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## Coffee Wilt Disease (*Gibberella xylarioides* Heim and Saccas) in Forest Coffee Systems of Southwest and Southeast Ethiopia

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**Abstract:** Coffee diseases are presumed to be less important in the forest coffee as compared to the garden and plantation systems of coffee production in Ethiopia. In this article, the results of a study conducted on the occurrence and incidence of Coffee Wilt Disease (CWD) and the major factors influencing the disease in four major forests coffee sites in southwest and southeast Ethiopia are discussed. In each forest coffee site, coffee wilt syndrome was assessed in three systematically selected sample plots during dry and wet seasons of 2008 and 2009. Concurrently, three to four samples of infected coffee trees were randomly collected from each plot and the causal pathogen was isolated and identified in the laboratory. The result indicated that CWD was prevalent in the four forest coffee sites, with mean incidence of 27.1 and 29.2% in Harenna during 2008 and 2009 wet seasons, respectively, followed by Berhane-Kontir with mean incidences of 22.1 (2008) and 27.7% (2009). Whereas, Bonga and Yayu forest coffees had comparatively low wilt severity (<10%). The wood samples of most of the infected coffee trees (90.6%) yielded *Gibberella xylarioides* in the laboratory proving that this pathogen is the main cause of coffee tree death in the forest. The difference in incidence of CWD across the four sites and among fields was strongly associated with human factors and variations in coffee populations. The forest coffee trees in Harenna and Berhane-Kontir (high CWD) are almost transformed to semiforest type by sub-planting coffee seedlings after thinning the dense vegetation cover. These activities are known to create wound to the host and disseminate the fungus spores from tree to tree and from one field to the other. The two independent seedling inoculation tests in the greenhouse evidenced that there were significant variations among coffee accessions in reactions to CWD though most accessions were susceptible. The study showed that CWD is one of the potential biotic factors threatening the genetic diversity of Arabica coffee in most forest coffee sites and thus the disease management practices should duly be considered in planning and implementing forest coffee conservation strategy.

**Key words:** *Coffea arabica*, coffee wilt disease, Ethiopia, forest coffee system, *Gibberella xylarioides*

### INTRODUCTION

Arabica coffee (*Coffea arabica* L.) has its centre of origin in the highlands of southwest and southeast Ethiopia where 'wild' coffee trees still grow naturally in the understory of the fragmented montane rainforests. Among the four coffee production systems known in the country, forest and semiforest coffees are believed to possess the largest coffee genetic resources (gene pool) followed by the 'landraces' with enormous potential to improve the crop (Sylvain, 1958; Meyer, 1965; Van der Graaff, 1981; Mesfin, 1991; Paulos and Demel, 2000; Gole *et al.*, 2002; Tesfaye, 2006; Tadesse *et al.*, 2008).

Nevertheless, settlement and agricultural land-use pressure have been persistently reducing the remaining

forest fragments inhabiting wild coffee populations and other invaluable biodiversity. Poverty and conflicting property rights make farmers convert forests into agricultural or pastoral land, thereby threatening the entire biodiversity of the forests. Consequently, coffee genetic erosion has gone far beyond the point of no return (Mesfin, 1991; Paulos and Demel, 2000; Tadesse *et al.*, 2008). Apart from threats posed by biological and ecological processes, the impact of insect pests and diseases on the forest coffee populations are little understood except presumption from limited observations that they are less important in the forests.

