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REVIEW ARTICLE

A REVIEW OF COFFEE WILT DISEASE, *GIBBERELLA XYLARIOIDES* (*FUSARIUM XYLARIOIDES*) IN AFRICA WITH SPECIAL REFERENCE TO ETHIOPIA

Tesfaye Alemu[1]

ABSTRACT: Coffee is vital to the economy of East and Central Africa, providing a major source of foreign exchange earnings and as a cash crop, supporting the livelihood of millions of people who are involved in cultivation, processing, marketing, and export. Coffee is attacked by various disease-causing organisms such as fungi, bacteria, viruses, nematodes, insects and weeds. One of the limiting factors for coffee production in Central and East African countries is tracheomycosis/vascular wilt disease caused by *Fusarium xylarioides* Steyaert imperfect stage (*Gibberella xylarioides* Heim and Saccas perfect stage). Coffee production and development is now threatened by coffee wilt disease (CWD). The major difference between tracheomycosis and many other coffee diseases is that it kills all affected trees at all stages of growth. Coffee wilt disease was first observed in 1927 in a plantation of *Coffea excelsa*, in the Central African Republic. Since then, CWD has re-emerged on *C. canephora*/*excelsa* in portions of the Democratic Republic of Congo in the mid-1980s, it affected up to 90% of plantations in 1993 in Uganda. The fungus lives in the soil, on infected debris, in alternative hosts or as resistant propagules of species, and enters the coffee tree through wounds at the base of the tree or on the roots. The outbreak of the pathogen has been reported throughout the major coffee-growing woredas in the south and south western parts of Ethiopia. The disease infestation incidence varied between 14.9 and 34.0%. The estimated annual coffee yield losses caused by CWD are about 7.4%, 1.6% and 2.6% in Uganda, Ethiopia and Tanzania, respectively. CWD is distributed, and caused coffee yield losses in major coffee-growing areas of western, southern and eastern parts of Ethiopia. The mean disease incidence ranged from 45% at Gera to 69% at Bebeka, with certain variations between coffee fields at each locality. The pathogen survives in the soil. It is difficult to control the pathogen by fungicides. However, the pathogen may be controlled by antagonistic biological control agents. *In vitro* evaluation of *Trichoderma* species has revealed up to 71% reduction of the mycelial growth of coffee wilt pathogen (*F. xylarioides*).

Key words/phrases: Biological control, Coffee, Coffee wilt disease, *Fusarium xylarioides* (*Gibberella xylarioides*).

[1] Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: talemu2000@yahoo.com or tesfayealemu932@gmail.com

INTRODUCTION

Coffee is a non-alcoholic and stimulant beverage crop and belongs to the family Rubiaceae and the genus *Coffea*, and is widely distributed throughout the tropical region. There are many species of coffee, but the only two commercially important ones are Arabica coffee (*C. arabica*, L.) and Robusta coffee (*C. canephora* Froener) (Pieters and van der Graaff, 1980). The major commercial species of coffee, *Coffea arabica* Linnaeus, is native to Africa. This has been introduced to many tropical areas of the world, especially in Central and South America, where it has become a major export crop. According to Kimani *et al*. (2002), about 60% of the world coffee production is from the former Arabica coffee, which is supposed to be of higher quality and can get much more in the market compared to Robusta coffee (*C. canephora*). Ethiopia is believed to be the country of origin of *C. arabica* that makes over 90% of the world's production (Paulos Dubale and Demel Teketay, 2000).

Coffee cultivation is confined to the tropical areas of the world consisting of over 80 developing countries, including Ethiopia. One of the most important gifts of Ethiopia is its coffee which has tremendous economic, social and spiritual impacts on many people of different geographical regions at cultural backgrounds (Tefestewold Biratu, 1995). Coffee is not only one of the highly preferred international beverages, but also one of the most important trade commodities in the world next to petroleum (Tefestewold Biratu, 1995). Coffee represents, for most coffee-growing countries, the major source of revenue for foreign exchange. The contributions of coffee in Ethiopian economy is more than 60% of the country's foreign exchange earnings, over 5% of the GDP, 12% of the agricultural output, and 10% of the government revenues (CSA, 2002). In Ethiopia, coffee provides employment for over 25 million people, who are involved in production, processing, marketing, and related services. It also employs 25% of the domestic labour force (IAR, 1996). About 55% of the production is exported and the rest is consumed locally (Mesfin Ameha, 1991). It is mainly cultivated in western, southern and eastern parts of Regional States of Oromia, SNNP, Gambella, Benishangul Gumuz and Amhara in Ethiopia. However, the production of coffee is hampered by various biotic factors and abiotic factors such as temperature, relative humidity, soil pH and mineral deficiency (Kimani *et al*., 2002; Ivey *et al.*, 2003; Agrios, 2005). Major biotic factors are fungal, bacterial, viral, nematodes, insect pests and weeds. Among the coffee diseases, fungi are the major constraints to the production, processing and quality of coffee. Being a perennial crop, the

coffee plant harbours many species of arthropods; over 850 species of insects are listed as feeding on coffee plants (Le Pelley, 1973). However, a relatively small number of the phytophagous species and some coffee diseases cause serious problems of reduction of coffee yield and deterioration of quality of coffee in an international market.

Among the major diseases, the coffee leaf rust (orange rust) and the coffee berry disease (CBD) caused by the fungus *Hemileia vastatrix* Berkeley and Broome and *Colletotrichum kahawae* Bridge and Waller, respectively, have major impacts. Other coffee rust diseases (powdery, yellow rust or grey rust) caused by the fungus, *H. coffeicola* Maubl and Rog. have not been considered as important economically. The symptoms of the disease are characterized by a dusty or powdery coating of yellow uredosori covering the underside of the leaves, in contrast to *H. vastatrix* that forms distinct blotches or pustules (Rodrigues, 1990; Adejumo, 2005). According to Waller (1985), *H. coffeicola* occurs only in West Africa, where it can be serious for some cultivars of *C. canephora* grown in very wet areas and it is favoured by hot humid conditions unsuitable for *C. arabica*.

There are more than 45 fungal pathogens of coffee reported in Ethiopia. Some of these are as follows: coffee berry disease (*Colletotrichum coffeanum/C. kahawae)*; coffee wilt disease/tracheomycosis (*Gibberella xylarioides*); coffee leaf rust (*Hemileia vastatrix*); brown eye spot/berry blotch or cherry blotch or berry spot (*Cercospora coffeicola*); stem blight dieback/ascochyta blight (*Ascochyta tarda)*; anthracnose/twig dieback or stalk rot of berries (*Colletotrichum gloeosporioides)*; damping off (*Rhizoctonia solani, Pythium* sp., *Fusarium* sp.); *Armillaria* root rot (*Armillaria mellea*); back rot/thread blight (*Corticium koleroga*); pink disease (*Corticium salmonicolor*); collar rot/bark diseases (*Fusarium lateritium, F. stilboides*); post-harvest fungal diseases (*Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Botrytis* sp. and *Alternaria* sp.). The coffee plant is seriously damaged by coffee berry disease (*C. kahawa*e) (Tesfaye Alemu and Ibrahim Sokar, 2000), coffee rust (*H. vastatrix*) and coffee wilt disease (*F. xylarioides*) (Walyaro, 1997; Hindorf, 1998). The brown eye spot or berry blotch (*C. coffeicola* Berk and Cooke) is a disease of widespread occurrence in nurseries and plantations, infecting the coffee leaves and the fruits as well (Waller, 1985; Wrigley, 1988; Chen, 2002; Geiser *et al*., 2005). Coffee berry disease is the most serious disease to *C. arabica* responsible on average for about 30% of the national yield losses to Ethiopia (Tefestewold Biratu, 1995; Eshetu Derso *et al.*, 2000). Coffee leaf rust occurs in Ethiopia at tolerable levels under a balanced path system and

it inflicts minor attack to the crop except in certain areas and some pocket fields planted with homogeneously susceptible cultivars at lower elevations (Meseret Wondimu *et al*., 1987; Eshetu Derso *et al*., 2000).

One of the limiting factors for coffee production in Central and East African countries is tracheomycosis/vascular wilt disease of coffee (CWD) caused by *F. xylarioides* Steyaert imperfect stage (*G. xylarioides* Heim and Saccas perfect stage). The coffee production and development is now threatened by coffee wilt disease (tracheomycosis). The major difference between tracheomycosis and many other coffee diseases is that it kills all infected plants at all stages of development. Coffee wilt disease (CWD) is a devastating disease spread across Africa reducing yields, destroying millions of coffee trees in affected countries and costing hundreds of millions of dollars in lost earnings to farmers. Farmers are unable to recover their lost income and need considerable resources to reinvest for replanting. Coffee wilt disease or tracheomycosis caused by *F. xylarioides* Steyaert (teleomorph: *G. xylarioides* Heim and Saccas) is becoming important in some regions of Central and West Africa, not only in *C. canephora* but also in *C. arabica*. It is a vascular disease causing yellowing and wilting of the trees. *F. stilboides* Wollenw (teleomorph: *G. stilboides*), the causal agent of the coffee bark disease, is also present in some African countries, particularly in Ethiopia, Kenya, Malawi and Tanzania. Its characteristic symptom is scaling of the bark leading to stem cankers and subsequently progresses to the death of the whole tree.

