms may be common in plantations, and such infection could cause a significant yield reduction.

Examination of roots (*Table 3*) revealed occlusion in less than 4% of xylem vessels in both healthy and diseased palms. This suggests that occlusion here was not due to the pathogen, and that blockage of the vascular elements in the roots is not a major contributor to symptom development in this disease. This latter theory is supported by previous observations that even severely diseased palms had many healthy roots (Wardlaw, 1950; Prendergast, 1957). In contrast, nearly half the trunk xylem vessels in diseased palms were occluded. This discrepancy between root and trunk colonization in diseased trees may be explained by the interconnecting and irreplaceable nature of xylem vessels in the palm trunk, where the level of occlusion represents the accumulation of infection over the life of the palm; whilst the constant replacement of old roots by new ones (Ruer, 1968, cited by

TABLE 2.
COMPARISON OF WILT CENSUS WITH AUGER SURVEY

Age of palms (years)	Cross or Clone	Wilt resistant (R) or susceptible (S)[a]	Wilt census results[b]	Auger results[c]	
				brown vessels	no brown vessels
6	UF4 (clonal)	S	Wilted	8	0
6	random selection	?	Wilted	4	1
12	109	S	Wilted	7	0
12	106	R	Wilted	1	0
16	29	?	Wilted	6	2
6	UF4 (clonal)	S	Not wilted	2	3
6	random selection	?	Not wilted	0	11
12	109	S	Not wilted	5	3
12	106	R	Not wilted	0	3
16	29	?	Not wilted	4	4
16	Dumpy x	R	Not wilted	0	6

KEY

[a]Clone UF4 had 64% wilted or dead palms after 6 years.
Cross 106 had 12% wilted or dead palms after 12 years.
Cross 109 had 50% wilted or dead palms after 12 years.
Dumpy x had 0% wilted or dead palms after 16 years.
[b]Palms categorized as diseased or healthy based upon foliar symptoms.
[c]Number of palms with or without brown vessels in the trunk.

TABLE 3.
OCCLUSION OF XYLEM VESSELS IN THE ROOTS OF HEALTHY AND DISEASED CLONAL PALMS

	Percentage of xylem vessels affected[a]	
	Uninfected palms[b]	Infected palms[b]
Roots[c]	3.2 a	3.85 a
Trunk[d]	0 a	42.7 b

KEY

[a] Affected vessels contained gels and/or tyloses.
[b] Based on external symptoms and browning of trunk xylem vessels in 13 6-yr old UF4 clonal palms.
[c] Mean of all vessels in 10 primary roots from the base of each palm.
[d] Mean of four cores per trunk: at least 20 vessels were examined in each core.

Within each column numbers with the same letter are not significantly different (U-Test, $p < 0.01$).

Dufrene, Ochs and Sangier, 1990) which grow directly from the stem base, means that cumulative infection of the root system is unlikely.

Thus, the critical point in symptom development appears to be colonization of the trunk and it is possible that roots may only be important as a site of initial infection. Nevertheless, although this pathogen has been shown to infect seedlings via the roots (Locke and Colhoun, 1977), in such plants occlusion of root xylem is conspicuous (Mepsted, unpublished); thus the absence of any obvious signs of root infection in wilted mature palms may indicate that roots are not the only infection site. Other points of entry may be via the fissure found in the central base of the palm trunk (Wardlaw, 1950), or through the disrupted tissue at the site of root eruption from the stem base.

In Malaysia, a total of 20 palms were sampled and in no case was *F. oxysporum* isolated from any cores, although the fungus was isolated from soil and root samples. These results support the findings of Ho and Varghese (1986) who suggested that although *F. oxysporum* is a common soil inhabitant there are no isolates pathogenic to oil palm in Malaysia.

CONCLUSIONS

The auger effectively removed cores from the palm trunk and brown xylem vessels found in these cylinders were shown to indicate infection with *Fusarium oxysporum* f. sp. *elaeidis*.

By estimating the percentage of affected vessels in auger cores it was possible, for the first time, to quantify infection and this could be related to symptom severity.

In diseased palms the high incidence of vascular occlusion in the trunk suggests that xylem dysfunction in this area and not in the roots is critical to symptom development.

For the majority of palms sampled the auger survey and wilt census were in agreement, thus demonstrating the reliability of the wilt census which provides a more convenient and rapid assessment method. However, the auger detected low levels of infection in some symptomless trees and was therefore more sensitive than the wilt census. Despite this increased sensivity the pathogen could not be detected in the trunks of palms in Malaysia.

At present, resistant seed is produced from palm crosses which have a low average incidence of wilt, but with the auger it will now be possible to quantify infection in individual seed producing trees and thus avoid collecting seed from infected but symptomless palms. This technique should also differentiate between resistant and tolerant breeding material.

The auger should also be of value in the non-destructive observation and isolation of other stem pathogens, for example *Ganoderma* and bud rot diseases. Indeed, during the course of this investigation *Pseudomonas putida* was isolated from abnormally soft trunk cores extracted from palms with several dead leaves.

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