An investigation into *Fusarium* spp. associated with coffee and banana plants as potential pathogens of robusta coffee

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Abstract

Studies were conducted on fungi isolated from coffee trees affected by *Fusarium xylarioides* (Heim & Saccas) Steyaert to establish their pathogenicity and identify possible alternate hosts. Coffee and banana plant samples were collected from individual coffee farmers from the coffee growing districts of Mukono, Mpigi, Mubende, Kabarole and Kibaale. The studies were aimed at isolating the type of *Fusarium* species affecting coffee trees, identifying and characterizing them. Various *Fusarium* species were found associated with robusta coffee berries, stems and roots. These included *Fusarium xylarioides*, *F. stilboides* Wollenwebber & Reiking, *F. solani* (Mart.) Saccas, *F. lateritium* Nees, F.semitectum Berk & Rav, *F. oxysporum* Schlecht, *F. moniliforme* Sheldon and *F. acuminatum* Ell. & Ev. Also recovered from banana roots and pseudo-stem were *F. xylarioides*, *F. oxysporum*, *F. solani*, *F. acuminatum* and *F. moniliforme*. *Fusarium xylarioides*, *F. oxysporum* and *F. solani* were isolated most frequently from roots followed by stems and rarely on berries. Both *F. stilboides* and *F. lateritium* were next in abundance and were isolated mostly from berries. In culture, *F. xylarioides* isolates showed two types of colonies: one was soft, slimy and smooth, while the other was wrinkled. The two *F. xylarioides* isolates were not compatible.The five commonly isolated *Fusarium* species from robusta coffee produced symptoms on inoculated robusta coffee clones but *F. xylarioides* isolates induced the more severe symptoms, killing the greatest number of shoots.

Key words: alternate hosts, coffee wilt, *Fusarium xylarioides*, pathogenicity, Uganda

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Introduction

Coffee contributes over 50% of Uganda's foreign export earnings. Presently, Uganda has a share of about 15% of world robusta coffee exports (Hakiza, 1997). Robusta and arabica coffee are grown in mixtures with food crops, mostly bananas (Ngambeki *et al.*, 1992). The most serious diseases are coffee leaf rust caused by *Hemileia vastatrix* Berk & Br, the coffee berry disease caused by *Colletotrichum kahawae* Waller & Bridge that can cause severe losses in arabica yields (Cambrony, 1992). Red blister disease caused by *Cercospora coffeicola* Berk & Br is a serious problem on the old seedling robusta coffee in Uganda. There is now the notorious vascular wilt disease (Tracheomycosis) caused by *Fusarium xylarioides* (Perseglove, 1977; Cambrony, 1992), which, in Uganda, is currently attacking only robusta coffee.

Tracheomycosis of coffee caused by *F. xylarioides* was first reported by Figueres in 1927 in a plantation of *Coffea excelsa* A. Chev in the Central African Republic (Hakiza, 1997). The disease was reported in Bundibugyo district in south-western Uganda and in 1995, the pathogen was isolated from coffee stem samples received from two farms in the region (G.J. Hakiza, personal communication). The disease is now found in all the robusta coffee-growing districts of the country. (Flood & Brayford, 1997; Hakiza, 1997; G.J. Hakiza, Personal communication).

Tracheomycosis of coffee which is caused by *F. xylarioides* is a fungal disease which attacks the vascular system of the plant and causes the plant to wilt and eventually die (Pieters & van der Graaff, 1980). When the bark is removed from the collar of the affected stem, a brown to violet streak is observed in the wood. Leaves can abscise rapidly while still green, or in some instances, they desiccate, turn brown and remain attached to the tree (Cambrony, 1992; Coste, 1992). The genus *Fusarium* causes disease on a wide range of crop plants. Some 20 species of *Fusarium* have been recorded on specimens from coffee in the Herbarium of the International Mycological Institute (Flood & Brayford, 1997). The pathogenic species of economic importance include *Fusarium stilboides*, *F. solani*, *F. xylarioides* and *F. oxysporum* (Waller & Holdeness, 1997). Canker and dieback are caused by *F. lateritium* (Holliday, 1980; Nelson, Toussoun & Cook, 1981).