Coffee wilt disease (CWD) was first observed in 1927 in a plantation of *C. excelsa*, in the Central African Republic (Figures, 1940). Between 1937 and 1939, the disease spread to *C. canephora* and *C. liberica* in Cameroon, Guinea, Côte d'Ivoire, and the Democratic Republic of Congo (DRC), where up to 40% of plantations were infected (Steyaert, 1948; Fraselle, 1950; Heim, 1950; Saccas, 1951; 1956). The disease has reached epidemic proportions twice during the last century, becoming a serious constraint during the 1930s, 1940s and 1950s and was responsible for the complete failure of excelsa coffee commercially (Flood, 2009). Since then, CWD has re-emerged on *C. canephora*/*excelsa* in portions of the DRC (mid-1980s), affecting up to 90% of plantations (Flood, 1996), in Uganda (Flood and Brayford, 1997), and the Lake Victoria region of Tanzania. On the basis of a probable initial misidentification as *F. oxysporum* (Fraselle, 1950) and *F. oxysporum* f.sp. *xylarioides* (Delassus, 1954), the pathogen was thought to be a saprophyte endemic in inter-tropical African soils invading coffee bushes through wounds.

Subsequently, many hectares of *C. excelsa* throughout West and Central Africa were destroyed by CWD (Guillemat, 1946). The pathogen was first described in Democratic Republic of Congo the former Zaire in 1948 (Steyaert, 1948). The perithecia represent the sexual stage of the fungus called *G. xylarioides* (Heim and Saccas, 1950). *C. arabica, C. canephora, C. excelsa* and wild *Coffea* species are all susceptible to this infestation. Saccas (1951) also gave some indication of the destruction caused by the disease in a well-maintained plantation of 280 hectares of Central African Republic, which was completely destroyed by the disease in one year. *C. canephora* provides about 27% of foreign exchange earnings in Uganda (Ivey *et al*., 2003). CWD is the most severe disease of Robusta coffee in Uganda and has resulted in the loss of an estimated 12 million Robusta coffee trees (Ivey *et al*., 2003). The CWD only occurred sporadically in Africa but in the last decade or so it has become virulent, sweeping across Cameroon, Congo and Uganda.

Occurrences of coffee wilt disease in Ethiopia

Historically, coffee wilt disease (CWD) on *C. arabica* was first observed in Ethiopia (Keffa province) by Stewart (1957), who described the wilting symptom and also identified the causal organism to be *Fusarium oxysporum* f.sp. *coffeae*. Lejeune (1958) also noted the presence of this disease on Arabica coffee. Later, based on comparative studies of the isolates collected from dying Arabica coffee trees from different origins and different *Coffea* spp., the causal organism was confirmed to be *Gibberella xylarioides* Heim and Saccas, of which *Fusarium xylarioides* Steyaert is the imperfect (conidial) state (Kranz and Mogk, 1973). Van der Graaff and Pieters (1978) reported that this pathogen caused a typical vascular wilt disease and was the main factor of coffee tree death in Ethiopia. Subsequent surveys accompanied by isolation and identification demonstrated occurrence of *G. xylarioides* (*F. xylarioides*) in major coffee-growing regions of south and south-west Ethiopia (van der Graaff and Pieters, 1978; Merdassa Ejetta, 1985; Girma Adugna, 1997; Eshetu Derso *et al*., 2000). Even in some localities like Bebeka and Teppi, CWD outbreaks were noticed in large-scale plantation coffee (Girma Adugna, 1997; Eshetu Derso *et al*., 2000). During recent years, the prevalence and importance of CWD have been markedly increasing throughout coffee-producing areas of the country (Girma Adugna and Hindorf, 2001; Girma Adugna *et al*., 2001; CABI, 2003; Girma Adugna, 2004; Oduor *et al*., 2004). The characteristic partial wilting symptom accompanied by discoloured internal tissues effectively facilitates diagnosis and recognition of infected coffee trees in the field. This

early detection allows rooting out of the infected trees early in the season before fungal sporulation at the advanced stage of pathogenesis (Girma Adugna and Hindorf, 2001).

Symptoms of CWD

The most characteristic symptom of infection on mature coffee and young coffee seedlings is partial wilting. Internally, dark reddish (brown) discolouration is commonly exhibited on the stem. The early symptoms of infection on mature and young coffee plants are epinasty of leaves on some branches in the lower coffee canopy. These leaves first appear chlorotic or necrotic that turn brownish or dark brownish within two weeks and finally drop off. These external symptoms most frequently begin on one side of a single-stemmed plant or on one of many verticals originated from a tree or on one of the multiple-stemmed coffee bushes (partial wilting) (Fig. 1), and then gradually progress upwards throughout the plant (complete wilting and death) (Fig. 2). It has been observed that the internal infection caused by *Fusarium xylarioides* on coffee stems showed dark reddish (brown) discolouration (Fig. 3). Affected branches may turn black brown or blackish and dry up (Ivey *et al*., 2002; Flood, 2003; Lepoint *et al.*, 2005; Rutherford, 2006). These signs are known as dieback, often start on the branches on one side of the tree, but rapidly spread to the whole tree. The disease was found to spread from initial infections to healthy neighbouring trees, resulting in an aggregated pattern. An infected tree could infect up to three healthy trees away, in any direction. The bark on the trunk, especially near the base of the tree, may become swollen and develop many vertical and spiral cranks. Towards the end of the rainy season, black structures resembling soil occur on the bark, usually at the base of the plant (Ivey *et al.*, 2002; NAO, 2003; Girma Adugna, 2004). These structures are dark-violet perithecia; contain spores (ascospores) of the fungus that enable it to spread to other coffee trees and to survive in the soil or on plant material (Miville-de-chene, 1999; Girma Adugna and Hindorf, 2001; Kimani *et al*., 2002). In the roots, a moist black rot is observed (Kimani *et al.*, 2002; NAO, 2003).

Another important early sign of CWD is that berries on infected trees turn red prematurely and appear to ripen early. Most affected trees die in 2-3 months after the first symptoms are observed (Rutherford, 2006). When symptoms are observed, it is difficult to save the plant from CWD. Although other symptoms are caused by other problems, only CWD causes the blue-black discolouration of the wood (Ivey *et al.*, 2003; Rutherford, 2006).

Fig.1. Partially wilted coffee

Fig. 2. Complete wilted and dead coffee

Fig. 3. Infected coffee stem

Morphology

The sporodochial are produced macroconidia and microconidia. Macroconidia are 1-3 septated, frequently falcate slightly curved with distinct visible foot and basal cells (Janzac *et al.*, 2005; Lepoint *et al.*, 2005). Microconida of the aerial mycelia are usually 0-1 septated, and often variable in shape from slightly curved to allantoidal, and comma or u-shaped (Ivey *et al.*, 2003; Geiser *et al.*, 2005; Janzac *et al.*, 2005; Lepoint *et al.*, 2005). The Uganda isolates of *F. xylarioides* produce both micro and macroconidia on potato dextrose agar (PDA) and synthetic low-nutrient agar (SNA). Microconidia were unicellular, allantoid and had 0-1 septae. Macroconidia were strongly curved with marked foot cells and had 1-4 septae. Chlamydospores and perithecia were not observed in culture (Ivey *et*

al., 2003). Colonies were pale to colourless becoming light orange with the onset of microconidia production. Microconidia were unicellular, allantoid, had 0-1 septae and were 5-10 µm in length. Macroconidia were strongly curved with marked foot cells, had 1-4 septae and were 12-45 µm in length. Chlamydospores (asexual resting spore) and perithecia (sexual fruiting structure) were not observed in culture. Ivey *et al.* (2003) have recorded that the mean growth rate for all isolates grown on both PDA and SNA media was 3.8-4.9 mm/day.

Three asexual spores (macroconidia, microconidia and chlamydospores) and the sexual spore (ascospores) allow the pathogen to produce highly variable populations, in addition to the parasexual cycle (Flood, 2003; Girma Adugna, 2004; Rutherford, 2006). The fungus only rarely produces special survival spores that are thick walled resting spores (chlamydospores) and survive for many years (Booth, 1971; Fisher *et al*., 1982; Girma Adugna, 2004; Rutherford, 2006). The sporulating stage of each fungus develops within one or two days on the split stem of diseased coffee, provided that stems are kept moist. *Fusarium* produces sickle-shaped multiseptate conidia on sprodochia. *F. xylarioides* survives for 2-11 years in the soil as "saprophyte" because it produces resting spores or chlamydospores that can survive for many years. Moreover, the sexual spores (ascospores) produced in the perithecia may be able to act as survival spores (Flood, 2003).

Infections

Although little is known about the fungus that causes tracheomycosis, it lives in the soil, on infected debris, in an alternative host or as resistant propagules of species and enters the coffee tree through wounds in the base of the tree or in the roots (Flood, 2003; Janzac *et al.*, 2005; Lepoint *et al.*, 2005). The disease was found to spread from initial infections to healthy neighbouring trees, resulting in an aggregated pattern. An infected tree could infect up to three healthy trees away, in any direction. Disease foci formed and expanded with time, coalescing but punctuated in spots planted with resistant hosts. There are varying levels of susceptibility among host genotypes, affecting the rates and levels of epidemic development. Girma Adugna (2004) has indicated that high infection of susceptible Arabica coffee seedlings was noticed after inoculating with field collected ascospores, suggesting that perithecial state is the primary source of inoculum in the field.