There are, however, many research findings documented on diseases and insect pest situations in semiforest, garden and plantation coffee production

systems, which originates from the 'wild' forests in Ethiopia (Van der Graaff, 1981; Merdassa, 1986; Eshetu *et al.*, 2000; Girma *et al.*, 2009a). Coffee wilt is one of the three economically important diseases dramatically limiting coffee production in the country. It is caused by a fungal pathogen commonly known by its teleomorphic name *Gibberella xylarioides* Heim and Saccas (*Fusarium xylarioides* Steyaert) that totally kills coffee plant at any growth stage in all production systems. The disease is more prevalent in plantation and garden coffee than semiforest coffee (Girma *et al.*, 2001; Girma *et al.*, 2009a). The disease incidence ranged from 3.6 to 15.5% in semiforest coffee of southwest coffee-producing areas, while about 18.6 and 25.4% was estimated in some garden coffee fields in the southern region (CAB International, 2003; Girma, 2004). In large-scale plantation, the mean incidence varied from 45% at Gera to about 69% at Bebeke and it is more serious in small-scale farmers' coffee plantations with mean incidence ranging from 21.7 to 77% (Girma *et al.*, 2009a). According to CAB International (2003), coffee production (yield) at the farm level decreased by 37% and this led to a decline in income of 67%. The national incidence and severity of CWD in Ethiopia were 27.9 and 5%, respectively, with estimated monetary loss of more than 3.8 million US\$ annually (CAB International, 2003; Girma *et al.*, 2009a).

The occurrence of CWD in the forest coffee systems was first noted by Arega (2006) with average tree death of 16.9%. This survey work was not, however, accompanied by disease sample collection, isolation and identification of the causal agents involved in the coffee tree death complexes including multitude of biotic and abiotic factors. Thus, the incidence and distribution of coffee wilt syndrome and its causal pathogen along with examining the important factors influencing the disease progress thereby impacting the forest coffee genetic resources in southwest and southeast Ethiopia is presented in this article.

## MATERIALS AND METHODS

**Descriptions of the study sites:** The study was conducted in the field, in the laboratory and in the greenhouse between the period 2008 and 2010. Coffee wilt disease surveys were carried out in sample fields at four rainforest coffee sites of Bonga, Berhane-Kontir and Yayu in the southwest and Harenna in the southeast Ethiopia. The laboratory and greenhouse studies were undertaken in Plant Pathology laboratory at Jimma Agricultural Research Center (JARC) situated 12 km away from Jimma town. Bonga forest coffee is found in Gimbo district of the Kafa zone while Berhane-Kontir forest coffee is located in

Sheko district of Bench Maji zone in Southern Nations Nationalities and Peoples' Regional state. Yayu forest coffee represents the major part of Geba-Dogi forest sites delineated in Yayu district of Illubabor zone. Harenna forest coffee is the major part of most eastern Afromontane rainforests that also constitutes the largest subsection of the Bale Mountains National Park in southeastern Ethiopia (Feyera, 2006). The detailed agroecological conditions of the four forest coffee sites and that of Jimma are well illustrated by Kufa and Burkhardt (2011a, b).

**Disease assessment and sample collection:** Three representative sample fields (20×20 m area/field) were randomly selected for coffee wilt disease (CWD) assessment and disease specimen collection in the four forest coffee sites. The disease assessment was conducted by diagnosing wilting/dying coffee trees for CWD, Armillaria root rot and other agents based on external and internal symptoms of the respective diseases and signs of the causal pathogens such as stromata and rhizomorph structures (Girma *et al.*, 2001; Girma, 2004; Girma *et al.*, 2009a). Finally the number of wilted/dead and healthy coffee trees were counted and recorded according to the observed symptom categories.

The disease distribution pattern and foci development in the forests was thoroughly studied in relation to topography, slope/gradient and above all the nature of the forests (intact or disturbed) based on the degree of human interferences measured by management activities such as thinning forest vegetation, weeding and sub-planting coffee seedlings. At same time, three stem pieces (20 cm long) were collected from three to four samples of infected coffee trees with wilting or die-back symptoms in each field, and kept in perforated plastic bags labeled with name of locality, sample number and collection date. The wood samples were transported to JARC Plant Pathology laboratory and maintained at 4°C until isolation (Girma and Mengistu, 2000; Girma, 2004). The disease survey was conducted twice, the first one was in January 2008 representing dry season soon after harvesting and the second was during the raining season in August 2009.

