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Evaluation of potential sources of inoculum for the coffee wilt epidemics in Uganda

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Abstract

Studies were conducted at Coffee Research Institute (CORI) to determine various sources of inoculum for *Fusarium xylarioides* (F.X), the causal agent of coffee wilt disease (CWD). Coffee seedlings of between 5-6 months were inoculated with *F. xylarioides* spores obtained in *situ* from perithecial stroma around the bases of infected coffee trees. *F. xylarioides* inoculum raised in culture initially obtained from infected coffee tissue was also used. Furthermore, an attempt was made to isolate *F. xylarioides* from the stalks of the coffee berries/seeds. Results suggest that disease was higher on seedlings inoculated with spores from perithecial stroma than inoculum raised under laboratory conditions from infected coffee tissue. Inoculation by root dip in spore suspensions gave highest disease incidence of up to 100% and was the most effective. In all cases where inoculation was done on leaves, no coffee wilt disease symptoms developed. However, when stems were inoculated by pricking above the level of the fourth leaf, wilt symptoms were observed only on the upper section and none on the lower. The section below the point of inoculation continued to produce healthy tillers even 100 days after inoculation. The studies concluded that *F. xylarioides* spores collected from infected coffee stems and inoculated in *situ* cause CWD and these together with infected coffee stalks could be the avenues through which the pathogen spreads.

Key words: *F. xylarioides, Coffee, Perithecia and conidia.*

Introduction

Coffee wilt disease (CWD) caused by *Fusarium xylarioides* (FX) remains a primary research concern in the major robusta coffee growing zones of Uganda, which constitutes more than 80% of foreign export earnings. The spread of the disease whose therapeutic cure is still unknown has reached alarming proportions. Cumulatively, this disease has destroyed a total of 14.5 million trees since it was first reported in 1993, representing 4.8% of losses and it is reported that 15 of the 24 main robusta growing districts in the country are affected (UCDA, 2000). At this rate of destruction, there is an urgent need to develop effective control measures to limit disease spread. The success of these measures would largely depend on understanding the mechanisms of spread and sources of inoculum.

Fusarium xylarioides is a vascular pathogen known to affect the entire vascular system of the coffee plant. Previous reports by Hakiiza et al (1999) suggested that recovery of the pathogen from infected coffee fruits and husks was considerably low. However, it was not clear whether isolation from fruit stalks, which constitute a *direct* attachment to the vascular system, was attempted. It was therefore presumed that, as a vascular pathogen, the possibility of isolating it from the berry stalks would be much higher than in the fruit coat or husks. In Uganda, most farmers occasionally take along fruit stalks while picking the coffee berries, which may remain attached to the berries during further processing into patchment and husks, and therefore becoming potential sources of inoculum.

Hakiiza et al (1999) confirmed that CWD could be incited into plantlets in green house trials by dipping roots into conidia suspensions of F.X. However, this report felt short of explaining mechanisms by which the disease spreads in the field. Observation on the bases of affected dry stems, especially during the wet season, clearly indicated massive production of perithecia, which are the sexual stage of the pathogen (also called Giberella xylarioides), and it was suspected that it was possibly through these propagules that the disease can spread from plant to plant.

This study was conducted at Coffee Research Institute (CORI) and Kawanda Agricultural Research Institute (KAR) to establish other potential sources of inoculum for spreading the disease and the significance of perithecial ascospores and conidia in its development.

Methodology

Research studies were undertaken at CORI to investigate the potential sources of inoculum for coffee wilt disease epidemics. Inoculum from the perithecia fruiting bodies of the pathogen in *situ* as well as that raised directly from infected coffee tissues were investigated. Additionally, stalks obtained from coffee berries born on infected primary branches were investigated.

Determination of primary sources of inoculum for CWD
Infected coffee stalks and stems were collected from the field at Kituza, Mukono district. Laboratory and green house studies were conducted at Coffee Research Institute (CORI) and Kawanda Agricultural Research Institute (KARI) respectively, to isolate the pathogen and conduct pathogenicity tests. Inoculum from the fruiting bodies *(perithecia)* of the pathogen were obtained in *situ* as well as raised by culturing infected coffee tissue on synthetic nutrient ager and Potato dextrose(PDA) agar media. An attempt was also made to isolate the pathogen from coffee berry stalks from plants heavily affected by the disease.