G. xylarioides is a vascular disease which plugs or stops water movement within the vascular system of coffee plants. The pathogen penetrates and infects the bark or rootlets of a coffee by knife injury or with digging/cutting tools during slashing, capping and or digging. The pathogen is more serious in humid and wet areas, where careless and frequent slashing is practised in the plantation of few earmarked varieties (uniform population). Tracheomycosis is one of vascular systemic diseases which affects the root lateral stem of the coffee and gradually kills the whole coffee plant. The disease usually kills the coffee plant in symmetrical pattern, i.e., at the early stage of the sign half of the tree bark will be green, while the other part shows a pinkish to tan browning discolouration. The conidial stage/imperfect/anamorph (asexual) stages of *F. xylarioides* is usually observed on culture media, whereas the sexual/perfect/teleomorph of *Gibberella xylarioides* and their ascospores in perithicium are usually observed on the collar bark of dead trees in wet and humid season. The death of the tree is indicated by leaf and branch blighting. Bark removal with sharp knife exhibits symmetrical death with bluish black streak ascospores on collar of the coffee bark. The longitudinal splitting of the tree in the internal parts reveals a violet brown to dark colour. The ascospores are usually observed in the wet rainy months of August and September on the collar barks. A dead plant which exhibits a cut at one of its lower stems by slashing knife is usually indicative to suspect the presence of *G. xylarioides*.

This disease can attack almost all aboveground parts of the plant. In young plants, dieback begins with the lower branches but spread to the whole plant as the disease develops. Stem tissues around the collar of the coffee plant are killed, and blue-black streaks appear in the stems, under the bark. In severe attacks, trees wilt and collapse. On berries, sunken brown lesions appear at the stalk end of the berry, which can cut off the flow of nutrients to the berries, causing them to die prematurely. Dark brown lesions may also appear elsewhere on the berries, especially where new infecting berry stage, which turn the infected berries red, so that they appear to ripen early. The fungus is soil-dwelling and enters the plant through wounds either above or below ground. CWD caused by *F. xylarioides*, considered to be a soil-inhabiting fungus, is endemic to several African countries, affecting commercially important coffee species and causing serious economic losses. The fungus is apparently not able to survive from one season to the next and is mainly transferred through asexual and sexual spores that contribute to the spread of the disease. There is no well-designed and finalized disease

cycle of *F. xylarioides*; however there is similarity with the *Fusarium* wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) that was described by Agrios (2005). The possible way of disease cycle/development of CWD is shown in Fig. 4.

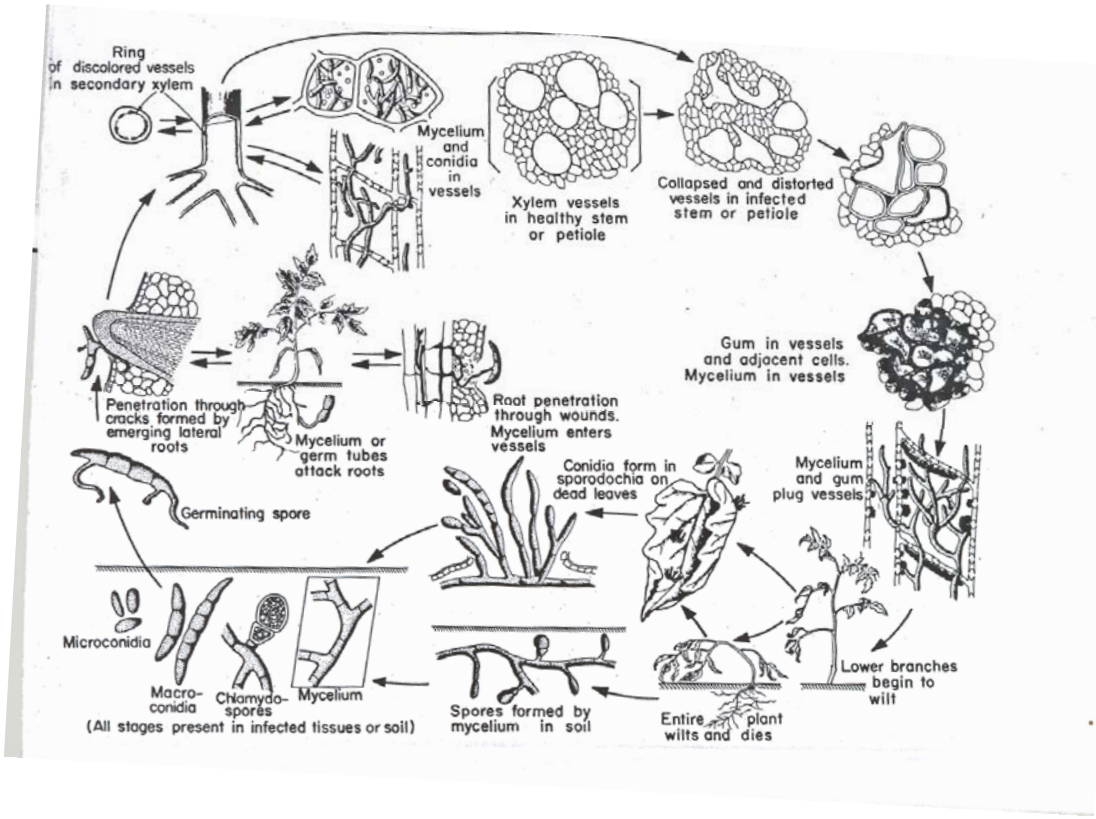

Fig. 4. Disease cycle of *Fusarium* wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) (After Agrios, 2005).

Phylogenetics of fusaria involved in coffee wilt disease

Coffee wilt historically has been associated with *F. xylarioides*/*G. xylarioides*, a species that has been classified as a member of *Fusarium* section *Lateritium*. Geiser *et al*. (2005) have investigated the molecular phylogenetics of fusarial coffee wilt isolates by generating partial DNA sequences from two protein-coding regions, translation elongation factor 1-α (tef) and beta-tubulin (*benA*), in 36 isolates previously identified as *F. xylarioides* and related fusaria from coffee and other woody hosts, as well as from twelve isolates associated with a current coffee wilt outbreak in Uganda. These isolates fell into two morphologically and phylogenetically distinct groups. The first group was found to represent previously unidentified members of the *G. fujikuroi* species complex (GFC). This

group of isolates fit the original description of *F. xylarioides*, thus connecting it to the GFC. The second group, which was diverse in its morphology and DNA sequences, comprised four distinct lineages related to *F. lateritium*.

One of the close relatives of *F. xylarioides* within the GFC, *F. udum* also causes vascular wilt diseases. True *F. xylarioides* isolates were found to possess two distinct alleles, with two isolates from West African countries possessing one, and isolates from East African countries possessing another. All isolates analyzed showed identical *benA* alleles. This raises the question as to whether these two groups represent distinct phylogenetic species (Geiser *et al*., 2005). The epidemiology and etiology of *Fusarium* infections and the biology of host-pathogen interactions, *Fusarium* taxonomy has been in a constant state of flux since the genus was first described by Link in 1809. These strains were characterized with vegetative spore morphology and vegetative growth, and by comparing DNA sequences from a portion of the translation elongation factor 1-alpha gene. DNA sequences from the Uganda isolates were further compared to those of seven *F. xylarioides* strains isolated from both *C. robusta* and *C. arabica* grown in South Africa, Zimbabwe, New Guinea and Ethiopia (Geiser *et al*., 2005).

The taxonomy of *Fusarium* species is based on the morphological characters including the presence or absence, the shape and the dimensions of microconidia, macroconidia basal cells and chlamydospores. The growth and colour development on different media are used as markers in practice (Gerlach, 1978; Flood and Brayford, 1997; Ivey *et al*., 2003; Geiser *et al*., 2005). When the fungus was identified by Steyaert (1948), the pathogen infected *Coffea* sp. was isolated and identified as *F. xylarioides* and the perithecia represent the sexual stage of the fungus called *G. xylarioides* Heim and Saccas. All *Gibberella* species are sexual states or teleomorphs of *Fusarium* species, which are destructive plant pathogens (Samuels *et al.*, 2001; Desjardins, 2003). The anamorphic stage, *F. xylarioides* was first described by Steyaert (1948) from stem samples of diseased coffee plants obtained from *C. excelsa* (Ivey *et al.*, 2003). The teleomorph form observed by Saccas in 1949 on dead trees of *C. noearnolandiana* was described and renamed as the *G. xylarioides* (Gerlach and Nirenberg, 1982; Nelson *et al.*, 1983). The fungus was indicated as heterothallic ascomycete having male and female strains, which can be identified based on the colony appearance and conidial morphology (Barnett and Hunter, 1972; Gerlach, 1978; Gerlach and Nirenberg, 1982; Nelson *et al.,* 1983; Girma Adugna and Mengsitu Hulluka, 2000). Over the last ten years, a tremendous amount of

phylogenetic data have been assembled that have revolutionized *Fusarium* taxonomy and identification (Synder and Hansen, 1945; O'Donnell, 1992; O'Donnell and Gray, 1995; O'Donnell and Cigelnik, 1997; O'Donnell *et al*., 1998; Aoki and O'Donnell, 1999; O'Donnell, 2000; O'Donnell *et al*., 2000; Baayen *et al*., 2000; Baayen *et al*., 2001; Geiser *et al*., 2001; Aoki *et al*., 2003).