**Isolation and identification of causal pathogens in the laboratory:** The disease causal pathogen was isolated and identified according to the standard laboratory procedures (Booth, 1971; Girma and Mengistu, 2000; Girma, 2004; Rutherford *et al.*, 2009). Four to five wood pieces (1 cm) were excised using scalpel after gently removing the bark from each of the samples and disinfected in plastic petri dish with 10% Clorox

(NaHCO<sub>3</sub>). The disinfected sections were plated on potato sucrose agar (PSA) and incubated for 5 to 7 days. The emerging colony out of the plated pieces were purified by hyphal tip method and sub-cultured on PSA and then incubated for 10 to 14 days under 12 h light and dark cycle. The cultural and morphological characteristics of the pure cultures were used to identify *Gibberella xylarioides* and other *Fusarium* spp. as described by Booth (1971), Girma and Mengistu (2000) and Rutherford *et al.* (2009) and in reference to the earlier *Fusarium* collections preserved in the laboratory at JARC.

**Seedling inoculation tests of coffee accessions collected in the forest sites:** There were two sets of seedling inoculation experiments conducted in the greenhouse to determine the diversity of forest coffee populations in reactions to the pathogen isolate following the recommended technique by Girma *et al.* (2009b). In set I experiment, the inoculation test was performed on 60 coffee accessions originated from three different fields (15 accessions/field) representing the four forests coffee sites (Bonga, Berhane-Kontir, Yayu and Harenna) and properly maintained at JARC for various research purposes. The second set consisted of 20 coffee accessions randomly collected from fruit bearing coffee trees in all forest sites except Berhane-Kontir in November 2008.

**Seed preparation and raising coffee seedlings:** Coffee seeds were prepared from each accession separately following the routine practices that fully ripe red cherries were picked, hand pulped and dried under lathouse shade. Seedlings were raised by sowing the coffee seeds in sterilized and moistened sandy soil in plastic pot with 5.8 L volume (20-25 seeds/pot and 3 pots/accession) after removing the parchment and soaking overnight. Coffee cultivars with known ranges of resistance to CWD (resistant, moderate and susceptible) were included as checks in each set of the experiments (Girma *et al.*, 2009b).

**Inoculum multiplication and inoculation of coffee seedlings:** Two *Gibberella xylarioides* isolates namely 'Gx2' and 'Y-21' were deliberately (known for pathogenicity) selected for inoculation tests in set I and set II experiments, respectively. The former isolate represented large *Fusarium* collections in plantation coffee while the latter was collected in Yayu forest coffee. At cotyledon stage of the seedlings (2 month after sowing), inocula of the two isolates were separately multiplied on sterile coffee twigs placed in test tubes. After 14 days incubation, conidia were harvested by scratching and rinsing from the branches with distilled sterile water and the concentration of spore suspension

was counted with haemocytometer and then adjusted to  $2.0 \times 10^6$  conidia mL<sup>-1</sup> (Girma and Mengistu, 2000; Girma *et al.*, 2009b). The seedlings of each coffee accession were subsequently inoculated with viable conidial suspension by stem nicking technique described by Pieters and van der Graaff, (1980) and Girma *et al.* (2009b). All the treated plants were then placed on experimental benches and immediately covered with plastic sheet in a growth room with high humidity and temperature of about 23°C to favour infection (Girma *et al.*, 2009b). After 10 days, the inoculated seedlings were transferred into greenhouse and the treatments were arranged in Randomized Complete Block Design (RCBD) with three replications (pots) having 20 inoculated seedlings per pot.

**Data collection and statistical analysis:** Based on the typical wilting symptoms and death of the seedlings, the number of wilted/dead and healthy seedlings were counted and recorded per pot every two weeks for six months starting a month after inoculation. Isolation from samples of inoculated seedlings was made when necessary. The percentages of dead seedlings were computed from the cumulative number of dead seedlings (during the 6 months period) over the total number of inoculated seedlings.