Fusarium xylarioides is an endemic saprophyte of intertropical African soils and attacks coffee bushes through wounds (Kranz & Mogk, 1993). Haarer (1963) regarded the fungus as an inhabitant of the soil. The pathogen may also survive in weeds as alternative hosts. There is a report of *F. xylarioides* being isolated from tomato roots in Nigeria which may indicate that solanaceous weeds or other plants may act as reservoirs for this pathogen (Flood & Brayford, 1997). The pathogen can be dispersed by coffee husk mulches, transportation of infected plants as fire wood, infected unhulled dry cherries (Kiboko) and infected transplants (Wrigley, 1986).

The spread of the disease on robusta coffee has caused a lot of concern among Ugandan farmers since it was first observed in 1993 in Bundibugyo district. It has become a major disease of robusta coffee in Uganda and the country now stands to lose a sizeable amount of foreign exchange due to the disease (Anon, 1997). The main aim of the research was to identify and characterize *Fusarium* species associated with coffee shrubs infected by wilt disease and to establish whether shade plants such as banana can be alternate hosts for the pathogen.

Materials and methods

Study area

Samples were collected from five districts, namely Mukono, Mpigi, Mubende, Kabarole and Kibaale. Thirty farms were visited and a total of 93 samples were analysed, of which 45 were from banana.

Sample analysis

The sample types included coffee berries, stem pieces and roots, and from banana corm and roots. Stem pieces were cut from different stems on the same tree shrub. Berries (green, ripe and dried) were hand-picked directly from the tree and wrapped separately. Samples of banana roots and corms were taken from banana next to the selected coffee bush. All the tools used were surface sterilized with absolute alcohol before using on the next tree sample. The soiled materials were washed with water and surface sterilized in 2% sodium hypochlorite for 5 min and rinsed in four sets of sterile distilled water. Before plating, excess water was mopped off and the pieces trimmed at the edges and cut into smaller size. The pieces were aseptically plated on tap water agar and potato sucrose agar (PSA) and incubated at 25°C for 3 days in the dark. The growing fungus was subcultured on media and identification of *Fusarium* species isolated was according to Booth (1971). All major *Fusarium* species isolated were studied for different growth characters and identified. Representative cultures of the species were sent to CABI-Bioscience for confirmation of identity.

Pathogenicity tests

This was performed following Koch's postulates. Six robusta coffee clones 4–5 months old were obtained from Coffee Research Institute (CORI) nursery at Kituza. The clones selected were $1^s/2$, $1^s/3$, $1^s/6$, 223/32, 257/53 and 258/24. The aim of the study was to determine the pathogenicity of the various *Fusarium* species isolated from coffee and banana. The question was, 'Which one of these *Fusarium* species isolated from coffee and from banana are a real danger to robusta coffee in Uganda?' Fungal isolates chosen were those *Fusarium* species previously reported to be pathogenic to coffee (Booth, 1975; Waller & Holdeness, 1997). Single spore cultures on Sucrose Nutrient Agar (SNA) of all isolates were used. Spore suspensions were prepared from 10-day-old cultures to a concentration adjusted to 1.3×10^6 spores ml^{-1} (by either dilution or addition of more inoculum). The root dip method of inoculation was used. After inoculation each plant was planted in a pot containing approximately 500 g of partially sterilized soil mixed with sand in the ratio 3 : 1. The pots were labelled and arranged in a completely randomized pattern. Laboratory conditions were kept at 25 ± 3°C under white florescent lights set at 12 h photoperiod during day and 22 ± 1°C at night. Symptom development was observed and assessed every after 2 weeks for 134 days. Percentage leaf loss and leaf gain was calculated from the original number of leaves. The number of dead infected plants per clone, per fungal isolate was recorded. The symptoms used to assess disease severity were necrosis of leaves, browning of stem and leaves and general wilting of the plant. The fungus was recovered from the infected plants.