Mating type and sexuality of the pathogen

G. xylarioides is considered as a heterothallic with sex-linked morphological characteristics (Booth, 1971). "Female" strains produce highly curved, 0-3-septate conidia, and masses of small bluish-black stromata, some of which represent perithecial initials. "Male" strains have a slimy appearance due to the presence of pionnote sporodochia containing long, thin, 5-7-septate conidia. Perithecia, occurring frequently in nature are produced *in vitro* if the correct mating types are brought together under suitable conditions (Steyaert, 1948; Fraselle, 1950; Heim, 1950; Saccas, 1951; Booth, 1971). However, representative mating type strains were never deposited in a culture collection and crossing conditions were not specified. As a consequence, von Blittersdorff and Kranz (1976) were unable to repeat *in vitro* production of the teleomorph, and the "male" strain was in fact re-identified as *F. stilboides* (Nelson *et al*., 1983; von Blittersdorff and Kranz, 1976) and more recently as belonging to the "*Lateritium* clade" (Geiser *et al*., 2004). Sexual reproduction in heterothallic filamentous ascomycetes is controlled by a single mating type (*MAT*) gene with two functional alleles/idiomorphs. The *MAT-1* idiomorph contains three open reading frames (ORFs), one of which (*MAT-1-1*) encodes a protein with a motif called the alpha-box, while the *MAT-2* idiomorph contains a single ORF (*MAT-2-1*) encoding a regulatory protein with a DNA-binding domain of the high mobility group (HMG) type (Coppin *et al*., 1997). The conservation of certain amino acids in these regions could enable PCR amplification of the as-yet-undescribed *G. xylarioides MAT-1*/*MAT-2* alleles using previously developed *G. fujikuroi* species and *F. oxysporum* primer pairs (Turgeon, 1998). Britz *et al*. (1999) have indicated that *F. subglutinans* f. sp. *pini* represents a distinct mating population in the *G. fujikuroi* species complex. Kistler (1997) also has studied the genetic diversity in the plant pathogenic fungus, *F. oxysporum*.

In addition to mating type, mating success in heterothallic fungi is also influenced by an isolate's ability to produce the required sexual structures. One of the parents must be "female fertile" i.e., capable of producing

perithecia, and the other parent must be "male fertile" i.e., capable of fertilizing the female structure. Self-sterile hermaphroditic individuals can function as either the male or female parent in a cross (Lepoint *et al*., 2005). *G. xylarioides* are produced in dark stromatic fruiting bodies in the barks of stems of dead coffee plants in the field after 2-3 months. These stromatic structures were mostly observed around the crown region of the plants within 30 to 50 cm above the ground level. *G. xylarioides* was presumed to be a heterothallic ascomycete (Booth, 1971), which is known to have a sexual/teleomorphic state in nature producing fertile perithecia in dead coffee plants (van der Graaff and Pieters, 1978; Flood, 1997; Girma Adugna *et al*., 2001). The perithecia were occasionally seen on the stem and branches of the coffee.

Chen and Mc Donald (1996) reported that genetic diversity is most likely expected in heterothallic microorganisms, which usually form their sexual stage in nature where recombination through meiosis would generate a large number of unique genotypes. The sexual reproduction also results in ascospores that can function as overwintering structures or infective propagules, and can be important component of the disease cycle (Glass and Kuldau, 1992; Anthony, 2006). This disease is clearly a serious threat to coffee production in Africa and the cause of its re-emergence is due to the arising of new, aggressive strain or biotypes of the pathogen. *Fusarium* isolates from other parts of Africa (Ivory Coast, Ethiopia) gave different band patterns. These results are surprising for a heterothallic fungus, which produces its sexual stage in nature and support the hypothesis that a new, more aggressive strain of the pathogen may have arisen within the wider gene pool of the pathogen population in Africa (Ivey *et al*., 2003; Geiser *et al*., 2005; Lepoint *et al*., 2005).

Losses

During the 1950s and 1960s, the disease was considered to be the most serious in Africa and destroyed millions of coffee plants (Oduor *et al*., 2003; Girma Adugna, 2004). Since 1993, the disease has become very serious in some Eastern and Central African countries (Flood, 1997; Flood and Brayford, 1997; Girma Adugna, 1997; Rutherford, 2006). The incidence increased dramatically and spread throughout Central and East Africa (Ivey *et al*., 2003; Rutherford, 2006). Since 1993, farmers began reporting a wilt disease of coffee in western Uganda near the border with the Democratic Republic of Congo and later in 1995, in Central African Republic (Ivey *et al*., 2003; Geiser *et al*., 2005; Rutherford, 2006). Disease foci formed and

expanded with time, coalescing but punctuated in spots planted with resistant hosts. There were varying levels of susceptibility among host genotypes, affecting the rates and levels of epidemic development (Musoli *et al*., 2008).

The fungus was earlier reported to be a well-known pathogen of other *Coffea* species in West and Central Africa in the 1950s (Booth, 1971; Coste, 1992). The disease was observed again in Zaire (Congo) in the early 1980s and noticed for the first time in Uganda in 1993, it is now causing economic losses to Robusta coffee in both countries (Flood, 1996; 1997; Lukwago and Birikunzira, 1997). According to the Uganda Coffee Development Authority (UCDA) the coffee wilt mainly affects the native, lowland Robusta variety and, since 1993, it has destroyed over 12 million coffee trees. In recent years, *F. xylarioides* has re-emerged in Central Africa, notably on Robusta coffee in Uganda and the Democratic Republic of Congo (DRC), although the disease is a continuing problem of *C. arabica* in Ethiopia. Economically, Uganda was affected by this coffee wilt disease and production declined from 4.4 million bags in 1996-97 to 3.6 million bags in 1997-98 (Flood, 1997).

The successful production of coffee in the Democratic Republic of Congo (DRC) is now threatened by CWD which has already caused enormous losses in the north-east of the country; over 40,000 hectares of coffee have been lost in the region of North Kivu alone (Congo). More plantations have been abandoned and smallholders are desperate as they see their only source of income disappearing. A decrease in coffee yield of over 50% has been observed in Haut Congo over the period from 1988 to 1995 (Flood and Brayford, 1997). The value of coffee exports of Rwanda declined from US $ 104 million in 1985 to just US $ 27 million in 1996. Losses of 1% per annum in coffee production since 1992 have already been reported spreading rapidly and observed some plantations with 90% infection (Flood, 1996). CWD due to *F. xylarioides* Steyaert on Robusta coffee was confirmed in Uganda in 1993 in two districts bordering the Democratic Republic of Congo (Zaire). Recently, the wilt occurred in 12 districts, to the south and east of the original foci. Disease incidence varied from a few infected trees to over 50% tree mortality (Flood, 1996).

In Ethiopia, the occurrence of *G. xylarioides* on *C. arabica* was established in the early 1970s by Kranz and Mogk (1973). Since then, survey works have demonstrated that the disease is becoming the main factor of coffee trees death in the country (van der Graaff and Pieters, 1978; Girma Adugna

et al., 2001). More recently, systematic surveys of tracheomycosis were conducted in coffee fields with known wilt disease history in some localities of south-western Ethiopia (Girma Adugna and Hindorf, 2001). CWD poses a considerable threat to the livelihood of millions of small farmers of Africa who are dependent on coffee for their income. In recent years, the emergence of *F. xylarioides* across East Africa has affected 90% and 30% of farms in Uganda and Ethiopia, respectively (CABI, 2005). According to CABI (2003), it has been estimated that affected coffee households are facing a reduction by a third of their income due to coffee wilt disease. The level of infection by this pathogen has confirmed the presence of tracheomycosis with an incidence of up to 40% (King`ori, 2001; Kimani *et al*., 2002; Rutherford, 2006). Vascular wilt, caused by *F. xylarioides* Steyaert (*G. xylarioides* Heim and Sacc.), is the most severe disease of Robusta coffee in Uganda and has resulted in the loss of an estimated 12 million Robusta coffee. Sixteen strains of *F. xylarioides* from nine districts within Uganda were isolated from Robusta coffee showing mild to severe symptoms of vascular wilt.

The disease was first recorded in Ethiopia (Kaffa Province) in 1957, and the causal organism was identified as *F. oxysporum* f. sp. *coffeae* (Stewart, 1957). Subsequently, the disease symptoms of coffee wilt disease on *C. arabica* were reported by Lejeune (1958) in the country. Similarly, Krantz and Mogk (1973) reported the occurrence of the pathogen, *G. xylarioides* on *C. arabica* and also isolated and confirmed the casual agent as *F. xylarioides* from diseased coffee in Ethiopia. The coffee industry of Ethiopia was severely affected by this pathogen and it was subsequently identified as the main cause of death of coffee tree in the late 1970s and early 1980s (van der Graaff and Pieters, 1978). Pieters and van der Graaff (1980) reported that the disease was endemic in all coffee-growing areas in Ethiopia and reached epidemic proportions in some areas. It has been reported by local authorities that the disease continues to affect coffee trees and constitutes a serious problem. Breeding programmes initiated for resistance to CWD in Ethiopia have not been successful (Pieters and van der Graaff, 1980). It was reported by Girma Adugna (2004) that CWD is found to be more prevalent in the plantations, either in small-scale farmers' fields, research plots or large-scale commercial farms followed by garden and semi-forest production systems. The disease is more severe in Yirgacheffe than in Kochore and Wenago areas of southern region. The highest disease intensity is in the garden-based coffee production systems of the aforementioned areas. The homogeneity population of coffee trees have

more infections, disease incidence and severity as well as susceptibility to the pathogen as has been observed during the field survey and sample collections of coffee wilt disease in major coffee-growing woredas of Jimma zone (Negash Hailu and Tesfaye Alemu, 2010). In addition to less heterogeneity in the local cultivars or landraces and relatively intensive agronomic activities, the fungus is more aggressive to cause high CWD incidence (Girma Adugna, 2004).