Similarly, the percentages of CWD infected coffee trees were calculated for the disease assessment in the fields, while proportions of isolation was calculated from the total number of collected and plated specimens from each forest coffee site. The percentage data sets were transformed to angular values before statistical analysis with SAS system for windows (9.2 version) (SAS, 2008). Treatment means are compared and separated based on LSD values when F-test showed significance.

## RESULTS

**Distributions of coffee wilt disease in forest coffee systems:** Coffee wilt disease was prevalent in forest coffee systems in the southwest and the southeast Ethiopia. The disease incidence varied from field to field and from one survey area to the other. During the dry season of 2008, the incidence ranged respectively from 0 to 15.4%, 7.3 to 37.6%, 0 to 22.3%, and 26.4 to 28.3% in Bonga, Berhane-Kontir, Yayu and Harenna (Fig. 1). During the wet season of 2009, the average disease incidence was 11.9, 29.2, 13.2 and 27.7% at the respective forest sites (Fig. 2).

**Isolation and identification of the fungus from collected samples:** *Gibberella xylarioides* was the predominant

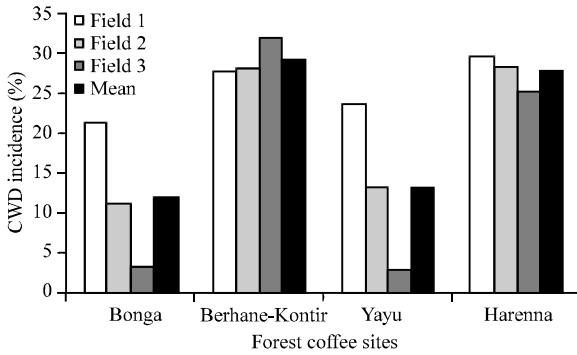


Fig. 1: Incidence of coffee wilt disease (CWD) in different fields of four forest coffee sites of southwest and southeast Ethiopia in dry season, 2008

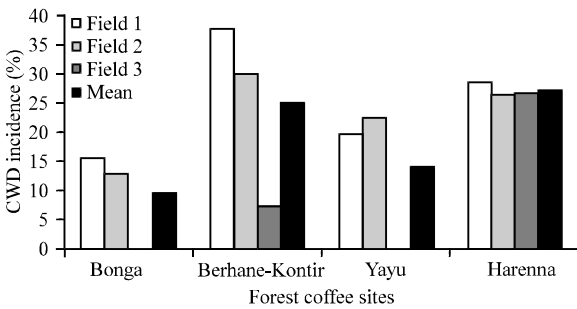


Fig. 2: Incidence of coffee wilt disease (CWD) in different fields of the forest coffee sites of southwest and southeast Ethiopia in wet season, 2009

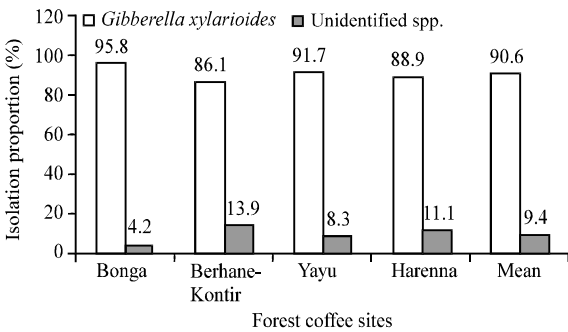


Fig. 3: Proportions (%) of *Gibberella xyloarioides* isolates and other unidentified spp. from wood samples of infected coffee trees collected in southwest and southeast forest coffee sites of Ethiopia

pathogen isolates (90.6%) identified from randomly collected samples of infected coffee trees in the four forest coffee sites of Bonga, Berhane-Kontir and Yayu in southwest and Harena in southeast Ethiopia. The remaining 9.4% of the samples produced unidentified microorganisms including other *Fusarium* spp. (Fig. 3).

