Appendix 4
Specialist Training: An International Course on Isolation and Identification of Fusaria from Coffee

A4.1 Introduction

Coffee wilt disease (CWD) is caused by the fungus, *Gibberella xylarioides (Fusarium xylarioides*). The disease affects *Coffea canephora* (in Democratic Republic of Congo (DRC), Uganda and Tanzania) and *C. arabica* (in Ethiopia only). In order to build the capacity of scientists from collaborating national programmes within the region, a specialist training was organized to equip the scientists in laboratory-based identification of *G. xylarioides*. Identification of *G. xylarioides* by many pathologists within the region is confused because of the alternative Fusaria occurring on the coffee trees, some of which cause similar wilting symptoms to *G. xylarioides*. The training, therefore, covers the identification of Fusaria occurring on coffee. The course focused on the identification of *G. xylarioides, F. oxysporium, F. solani, F. latelitium* and *F. stilboides*. Diseases and symptoms caused by these pathogens on coffee were also taught. Isolation and identification procedures were covered in detail, including types of media used for isolation and identification. Characteristics of the pathogens were based on illustrations developed by Booth (1971).

The training took place at the Coffee Research Institute (CORI), Kituza, Mukono, Uganda, from 26 to 27 February 2003. It included lectures and practicals.

A4.2 Activities

Dr Mike Rutherford (CAB International – UK Centre), conducted the course with the support of Dr Georgina Hakiza (CORI) and Dr Noah Phiri (CAB International – Africa Regional Centre).

A4.3 Topics Covered

A4.3.1 Classroom/laboratory-based

- *Fusaria in plant pathology* – an overview was given of *Fusarium* spp., which may cause diseases or can act as secondary invaders. A number of symptoms or diseases caused by different *Fusarium* spp. were presented. Examples of diseases caused by *Fusarium* spp. are as follows:

    - seedling blights (*F. solani, F. oxysporum, F. avenaceum, F. lateritium F. culmorum, F. graminearum, F. moniliforme* and *F. sambucinum*);
    - root rots (*F. solani, F. oxysporum, F. avenaceum* and *F. culmorum*);
    - basal rot of bulbs (*F. oxysporum*);
    - vascular wilts (*F. oxysporum* – can also be a soil saprophyte or soil invader);
    - stem, leaf and head blights of cereals (*F. culmorum, F. graminearum, F. avenaceum, F. heterosporum* and *Microdochium* (*Fusarium*) *nivale*);

- post-harvest or storage rots (*F. sambucinum*, *F. solani* var. *coeruleum*, and in the tropics – *F. equiseti*, *F. acuminatum*, *F. pallidoroseum* (= *F. semitectum*) and *F. moniliforme*);
- cankering of woody plants (*F. lateritium*, *F. sambucinum*, *F. solani*, *F. sacchari*, *F. moniliforme* var. *subglutinans* and *F. stilboides* causes bark disease of coffee);
- others included seed contamination, animal infection and industrial contamination.

- *Fusaria found on coffee and the associated symptoms* – diseases caused by *Fusarium* spp. were presented. Identification of the diseases, at this stage of the course, was based on symptoms caused. Emphasis was placed on diseases and symptoms caused by the following *Fusarium* spp.:

  - *F. xylarioides* – causing CWD, the most important disease caused by *Fusarium* spp. on coffee;
  - *F. oxysporum* – causing vascular wilt and root diseases;
  - *F. solani* – causing dry root rot and wilt;
  - *F. stilboides* – causing Storey's bark disease, collar rot and scaly bark disease;
  - *F. lateritium* – causing collar rot;
  - *F. pallidoroseum* – considered non-pathogenic or a weak parasite, often a secondary invader. Participants had to learn about this *Fusarium* sp. to avoid confusing it with other pathogenic species occurring on coffee.

- *Collection of plant specimens* – principles of collecting plant specimen in the field were covered.

- *Fusaria growth media* – the following media were covered: tap water agar (TWA), special nutrition weak agar (SNA), potato sucrose agar (PSA) and carnation leaf agar (CLA).