Coffee wilt disease was reported to be prevalent on *C. excelsa* in Central African Republic and Cameroon, on Robusta varieties in Zaire and Ivory Coast (Booth, 1971; Coste, 1992) and found on Arabica coffee in Ethiopia, mainly in plantations near Agaro, Jimma and Bonga in early 1970s (Kranz and Mogk, 1973). The disease incidence is high where coffee is grown under advanced cultural practices and minimal in the less-managed forest coffee (van der Graaff, 1983). During initial surveys made in 1973, the presence of *G. xylarioides* was confirmed from Jimma, Gera, Manna, Gomma, Mettu, Sidamo (IAR, 1974); Dembidollo and Wondoguenet (IAR, 1980). According to Girma Adugna (1997), disease outbreaks were observed on some trees at Bekeka and in the Baya at Tepi in 1992. Later, the disease was distributed to Chira, Gechi, Choora, Yayo districts (Girma Adugna, 2004) and CWD became endemic to *C. arabica*. Van der Plank (1975) has indicated that the endemic of *F. xylarioides* was always present, but the extent of the damage of the crop was little, because of the horizontal resistance in the host and relatively low level of virulence of the pathogen. CWD is known to attack all species of *Coffea*, including the wild indigenous lines in Tropical Africa (Wrigley, 1988; Coste, 1992). The pathogen is endemic in all coffee-growing areas of Ethiopia (Flood, 1997; Girma Adugna and Hindorf, 2001; Girma Adugna, 2004; Lepoint *et al.,* 2005). The annual national crop losses attributed to CWD was 3,360 tonnes amounting to US $3,750,976 in Ethiopia (CABI, 2003). According to CABI (2003), most farmers observed the disease 40 years ago and since then the pathogen has caused considerable losses to coffee trees. Its control methods are not well known by farmers, extension workers and agricultural officers in the country to control the pathogen. The systematic surveys of CWD conducted in coffee fields in some localities of south-western Ethiopia have observed that the variations and aggressiveness of the test pathogen isolates are due to susceptibility of coffee cultivars, intensity of cultural practices and environmental factors that prevail for the disease development in the field conditions. In Ethiopia, *F. xylarioides* is becoming severe in coffee state farms, at Teppi/Bebeka and Gera and the mean prevalence of the disease

severity was recorded at 35.09% (Eshetu Derso *et al*., 2000). It is apparent that CWD is increasingly becoming important, especially in coffee plantations. The mean disease incidence ranged from 45% at Gera to 69% at Bebeka, with certain variations between coffee fields at each locality. CABI (2003) has indicated that the incidence of CWD is higher on coffee trees that are older, shaded, planted on loamy soil and weeded by slashing.

Susceptibility of coffee to CWD

In addition there were certain variations in the incidence of CWD between coffee fields at each locality that may be ascribed to differences in their genetic makeup and age of coffee cultivars, cultural practices and environmental conditions at specific locations. The disease spreading mechanisms mainly involve human activities including pruning, stumping, slashing and hoeing to control weeds and transporting infected trees from diseased fields to healthy fields (CABI, 2003). The mechanism of change in pathogen population and the basis for the disease management strategies including development and use of resistant coffee varieties have been reviewed by McDonald and Linde (2002) who have indicated the amount of genetic variation among individuals in a population, the ways in which this variation is partitioned in time and space and phylogenetic relationships along individuals within and between sub-populations.

The hitherto research activities on coffee wilt disease have been concentrated on selection of resistant coffee varieties due to the severity of the disease and difficulties encountered in applying chemical treatments (Coste, 1992). The development and use of resistant germplasms through selection, from an enormously heterogeneous population of Arabica coffee, is the only long-term solution to the tracheomycosis problem. Thus, in order to achieve reliable results in wilt disease resistance, field evidence on mature coffee trees should be supported by intensive greenhouse seedling tests. Conversely, coffee cultivars known to have promising performance in the seedling test must be observed for certain periods of time under field conditions essentially in areas where they will be released (Girma Adugna, 1997). According to Girma Adugna *et al*. (2001), significant difference was observed among the cultivars in percent tree death caused by *G. xylarioides* under field conditions. Resistance to *G. xylarioides* in *Coffea arabica* is of a horizontal nature (Pieters and van der Graaff, 1980; Girma Adugna *et al*., 2005). Cultural comparisons, pathogenicity tests and RAPD-PCR markers corroborated existence of host specialization into at least two pathogenic forms within *Gibberella xylarioides* populations. Thus, two formae speciales, namely, *G. xylarioides* f.sp. *abyssiniae* (anamorph: *F. xylarioides*

f.sp. *abyssiniae*) for the fungal strains attacking only *Coffea arabica* and *G. xylarioides* f.sp. *canephorae* (anamorph: *F. xylarioides* f.sp. *canephorae*) pathogenic to *C. canephora* and *C. excelsa* are known (Girma Adugna *et al*., 2005). According to Girma Adugna *et al*. (2005), this subdivision enables to design effective coffee wilt disease management strategies, develop resistant cultivars/lines and formulate further breeding programs towards each population group.

***Fusarium* species causes plant diseases**

Fusarium is one of the most important plant pathogenic fungal genera, causing a wide variety of diseases on many different hosts. *Fusarium* diseases of grains and greenhouse crops have a particular impact on African agriculture. *Fusarium* species may be primary pathogens of plants or act as components of disease complexes together with other fungi, bacteria, nematodes, diseases in pot culture and field conditions. These pathogens are also common secondary invaders of plant tissues, which have been weakened by factors such as environmental stress or which are dying due to other pests, or diseases of plants. *Fusarium* diseases of grains and greenhouse crops have a particular impact on cultivated and plantation plants in agriculture. Included are vascular wilts caused by *F. oxysporum*, head blight of grains caused by *F. graminearum*, and rots of tubers, fruits and stems caused by a variety of species. *Fusarium* species also produce toxic compounds in association with plants, thus causing a direct threat to human and animal health. If we wish to understand the epidemiology and aetiology of these plant-fungus interactions, as well as control them, we need to accurately identify the causative fungi to the species level.

A broad range of disease symptoms may be produced by *Fusarium* species as has been indicated below: (1) Seedling blights of many herbaceous and woody plants can be affected and several different fusaria may be involved; e.g. *F. solani*, *F. oxysporum*, *F. avenaceum, F. lateritium*, *F. culmorum, F. graminearum*, *F. moniliforme*, *F. sambucinum* (Rutherford, 2003); (2) The root rots of several plant pathogens are caused by *F. solani*, *F. oxysporum*, *F. avenaceum* and *F. culmorum* and also with synergistic interactions of plant parasitic nematodes; (3) Basal rot of bulbs are commonly caused by *F. oxysporum*; (4) Vascular wilts *F. oxysporum*, can be a soil saprophyte or secondary invader, so it must be isolated from the vascular tract of the stem and re-inoculated to be sure that it is responsible for vascular wilting; (5) Stem leaf and head blights of cereals of a wide range of fusaria may be involved: *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. heterosporum* and *F. nivale*. These *Fusarium* species often occur with other genera such as

Helminthosporium, *Septoria* and other species; (6) Post-harvest/storage rots may be caused by *F. sambucinum*, *F. solani var cocruleum*, and in the tropics by *F. equiseti*, *F. acumunatum*, *F. pallidoroseum* (=*F. semitechum*) and *F. moniliforme*; and (7) Cankering of woody hosts is caused by *F. lateritium*, *F. sambucinum*, *F. solani*, *F. sacchari* and *F. moniliforme var subglutinans*. Also, *F. stilboides* causes bark disease of coffee. Therefore, care must be taken in interpreting the pathological significance of fusaria isolated from diseased plants (Rutherford, 2003). Included are vascular wilts caused by *F. oxysporum*, head blight of grains caused by *F. graminearum*, and rots of tubers, fruits and stems caused by a variety of species. *Fusarium* species also produce toxic compounds in association with plants, thus causing a direct threat to human and animal health.