Results

Field observations

Symptoms observed in the field included wilting, chlorosis and yellowing with defoliation of leaves and dieback. Blue-black coloration of the wood was observed under the bark. Berries on affected trees ripened prematurely and dried out but remained attached to the plant. The symptoms were as those described by Hakiza (1995). Symptoms of infected banana plants observed in the field were yellowing of leaves. Internal symptoms when the corm or pseudo-stem was split showed brown lesions.

Fusarium *species isolated*

Five districts were visited and a total of 93 coffee and 45 banana samples were collected. The commonly isolated species from coffee were *F. xylarioides* and *F. stilboides* in all

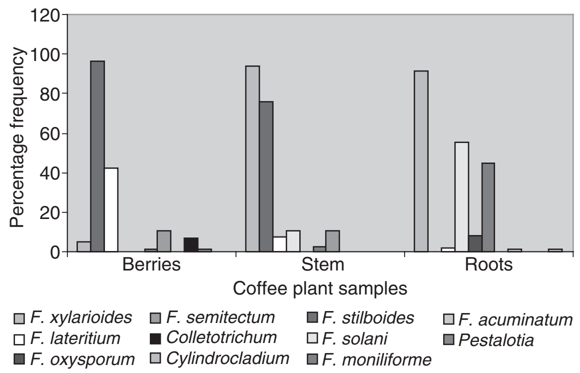

Fig 1 Average frequency of fungi isolates from coffee plant parts in the five districts

the five districts. As shown in Fig. 1, *F. xylarioides* was more commonly isolated from roots, followed by stem with 100% occurrence. However, its occurrence on berries was low with 13% isolation frequency. It was observed that *F. stilboides* was most frequently isolated on berries. All the five districts were affected with *F. xylarioides*. The *F. xylarioides* isolates from coffee and banana had some cultural

Table 1 Percentage number of leaves lost from coffee plants inoculated by various *Fusarium* species

	Coffee clones					
Fungal isolates	$1^s/2$	$1^s/3$	$1^s/6$	223/32	257/53	258/24
F. xylarioides (S)	98.00	75.0	97.36	86.79	98.7	63.33
F. xylarioides (W)	88.52	75.0	93.54	88.70	89.47	52.63
F. xylarioides (B)	72.13	54.23	88.52	83.33	82.75	38.59
F. stilboides	38.70	27.58	36.20	27.86	31.25	17.54
F. solani	35.59	27.86	33.92	25.00	30.00	11.66
F. lateritium	69.69	48.27	39.29	36.20	46.77	22.58
F. oxysporum	65.51	38.59	33.33	31.03	46.55	22.58
Control	16.66	12.28	20.0	15.38	26.98	10.0

(S), slimy isolate; (W), wrinkling of culture; (B), isolate from banana.

Table 2 Number of dead plants per clone per fungal isolate

	Clones						
Isolates	$1^s/2$	$1^s/3$	$1^s/6$	223/32	257/53	258/24	Total
F. xylarioides (S)	6	6	6	6	7	5	36
F. xylarioides (W)	7	5	7	7	7	4	37
F. xylarioides (B)	5	4	7	7	7	3	33
F. stilboides	0	0	1	1	1	0	3
F. solani	2	2	0	1	1	1	5
F. lateritium	3	3	3	1	4	2	13
F. oxysporum	1	0	2	0	0	0	2
Control	0	0	0	0	0	0	0
Total	24	20	26	23	27	15	

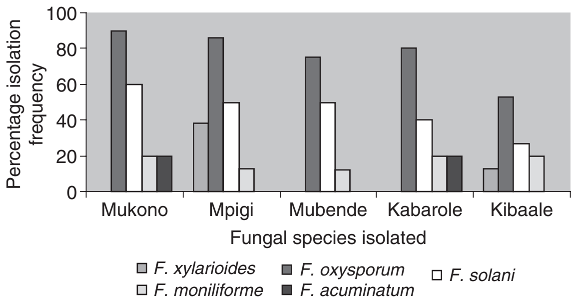

Fig 2 *Fusarium* species isolated from banana from the different sampling districts

differences. One isolate showed wrinkling of culture denoted by *F. xylarioides* (W), another grew soft and smooth with a wet appearance in culture due to the presence of pionnote sporodochia denoted by *F. xylarioides* (S). *F. xylarioides* (B) from banana appeared soft and smooth on culture with a pale pinkish pigment. These characters were consistent for the isolates from the different districts.