- *Isolation of Fusaria from plant material* – this topic covered the primary isolation of Fusaria from wood.

- *Selective media for Fusaria* – this covered the following media: Nash and Snyder's peptone PCNB agar, selective fusarium agar (SFA), Rose Bengal, glycerine and urea medium.

- *Purification of Fusaria* – participants were taught single-sporing techniques, which involved streaking conidia on to TWA, picking out single spores or conidia after incubation and transferring the picked spore on to SNA medium. The resultant cultures should then be pure cultures.

- *Early recognition of* F. xylarioides – it is characterized by relatively slow growth, producing sparse hyaline hyphal growth, and the formation of small slimy clusters of microconidia on the agar surface of TWA. The microconidia arise from short, cylindrical conidia (forming cells borne on the vegetative mycelium, and are strongly curved).

- *Storage and preservation of Fusarium cultures are as follows*:

  - Short term – storage on slopes of SNA or CLA.
  - Long term – storage by lyophilization, storage in liquid nitrogen, or on pieces of sterile filter paper. Cheaper methods include storing isolates by refrigerating them in vials of sterile silica gel or soil.

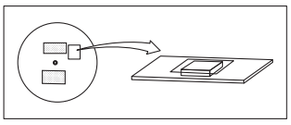
- *Principal characters used to identify Fusarium species are as follows*:
  - microconidia – presence or absence, shape, formation in chains or only in slimy droplets and formation in dry heads or only in slimy masses/droplets;
  - macroconidia – shape, size and formation in sporodochia or dry heads;
  - chlamydospores – presence or absence, shape and where formed;
  - colony morphology and growth rate – colony appearance on PSA;
  - colony pigmentation – distinctive pigments on PSA;
  - conidiogenous cells – size, shape and type;
  - teleomorph – presence or absence;
  - association with the plant disease symptoms – vascular wilt and head blight, but not reliable.

- *Methodology of Fusaria identification is as follows*:
  - Examination of cultures on PSA plates – noting the colour; see examples of colours below.

  - Examination of cultures on SNA plate under low-power microscope objective.

  - If elongated conidiogenous cells bearing small, colourless and slimy droplets of spores are visible on SNA, the fungus is possibly *F. solani*, but could also be a *Cylindrocarpon* or *Acremonium* sp. Therefore, one has to look for Fusarium macroconidia by making a slide from any sporodochia present either on SNA or PSA. These are most likely to be found close to the inoculum block, or close to the filter paper on SNA. In addition, one has to look for macroconidia with 'Fusarium' foot cells and with apical cells tapering to a point. If macroconidia have rounded ends, check *Cylindrocarpon* spp.

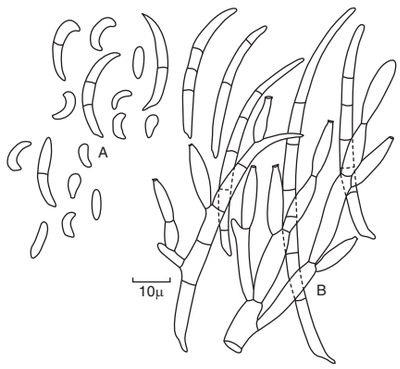
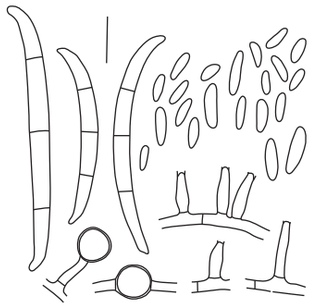
  - If elongated conidiogenous cells are not seen, using a sterile scalpel one has to cut a block of agar approximately 1 cm square from a region of the SNA plate where one can see spores existing. Adjacent to the filter paper is often a good region to choose. The block should be placed on to a microscope slide, a small drop of water added and a cover slip gently placed on top. Air bubbles are removed by gently tapping the cover slip.