Fusarium species causes coffee wilt diseases

Twenty species of *Fusarium* have been recorded from coffee in the Common Agricultural Bureaus of International (CABI) Bioscience herbarium, but are not associated with specific diseases. Four species are known to be pathogenic to coffee (*F. xylarioides*, *F. solani*, *F. oxysporum* and *F. stilboides*), while the status of *F. lateritium* is still under debate (Rutherford, 2003). *F. solani* causes lethal root disease, *F. stilboides* incites bark disease, *F. oxysporum* and *F. xylarioides* cause wilt diseases in coffee (Waller and Brayford, 1990; Stover, 1992; Waller and Holderness, 1997; Geiser *et al.*, 2005). Formae speciales of *F. solani* and *F. oxysporum* can be recovered from coffee root, husks and soil samples obtained from infected trees with wilt disease (Flood, 1997) and inducing different types of wilting on coffee in different geographical regions. *F. stilboides* was identified from dead suckers from newly stamped coffee. *F. solani* was observed on young coffee weakened by moisture stress, deformed root and parts damaged by herbicides. *Fusarium* species were observed from discoloured beans, at nursery stage and from over riped or dieback berries. Fusarial bark diseases of coffee caused by *F. stilboides* is an important factor limiting Arabica coffee production in the low and medium-altitude districts of Kenya (Flood and Brayford, 1997; King'ori, 2001).

C. liberica was seriously infected and high incidences were observed in West Africa. In Ethiopia, large areas of old plantation from Gera, Kossa and Gummer State Farms were uprooted due to CWD. Observations in West Africa and Ethiopia have revealed that coffee exhibits the last symptom of blighting and death after 2-3 years. The pathogen has already spread in coffee-growing countries such as Central African Republic, Congo, Guinea,

Ivory Coast, Ethiopia, El Salvador, Zimbabwe, Tanzania, Uganda, Malawi, and Zaire (Flood, 1997).

Table 1. The *Fusarium* species, symptoms and distribution on coffee in Africa.

***Fusarium* species**	**Common name of disease**	**Geographical distribution**	**Symptoms**
F. xylarioides	Coffee wilt, vascular wilt or tracheomycosis	Widespread in Africa, also El Salvador	Wilting, chlorosis and defoliation of the aerial parts of the crop, vertical and spiral cracks in bark of trunk. Characteristic blue-black streak in the wood under bark, usually near collar. Stromata producing perithecia may be observed in bark. Infected berries turn red and appear to ripen early. Seed infection causes blue-bark discolouration of parchment and silver skin.
F. solani	Dry root rot and root disease	East Africa	Sudden wilting and death of entire tree, brown to purple discolouration of central root and stem tissues.
F. oxysporum	Vascular wilt and root disease	Central America, India and Brazil	Gradual wilting, chlorosis and necrosis. Defoliation of the aerial parts of the crop occurs but is slower than in *F. xylarioides*, leaves remaining intact for some time. Characteristic black streaks in the wood under bark.
F. stilboides	Storey's bark Disease, collar rot, Scaly bark disease	Mainly southern and Eastern African	Storey's bark-cankers of young sucker stems. Constriction at base, stem girdling, dieback. Collar rot-girdling of older stems near soil level. Scaly bark- flaking of mature bark over whole trees.
F. lateritium	Collar rot	????	Collar rot leading to wilting and death of entire tree.

????- Not specified Source: Rutherford (2003)

Survival and spread of CWD

The pathogen survives in the soil in the form of microconidia, macroconidia, chlamydospores and perithicium with ascospores. The pathogen appears to be a soil-inhabiting fungus, which can penetrate through wounds either above or below ground. Inside the coffee, the fungus invades the water conducting system/xylem and blocks the movement of water upwards from the roots to the rest of the plant. The timing from first symptoms to death of the coffee varies from days in young plants to eight months in coffee more than ten years old (Girma Adugna, 2004). Once the fungus affects the coffee, all affected trees eventually die. This disease can attack almost all aboveground parts of plant, and it is most common in young coffee. Dieback begins with the lower branches but may spread to the whole coffee as the disease develops. Stem tissues around the collar of the coffee are killed, and blue-black streaks appear in the wood, under the bark. Infected coffee trees lead to defoliation of leaves and wilting of branches and subsequently gradual death of coffee trees is commonly found in the

field conditions (Negash Hailu and Tesfaye Alemu, 2010). The fungus is apparently not able to survive long in the soil and survival from one season to the next is mainly through seed from infected berries, however, insects and rain splash may also contribute to the spread of the disease.

According to Wrigley (1988), the lateral and feeder roots of coffee spread on the surface plate parallel to soil surface for a distance of 1.2 to 1.8 metres from the trunk. It has been observed that *F. xylarioides* is abundantly recovered from root parts of symptomatic and asymptomatic coffee (Flood, 1997; Flood and Brayford, 1997; Girma Adugna *et al*., 2001; Girma Adugna, 2004). The pathogen spreads 2 metres up to four plants on either sides of the inoculated focus plant through the infection of the roots in greenhouse (Ivey *et al*., 2002; Rutherford, 2006). Closely spaced coffee is more liable to wounding and cross inoculation while slashing or hoeing coffee fields. According to Girma Adugna (2004), almost all coffee plants have wounds at the crown level or few centimetres above, and on average healthy trees have 1-3 wounds per coffee stem. The wounds arise from intensive slashing of weeds in coffee fields by hoeing (Rutherford, 2006). Most coffee plants are found with wound at least once at all locations, where slashing is employed to control coffee weeds (Girma Adugna, 2004). The stem nicking or root drenching inoculation methods also elaborate the role of contaminated farm implements in crosses inoculating coffee plants as well as disseminating the coffee wilt pathogen in the field (CABI, 2003). It has been indicated that it is necessary to evaluate and know the proper stage of coffee seedlings, amount of inoculum and concentration of spores and method of inoculation in order to determine the effect of wounding on young coffee seedlings and the infection process of the test pathogen (*F. xylarioides*) in pot cultures under greenhouse condition (Girma Adugna, 2004; Negash Hailu and Tesfaye Alemu, 2010).

Replanting susceptible cultivars in the infected field increases the fungus inoculum density (CABI, 2003). Pieters and van der Graaff (1980) reported that among socioeconomic factors contributing to the spread of CWD, particularly in Ethiopia, frequent replacing with several seedlings (3-8) per uprooted wilted coffee is one. The infection of the young replants undoubtedly suggests that the fungus survives in stumps, root debris or in the soil for 2-3 years (Stover, 1992; Miville-de-chene, 1999; Kimani *et al.*, 2002). Perithecia of *F. xylarioides* contain great number of viable ascospores with 95% germinating rate and are abundant in the soil, so that these sexual structures are the most important source of inoculum in the CWD epidemics (CABI, 2003). The major function of the sexual state of the

fungus is largely to serve as a survival mechanism, rather than maintaining diversity in the population structure. The cut wilted trees and remaining stumps harbour the fungal fruiting bodies such as perithecia with ascospores that serve as inoculum source for further infection and initiate disease epidemics (Girma Adugna, 2004). The spores of the fungus can be carried by wind and water (rain splash and flooding) to spread the disease from tree to tree. Wind spread may occur over long distances (Flood, 1997; Flood, 2003; Rutherford, 2006). Human activities, such as pruning, weeding with a hoe and transporting affected trees for use as firewood or fencing can also spread the fungus (Flood, 2003; Rutherford, 2006). When a tree is mechanically or accidentally wounded, during pruning, weeding around the trees and even harvesting, the fungus may enter and cause disease (Geiser *et al.*, 2005; Rutherford, 2006).

Isolation

The process of isolation begins with the collection of symptomatic diseased parts of coffee (branches, twigs, stems and roots). Diseased coffee tissues are first washed in sterile distilled water (SDW) and surface sterilized in 2% Sodium hypochlorite for one to two minutes or 96% ethanol for ten seconds or 0.5% cupric chloride, 70% alcohol each for one minute (Geiser *et al.*, 2005; Summerell *et al.*, 2006). This is usually followed by rinsing the plant material in sterile distilled water and allowing it to dry on sterile filter paper (Dhingra and Sinclair, 1993; Aneja, 2005; Geiser *et al*., 2005).

There are two types of isolation methods proposed, which are direct, indirect or both. In direct ways of isolation, the fruiting bodies or small excised plant parts from the margins of healthy and infected part will be transferred into general or selective media (Vannini and Vettraino, 2000). The indirect means of isolation can be done at least by two ways. The first one is done by attacking symptomatic organs disc to the cover of Petri plates with the perithecia facing downwards in order to release its spores directly to the isolation media (Ivey *et al.*, 2003; Jansen, 2005). Secondly, segments from infected plant parts are transferred into moist chambers to promote growth of mycelium, fruiting bodies or to sporulate out of infected tissue (Roux *et al.*, 2004; Geiser *et al*., 2005). Finally, the isolates of *F. xylarioides* are purified and maintained on separate Petri plates which contain suitable PDA/MEA media and are incubated at 25°C for 7 days and the pure cultures of the test pathogen kept at 4°C for further study.

Sporulation

This is one of the important steps in the process of identification, characterization and preparation of spores for pathogenicity tests. Spores often are produced under conditions that are not suitable for vegetative growth (Nelson *et al.*, 1983; Dhingra and Sinclair, 1993; CABI, 2005). Some of the factors affecting sporulation are ultraviolet light, fluctuating temperature conditions (Nelson *et al*., 1983) and high humidity or nutrient poor medium (Nelson *et al*., 1983; Vettraino *et al.*, 2002). The use of near-ultra violet (black light) would be best if it was alternatively used on the basis of 12:12 hours light and dark in an incubator (Nelson *et al*., 1983). Regarding the sporulation media, many species sporulate better on natural substrata such as macerated leaves and lupine stems than agar (Ivey *et al*., 2003; Geiser *et al*., 2004). In addition to this, nutritionally weak media such as potato carrot agar, oat agar, and tap water agar are recommended for many fungi to sporulate successfully (CABI, 2005).