Isolations from banana

One of the questions posed when this work was being planned was whether the coffee shade plants (banana) acted as alternate hosts of *F. xylarioides*. Isolations made from roots of 45 samples from the five districts revealed that *F. xylarioides* was present in roots of *Musa* sp. (the beer type banana) at Mpigi and Kibaale districts (Fig. 2). However, *F. oxysporum* f.sp *cubense* and *F. solani* were the most abundant from the banana samples (Fig. 2).

Pathogenicity tests

The *Fusarium* species were further characterized according to pathogenicity tests. The control experimental plants showed no symptoms. Plants inoculated with *F. xylarioides* developed symptoms similar to those observed on coffee growing in naturally infested soils in the field. The other fungal species showed general wilting and stunted growth. All the fungal isolates caused defoliation and wilting of plants to some extent. It was therefore evident that *F. xylarioides* was the pathogen destroying coffee in Uganda. The percentage leaf loss was calculated from the original number of leaves (Table 1). The percentage number of dead plants per clone, per fungal isolate was calculated as given in table 2.

Discussion

The fungal species isolated from coffee were established as monosporous cultures on SNA and PSA as the first step in the identification process. They were identified as *F. xylarioides*, *F. stilboides*, *F. solani*, *F. oxysporum*, *F. lateritium*, *F. semitectum*, *F. acuminatum*, *F. monoliforme*, *Colletotrichum*, *Pestalotia* and *Cylindrocladium*. Of the eight species of *Fusarium* isolated from coffee during the study, six had been reported previously as pathogenic to coffee (Waller & Holdeness, 1997) among which were those isolated from banana. In the case of coffee *F. xylarioides* was isolated mostly from roots, stem and rarely from berries. Wrigley (1986) reported the presence of *F. xylarioides* on coffee berries. This supports the results, which show 13% isolation frequency of *F. xylarioides* on berries. *Fusarium xylarioides* was isolated from some samples of coffee husks (G.J. Hakiza, personal communication).

Other *Fusarium* species isolated from the study that have been known to be pathogenic to coffee include *F. stilboides* mostly isolated from berries as reported by Gordon (1956), *F. lateritium* (Wrigley, 1988) commonly found on berries. *Fusarium oxysporum* are reported to be pathogenic to coffee (Wellman, 1954). *Fusarium solani* f.s *coffea* produces a dry root rot and wilt of coffee (Baker, 1972). *Fusarium semitectum* was another species isolated from coffee and also reported by Waller & Holdeness (1997). *Fusarium monoliforme* was isolated from coffee roots but the results show that it may not be pathogenic to coffee.

Pathogenicity of Fusarium *species on robusta coffee*

Heim & Saccas (1950) reported that all infected trees had obvious wounds, either on the upper parts of the plant or on big roots. *Fusarium xylarioides* isolates were observed to cause more deaths of young plants of coffee clones than any of the other fusaria. The control plants were also uprooted and inoculated in sterile distilled water. It was observed that none of the plants died.

Conclusion

A fungus similar to *F. xylarioides* was isolated from banana (*Musa* sp.) in the districts of Mpigi and Kibaale. A report from Nigeria indicated that *F. xylarioides* was also recovered from tomatoes, which indicates that solanaceous weeds may act as reservoirs of inoculum for coffee wilt disease (Onesirosan & Fatunla, 1976). Banana may be one of the alternative host plants of *F. xylarioides*. The isolation of *F. xylarioides* from banana (*Musa* sp.) will provide a basis for further research on this pathogen.

The re-isolation of *F. xylarioides* from artificially inoculated plants is proof that the disease symptom on such plants was caused by *F. xylarioides*. This confirms that the vascular coffee wilt experienced in Uganda is mainly caused by *F. xylarioides* strain.

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