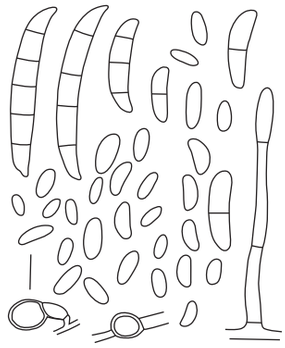
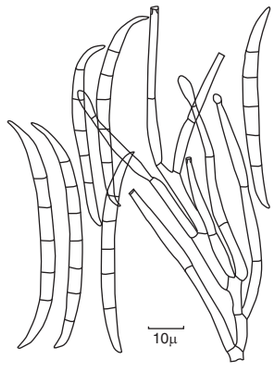
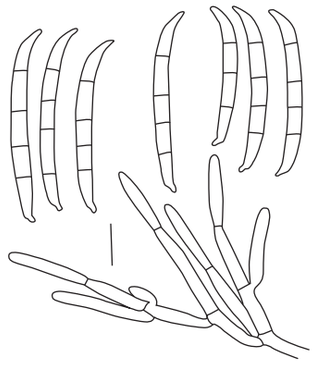
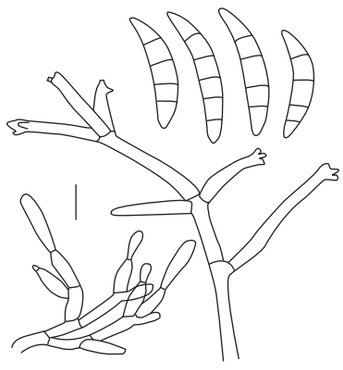


- The slide is examined with 10× and 40× objectives, focusing on the agar surface. Production of sparse or abundant microconidia is noted. If abundant, their shape is noted. Conidiogenous cells are located, noting whether they were monophialides, polyphialides or polyblastic cells. If macroconidia are present, their shape is noted. The presence or absence of the thick-walled, globose chlamydospores is noted. These may occur at the agar surface or form deeper down in the agar.

Key characteristics of each *Fusarium* sp. are presented in Table A4.1.

Table A4.1: Characteristics of common Fusaria occurring on coffee. (Illustrations from Booth, 1971.)

***Fusarium* sp.**	**Description**	**Illustration**
F. xylarioides	Small slimy clusters of microconidia at the apex of *short*, cylindrical conidiogenous cells borne on the vegetative mycelium. Microconidia are usually abundant and *strongly curved*	
F. oxysporum	Microconidia in slimy droplets on *short* conidiogenous cells (monophialides) similar to those of *F. xylarioides*, but microconidia *ovoid*, not strongly curved	

F. solani	Microconidia in colourless, slimy droplets at the apex of the *elongated* conidiogenous cells; microconidia *ovoid*, not curved	
F. stilboides	*Microconidia absent or very sparse*. Macroconidia long, narrow, multiseptate, straight or cylindrical with curved apical cell and formed in slimy droplets or masses. Colonies have reddish pigment on PSA	
F. lateritium	*Microconidia absent*. Macroconidia in slimy masses or in sporodochia. Apical cell of macroconidia often hooked or beaked, foot cell very obvious. Orange sporodochia abundant on agar surface. Chlamydospores absent or sparse. *Slow growing on PSA with lobed margin*	
F. pallidoroseum (= *F. semitectum*)	*Microconidia absent or very sparse*. Macroconidia multiseptate and usually formed singly, *dry* and not in slimy droplets	

A4.3.2 Practicals

- Used teaching key to common species of *Fusarium*.
- Focused on field identification of CWD and coffee diseases caused by other Fusaria by assessing symptoms observed (see below).

Field discussion of coffee diseases.

Characteristic blue-black coloration of CWD under a coffee bark when scraped with a knife.

A4.3.2.1 Laboratory-based practicals

Participants learnt how to identify cultures of *Fusarium* spp., both visually and with the aid of microscopes (dissecting and compound) (see below).

Laboratory-based practical session on identification of *Fusarium* spp.

A4.4 Outputs

A total of nine participants, who came from the following countries: DRC (two), Ethiopia (two), Rwanda (two), Tanzania (two) and Uganda (one), were trained in the identification of CWD and other coffee diseases caused by *Fusarium* spp.