Pathogenicity test

In order to substantiate an organism, group of organisms or combination of organisms and environmental factors as the causal agents for a disease, they must fulfill Koch's postulates (Patridge, 1999; Agrios, 2005; Pethybridge *et al*., 2004; Aneja, 2005). The pathogenicity test begins with preparation of sporulate spore or raw mycelium from *F. xylarioides*. Different authors have used different amount of spore loads during this test: 2-4 $x10^5$ spores/ml of *Phythophthora ramorum* (Denman *et al*., 2005), $5x10^5$ spores/ml of *Alternaria* blight of Paulownia spp. (Ray *et al.*, 2005), 10^4-10^5 conidia/ml of *Alternaria alternata* (Belisario *et al.*, 1999), $5x10^4$ (Bohar and Schwazinger, 1999), 10^4-10^5 conidia/ml (Holdenrieder and Kowalski, 1989) and $5x10^4$ propagules/ml (Hutton and Mayers, 1988). The given spore or mycelium could be loaded with or without wounding the plant (Denman *et al.*, 2005). The non-wound trial helps to determine whether infection could develop without previous damage (Luque *et al*., 2000; Geiser *et al*., 2005). During inoculation of stems, the adjusted spore suspension should be placed on the surface of the soil by little wounding of feeder roots of coffee.

Pathogenicity of each isolate of *F. xylarioides* was tested on coffee seedlings which were planted in polyethylene bags in soil inoculated with the test isolates of spore concentration of $3.93x10^5$ spores/ml. The application of this spore concentration confirmed the coffee seedling foliage symptoms, disease development, mortality of coffee seedlings and its virulence by three isolates of *F. xylarioides* (Negash Hailu and Tesfaye

Alemu, 2010). The result of the experiment has shown that the disease symptoms and development of *F. xylarioides* isolates increased up to 90 days of drenching of the inocula of three isolates. The development lesions were observed on all of the coffee seedling treatments and mycelial growth were observed on stem pieces kept in growth chamber experiment (Fig. 5). Since the feeder roots were wounded, positive re-isolations coincided from all parts of the coffee seedlings and from the soil, where the seedlings were grown (Negash Hailu, 2007). Studies on pathogenicity tests proved the host specificity and diversity in aggressiveness of *G. xylarioides* populations obtained from *C. arabica, C. canephora*, and *C. excelsa* (Girma Adugna and Mengistu Hulluka, 2000; Girma Adugna *et al*., 2005; 2007).

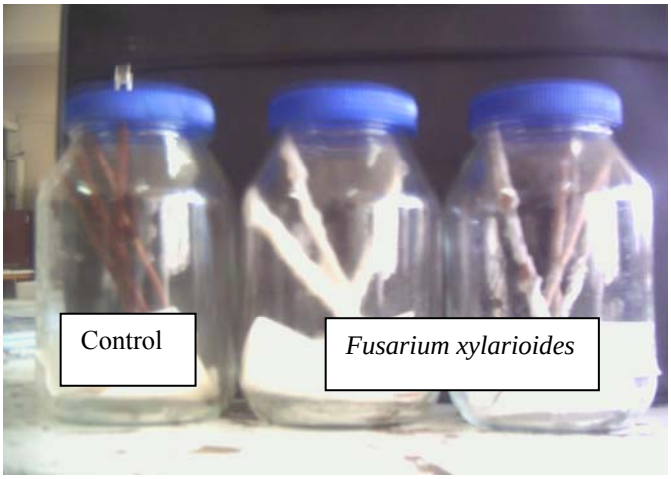

Fig. 5. The mycelial growth of five isolates of *F. xylarioides* in the growth chamber.

Factors influencing incidence and prevalence of the pathogen

Although the biology of *G. xylarioides* as well as the disease cycle is not well known, the pathogen is soil-borne and infects coffee trees through wounds or natural openings (Miville-de-chene, 1999; Kimani *et al.*, 2002). Some tree predisposing factors aggravate the CWD incidence and prevalence. The CWD outbreak started mostly at the center of the plots where trees are planted very close to each other (1x1 m) and radiate towards as pacing distances between trees increased (2x2 m) (Girma Adugna, 2004). The incidence was significantly higher in closely spaced trees than in widely spaced plots. This is due to crossing over of roots, or root-to-root contact through which the fungus can transmit from infected to nearby healthy coffee trees.

Factors affecting the growth of the pathogen

The major factors affecting growth of the pathogens are medium, temperature, light, aeration, pH and water activity.

Culture media

The growth requirements for fungi may vary from strain to strain, although cultures of the same species and genera tend to grow best on similar media (Dhingra and Sinclair, 1993; Aneja, 2005). The source of isolates can give an indication of suitable growth conditions. Cultures grow more satisfactorily on media freshly prepared in the laboratory, especially natural media such as vegetable decoctions. However, synthetically prepared media are often useful and can be very important in using different culture media for the growth and maintenance of the pathogen. The standardization of media formulae is necessary for most work. Media will affect colony morphology and colour, whether particular structures are formed and may affect the retention of properties (Dhingra and Sinclair, 1993; Burgess *et al*., 1994; Burgess and Wingfield, 2002; Aneja, 2005). The best media for mycelia growth of *F. xylarioides* are potato dextrose medium (PDA), malt extract medium (MEA) and Fusarium sporulation medium (FSA) and for sporulation synthetic low-nutrient agar medium (SNA).

Temperature

The majority of filamentous fungi are mesophilic, growing at temperatures within the range of 10-35°C, and most grow within temperatures between 15 and 30°C (Kapoor and Kar, 1989; Dhingra and Sinclair, 1993; Smith and Onions, 1994; Burgess and Wingfield, 2002; Negash Hailu and Tesfaye Alemu, 2010). According to Nelson *et al.* (1983) the suitable temperatures for the growth of *F. xylarioides* is 25±1°C.

pH

Filamentous fungi vary in pH requirements. Most common fungi grow well over the range of pH 3 to 7, although some can grow at pH 2 (Smith and Onions, 1994; Burgess *et al.*, 1994; Aneja, 2005). The best pH for *F. xylarioides* was found to be 5.5 during an investigation by Negash Hailu and Tesfaye Alemu (2010).

Management of Coffee Wilt Disease

Quarantine measures

For the coffee trees currently free from the pathogen (*F. xylarioides*) strict quarantine measures, which help to prevent its entry and spread, are necessary. Movement of coffee materials (seedlings, husks) between affected and unaffected areas should be restricted as much as possible (Hakiza and Mwebesa, 1997). These measures need to be backed up with dissemination of information about the disease to farmers, extension workers and the general public. This involves the destruction of all affected coffee in border areas and encouragement of farmers to grow crops other than coffee (Flood, 1997; Girma Adugna, 2004; Rutherford, 2006).

Resistant varieties

Production of resistant cultivars is the best option for controlling CWD in the long term. This method was very successful in controlling outbreaks of the disease in 1950s and 1960s in West and Central Africa, where affected coffee was uprooted and destroyed and the fields replanted with resistant cultivars of *C. canephora* such as cultivar `robust`, but recently resistance has broken down due to emergence of a new isolate of the fungus (Meseret Wondimu *et al.*, 1987; Flood and Brayford, 1997). In Ethiopia, breeding programmes were initiated for *C. arabica* (van der Graaff and Pieters, 1978; Pieters and van der Graaff, 1980), but the disease remains a problem in some areas of the country. Some farmers are conducting their own selection since, even in very badly affected areas, a few trees may survive. Some farmers are trying to replant with coffee seedlings, in order to fill in the site of infected coffee trees of their farms.

Cultural practices

Affected trees and trees adjacent to affected trees should also be uprooted and burnt although they appear healthy because while symptoms of the disease may not be visible, the fungus may be inside the plant (Rutherford, 2006). When symptoms are recognized quickly and uprooting and burning is done efficiently, farmers may save some of the crops (Flood and Brayford, 1997; Girma Adugna, 1997; Lepoint *et al.*, 2005; Leslie *et al.*, 2005). If the farmers delay, the infected trees act as source of inoculum to other coffee and this leads to whole crop losses. Coffee plants cut down as control measure should not be used as fuel as affected coffee dragged through healthy trees in the farm will aggravate the spread of the disease. Diseased trees must be burnt where they are uprooted. To prevent spread

from one field to another in large plantation, it is recommended that a 300 m strip of land be cleared of coffee (by uprooting and burning) ahead of the disease front (Girma Adugna, 1997; Hakiza and Mwebesa, 1997; Flood, 2003; Rutherford, 2006).

Mulches and soil amendments including cow dung and urine have been claimed to control the disease, but bring only temporary improvement to infected coffee by increasing plant vigour and stimulating new growth of roots, shoots, and leaves (Flood, 1997; Hakiza and Mwebesa, 1997; Rutherford, 2006). Improvement will also be partially due to the encouragement of organisms such as *Trichoderma* spp. and *Aspergillus* spp. in the soil that compete with the wilt fungus (Thomashow, 2002). Mulches and soil amendments are therefore unlikely to control the disease in already infected coffee, but may be useful in preparing the land for replanting after affected trees have been uprooted and burnt (Cooney and Lauren, 1998; Rutherford, 2006).