Sources of inoculum used for pathogenicity tests
The sources of inoculum used for pathogenicity tests were: i) a mixture of ascosore/conidia (*in situ*) ii) conidia raised by culturing perithecial stroma on agar media iii) conidia raised by culturing pieces of infected tissue on agar media.

i) Perithecial stroma
Fruiting bodies (perithecial stroma) of *F. xylarioides* were scraped from the base of heavily infected coffee trees using scalpels. They were crashed directly in a mortar to release the ascospores and conidia and diluted with water to a concentration of between 5 x 10^5 ml^{-1} ascospores and 2.5 x 10^5 ml^{-1} conidia. Young coffee seedlings (5-6 months) were inoculated with both ascospores and conidia using the following methodologies: root dip, leaf spray and stem pricking (above the 4th leaf) of the coffee seedling. Prior to inoculation, observation under the microscope revealed that both ascospores and conidia were abundantly present in the perithecial stroma although conidia were relatively fewer than ascospores.

ii) Conidia raised on agar, from both infected plant tissue and perithecial stroma.
Small pieces (3 mm^2) of infected coffee tissue were taken from underneath the bark of affected coffee stems and plated on synthetic nutrient agar (SNA) or potato dextrose agar(PDA). The perithecia were also plated on the same medium and in both cases, the incubation was at 25°C for 7 days. The resulting conidia from the two sources were diluted to about 1.3 x 10^6 and inoculated to the young coffee seedling as described in (i) above. Recording of disease incidence and severity started 40 days after inoculation.

Isolation of F. xylarioides from stalks of coffee berries.
The possibility of recovering *F. xylarioides* from the stalks of infected coffee berries was investigated over two occasions. The primary branches together with berries from affected coffee trees were taken to KARI for isolation of the pathogen. The stalks were carefully separated from the mother branches and berries using a sterilized pair of scissors. They were then surface sterilized in 2% Jik (Sodium hypochlorite) for three minutes, double rinsed in sterile water and plated on PDA with 100 mg/l streptomycin sulphate before incubation at 25°C for 7 days. The developing fungal colonies were transferred to fresh media, incubated and identified on basis of morphological characteristics.

Results and discussion

Potential sources of inoculum
These results confirmed that coffee plants inoculated by root dipping in ascospores and/or conidia suspensions, from either perithecial stroma (in situ) or raised on agar medium under laboratory conditions could develop CWD symptoms. Earlier studies by Hakiza et al (1999) had used *F. xylarioides* suspensions obtained only from infected coffee tissues to reproduce wilt symptoms after inoculation by root dipping. However, according to these results, spores from either perithecial stroma (in situ) or raised on agar medium from infected coffee tissues under laboratory conditions incited CWD symptoms. In most cases however, inoculum from perithecial stroma either direct (in situ),

Figure 1a. WD incidence on seedlings after inoculation with *F.xylarioids* spores extracted from different sources.

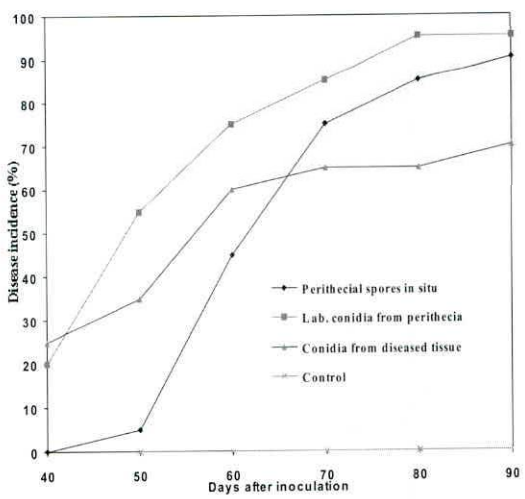


Figure 1b. CWD severity on seedlings inoculated with F. xylarioides spores from various sources

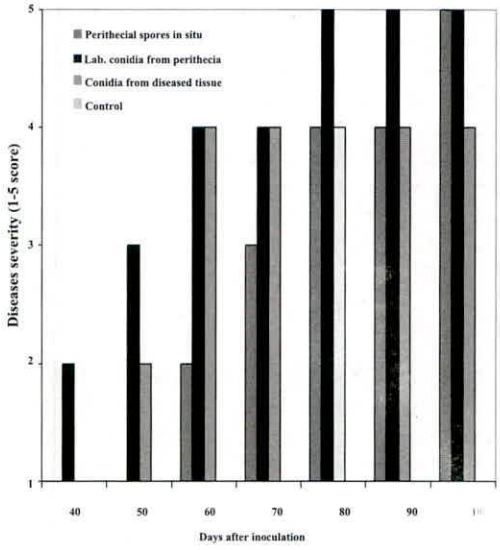


Figure. 3. Severity of coffee wilt disease on seedlings inoculated with F. xylarioides