Following destruction of the diseased coffee and preparation of the land, replanting should not be carried out for at least two years to allow the inoculum of the fungus in the soil to decrease (Girma Adugna and Mengistu Hulluka, 2000; Girma Adugna, 2004; Rutherford, 2006). Replanting should be done with seedlings raised from the disease-free cuttings and seeds of coffee collected from areas that are free from CWD (Girma Adugna and Mengistu Hulluka, 2000). The control measures being implemented are sensitization of farmers and civic leaders about the disease, urging farmers to cut and burn affected trees *in situ*, restriction on movement of unhulled coffee, a ban on use of coffee husks as mulch in coffee, and replanting on new land. The successful management of CWD depends on the principles of disease prevention that is to avoid wounding of any part of the plant and phyto-sanitation measures.

Chemical control

The pathogen is thought to live in the soil and inside the plant, making it hard to target the fungus even with systemic fungicides (Tesfaye Alemu and Kapoor, 2004). The usage of fungicides will not be economic either in a plantation or in smallholder farms and will have undesirable environmental effects. If the fungus is carried on coffee seeds, then the treatment of seeds with fungicides may be beneficial (Ivey *et al.*, 2003; Rutherford, 2006). There is an urgent need to intensify research in these areas and to identify sources of resistance, which are basic in formulating effective control strategies.

It is very difficult to provide rational solutions to the problem when detailed knowledge of the epidemiology and variability of the pathogen is not available and likewise even the basic biology of the fungus such as its survival in soil, or in seed, is unknown. The disease continues to be a problem of *C. arabica* in coffee-growing regions of Ethiopia. However, there is no resistant variety that stands against *F. xylarioides*. There are no curative control methods and current recommendations for control are limited to phyto-sanitary measures against the source and spread of diseased material from infected trees. The fungus life cycle and the disease epidemiology are little understood and this has hampered coffee wilt management.

Biological control

However, to overcome the existing problem of the CWD, there is one possible control, the use of biocontrol agents. Biological control is the strategy for reducing disease incidence or severity by direct or indirect manipulation of antagonists (Papavizas, 1985; Zhang *et al*., 1994; Paullitz and Bekanger, 2000; Tesfaye Alemu and Kapoor, 2004). The result of *in vitro* evaluation observation indicated that the antagonistic effects of some Rhizobacteria against the three *Fusarium* pathogens including *F. oxysporum*, *Fusarium stilboides* and *F. xylarioides* were promising (Diriba Muleta *et al*., 2007). Some antagonists occupy the niches and exclude pathogens from becoming established, thereby protecting plants from infection. Biological control is especially attractive for soil-borne diseases because the pathogens are difficult to reach with specific fungicides (Montealegre *et al*., 2003). A number of *Trichoderma* spp. have promising potential for biological control of plant pathogenic fungi (Lin *et al*., 1994; Huang *et al*., 2000; Janisiewicz and Korsten, 2002; Mendoza Garcia *et al*., 2003; Mazzola, 2004; Garbeva *et al*., 2004; Fravel, 2005). *T. harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* are the most studied of all antagonists for biological control and the most effective in reducing diseases caused by soil-borne plant pathogens (Baker, 1987; Baker and Cook, 1974; Thomashow, 2002; Li *et al*., 2004; Martin and Bull, 2002; Tesfaye Alemu and Kapoor, 2004; 2007). The success of *Trichoderma* spp. as a biocontrol agent is believed to involve various modes of action, including antibiotic production, secretion of lytic-enzymes, mycoparasitism, competition for space and nutrients, and induction of systemic resistance (Cortes *et al*., 1998; Rocco and Perez, 2001). Rapid advances in exploiting the potential of *Trichoderma* and *Gliocladium* species for control of plant disease is evident from the fact that international workshops on

Trichoderma and *Gliocladium* species have been held in USA (1984), UK (1987), Italy (1991) and USA (1995). Currently, more than 13 fungal and 12 bacterial commercial formulations of biocontrol products are available for control of various plant pathogens. Some of such commercial biocontrol product formulations are: F. stop (*T. harzianum)*; Binab T (*T. viride*); Trichodermin (*Trichoderma* spp.); Trichodex (*T. harzianum*); Antagon Tv (*T. viride*); Trichosan (*T. viride*); Antagon combi (*T. viride* + *P. fluorescens*); Gliogard (*Gliocladium virens*); Biocare (*T. viride*); Dagger G (*P. flourescens*); Conquerer (*P. fluorescens*); Galltro (*Agrobacterium radiobacter*); Kodiac (*Bacillus subtilis*); Quantum 4000 (*B. subtilis*) and Mycostop (*Streptomyces griseoviridis*) (Jayarajan, 1996) and Funginil (*Trichoderma* formulation) (Tesfaye Alemu and Kapoor, 2010). When the biocontrol antagonists are found to be potential in laboratory condition, it is necessary to develop easy, economically feasible and environmentally safe substrates for the delivery of biocontrol agents. Application of biological antagonists against coffee wilt disease (*F. xylarioides*) will reduce disease incidence and severity and also increase the yield and quality of coffee beans in the country. *Trichoderma* spp. has been used against the wilt diseases of tomato, melon and cotton, and *Fusarium culmorum* on wheat (Table 2).

Table 2. Biological control of *Fusarium* species by *T. harzianum.*

Pathogen	Crop	Per cent of disease reduction
Fusarium oxysporum f. sp. *vasinfectum*	Cotton	80
Fusarium oxysporum f. sp. *melonis*	Melon	60
Fusarium oxysporum f. sp. *radicis-lycopersici*	Tomato	80
Fusarium culmorum	Wheat	83

Source: Sivan and Chet (1986)

It has been observed that the potential efficacy of *T. harzianum* showed 60 to 83% for the control of *Fusarium* spp. in naturally infected soil in the field condition (Sivan and Chet, 1986). There is no clear information on the mode of action in the studies so far conducted with *Fusarium*. In this regard, Negash Hailu and Tesfaye Alemu (2010) investigated *in vitro* evaluation of biological control agents of fungal antagonists against *F. xylarioides* and found reduction of 66.2% and 70.9% of the mycelial growth of the test pathogen by *T. harzianum* and *T. viride*, respectively. *In vitro* evaluation of antagonistic activity of *T. harzianum* and *T. viride* against *F. xylarioides* isolates have shown a radial growth reduction and inhibition of the test pathogen by 66.2% (Fx.20) and 70.9% (Fx.22) isolates of *F. xylarioides*, respectively. The minimum per cent of mycelial growth inhibition (53.7%) was observed by *T. viride* on isolate Fx.16 (Negash Hailu and Tesfaye

Alemu, 2010). The cell free culture filtrates of *T. harzianum* and *T. viride* having different concentrations (3 ml, 4 ml and 5 ml) also showed the radial growth inhibition of the test pathogen isolates. *T. viride* was found to be more efficient than *T. harzianum*. Similarly, *in vitro* evaluation of the mean of the culture filtrates have shown an average 63.6% of mycelial growth inhibition by *T. viride* over the control against isolate Fx.22 of *F. xylarioides*. With *F. xylarioides* causing CWD a clear evidence of inhibition of the mycelial growth of the pathogen *in vitro* condition was evaluated (Negash Hailu and Tesfaye Alemu, 2010). The study conducted by Addis Ababa University and Jimma Agricultural Research Centre from 2010 to 2012 has indicated that *in vivo* and field application of Trichoderma isolates against *F. xylarioides,* in Jimma zone, coffee-growing woredas have reduced the disease incidence and severity to some extent.

CONCLUSION

This review of the causal agent of coffee wilt disease (*F. xylarioides*) was based on various investigations that were carried out using the morphologically, genetically and biologically distinct forms of the pathogenic characteristics of species and isolates of *Fusarium xylarioides* which were reported and shown to be responsible for the current epidemics across East and Central Africa. CWD caused by *F. xylarioides*, a soil-inhabiting fungus, is endemic in several African countries, affecting commercially important coffee species and causing serious economic losses to coffee producers. The disease symptoms on young and mature *C. arabica*, which is similar to most symptoms of vascular wilt diseases of crops, were similar with that of CWD caused by *G. xylarioides* infection. The characteristic partially wilting symptom accompanied by discoloured internal tissues can effectively facilitate diagnosis and recognition of infected coffee in the field. The control measures being implemented are sensitization of farmers and civic leaders about the disease, urging farmers to cut and burn affected trees *in situ*, restriction on movement of unhulled coffee, a ban on use of coffee husks as mulch material in coffee plantation under new planting and replanting areas of coffee seedlings. There is an urgent need to intensify research in these areas and to identify sources of resistance, which are basic in formulating effective control strategies. The disease has also contributed to a decline in revenue for several African nations due to reduced coffee production and also will threaten the African coffee sector in the future. The disease affects plantations and small holders of Arabica and Robusta coffee although its economic impact is far greater on Robusta coffee. A number of husbandry practices have been shown to

reduce the number of newly-infected coffee trees. Integrated disease management strategies and biological control agents are required to combat the CWD in coffee-producing African countries.

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