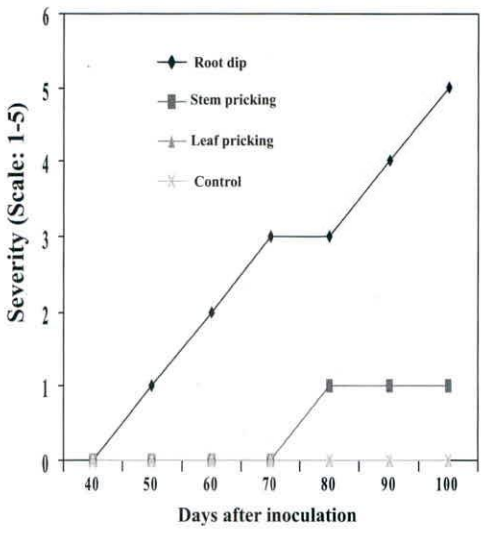
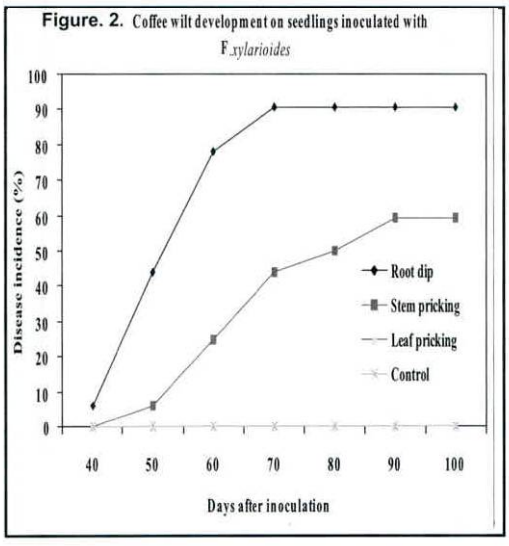


Figure. 2. Coffee wilt development on seedlings inoculated with *F. xylarioides*



or indirectly raised in culture medium caused higher disease incidence and severity than that raised from infected plant tissue (Figures 1a & b).

Nevertheless, all plants that were inoculated through wounding (pricking) the stems, developed wilt symptoms only in the region above the wound (prick) and never below the wound. The region below the wounds increasingly proliferated into healthy tillers even after 100 days following inoculation suggesting that the pathogen was not inciting symptoms in this region.

Table 1. Species of fungi isolated from stalks of coffee berries collected from wilted branches

Fungus species	First isolation occasion (%)	Second isolation occasion (%)
Fusarium xylarioides	9	3.5
Fusarium stilboides	18	4.5
Aspergilus niger	43	3.5
Aspergilus fluvus	11	0
Aspergilus ochracious	3	0
Fusarium semitectum	6	3.5
Colletotricum	6	.5
Cgleosporioides ladosporium sp.	4	2.5
Unidentified fungus	-	10.5

However, the incubation period of the disease was also longer in such plants than those inoculated by root dip (figures 2 and 3). All plants inoculated exclusively by leaf spray, with or without wounding and using spores from any of the sources mentioned, did not develop any wilt symptoms even after 100 days, suggesting that the pathogen does not enter through leaves at least under green house conditions.

Transmission of F. xylarioides on coffee berry stalk

Results suggest that 3.5-9.0% recovery of the pathogen from coffee berry stalks is possible (Table 1). Although other fungal organisms were also recovered from the samples, their pathogenicity was not verified and were considered to be saprophytes with the exception of Fusarium stilboides which is known.

These studies concluded that *F. xylarioides* spores collected from infected coffee stems and inoculated in situ cause CWD and together with infected coffee stalks could be the avenues through which the pathogen spreads from plant to plant or place to place. Earlier studies had shown that isolation of *F. xylarioides* from coffee husks was minimal and therefore not considered a major source of inoculum. This was evident in the report by Hakiza et al (1999) where less than 1% recovery of the pathogen was given. However, these results suggest that 3.5-9.0% recovery of the pathogen from coffee berry stalks is possible. The authors consider this percentage quite significant, bearing in mind that most farmers pick coffee berries and deliver these together with their stalks attached for further processing. Since coffee husks resulting from the processed coffee can be used as mulch in coffee fields, and may still remain with some stalks attached, it is possible that they can serve as the foci for disease infection.

It was evident that while the pathogen does not incite symptoms through leaves, incidence and severity was low when infection was initiated through stems, at least in green house experiments. On several occasions, it has been observed in the field that the disease may attack a single branch of a coffee tree and for a while other branches remain healthy. If it is true that movement of the pathogen through the plant tissues is slower downwards than towards the apex of the plant, in cases where infection originates through the stems, there is a possibility that removing affected branches immediately after the disease is detected in the field, may give the plant considerable protection. However, further research is needed to establish this relationship.

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