Table 1: Reactions of forest coffee accessions to *Gibberella xyloarioides* 'Gx2' isolate in seedling inoculation test under greenhouse conditions at Jimma Research Centre (set I experiment)

Coffee accession*	Mean seedling death (%)		
	Mean seedling death (%)	Mean seedling death (%)	
P111	0.0 <sup>f</sup>	P313	79.4 <sup>g</sup>
P112	14.9 <sup>g</sup>	P314	78.2 <sup>h</sup>
P113	1.4 <sup>f</sup>	P315	95.6 <sup>g</sup>
P114	0.0 <sup>f</sup>	P321	98.3 <sup>h</sup>
P115	2.1 <sup>f</sup>	P322	100.0 <sup>g</sup>
P121	1.7 <sup>f</sup>	P323	98.6 <sup>h</sup>
P122	18.4 <sup>g</sup>	P324	91.9 <sup>g</sup>
P123	25.7 <sup>h</sup>	P325	92.6 <sup>g</sup>
P124	2.7 <sup>f</sup>	P331	94.4 <sup>g</sup>
P125	0.0 <sup>f</sup>	P332	98.9 <sup>g</sup>
P131	0.0 <sup>f</sup>	P333	83.8 <sup>g</sup>
P132	0.0 <sup>f</sup>	P334	98.6 <sup>h</sup>
P133	4.0 <sup>f</sup>	P335	63.1 <sup>f</sup>
P134	0.0 <sup>f</sup>	P411	94.9 <sup>g</sup>
P135	2.3 <sup>f</sup>	P412	81.6 <sup>g</sup>
P211	91.5 <sup>g</sup>	P413	94.5 <sup>g</sup>
P212	94.9 <sup>g</sup>	P414	56.8 <sup>k</sup>
P213	97.1 <sup>g</sup>	P415	98.5 <sup>h</sup>
P214	72.1 <sup>d</sup>	P421	89.7 <sup>g</sup>
P215	89.7 <sup>g</sup>	P422	97.5 <sup>g</sup>
P221	67.5 <sup>e</sup>	P423	96.3 <sup>g</sup>
P222	60.6 <sup>g</sup>	P424	100.0 <sup>g</sup>
P223	52.8 <sup>g</sup>	P425	96.2 <sup>g</sup>
P224	62.6 <sup>f</sup>	P431	36.2 <sup>b</sup>
P225	42.8 <sup>h</sup>	P432	98.6 <sup>h</sup>
P231	69.5 <sup>e</sup>	P433	92.9 <sup>g</sup>
P232	41.4 <sup>h</sup>	P434	100.0 <sup>g</sup>
P233	97.2 <sup>g</sup>	P435	90.9 <sup>g</sup>
P234	94.2 <sup>g</sup>	SN5 <sup>1</sup>	58.9 <sup>h</sup>
P235	92.6 <sup>g</sup>	7440 <sup>2</sup>	31.3 <sup>g</sup>
P311	93.7 <sup>g</sup>	Catimor J-19 <sup>3</sup>	2.9 <sup>f</sup>
P312	85.7 <sup>g</sup>	Catimor J-21 <sup>3</sup>	10.5 <sup>g</sup>
Mean	62.0		
CV (%)	19.3		
LSD value (p<0.05)	13.3		

<sup>1</sup>Susceptible, <sup>2</sup>Moderately resistant and <sup>3</sup>Resistant Arabica coffee varieties to CWD in seedling tests (Girma *et al.*, 2009b) used as check, \*Coffee accessions coded as P111-P135, P211-P235, P311-P335, P411-P435 were respectively collected from Harena, Bonga, Berhane-Kontir and Yayu forest coffee sites. Means with the same letter(s) are not significantly (p<0.05) different from each other

Isolation and identification results demonstrated that the highest proportion of coffee tree deaths are largely caused by coffee wilt disease infected by *Gibberella xyloarioides* proving that this disease is significantly threatening the forest coffee populations. There were, however, some died coffee trees caused by *Armillaria* root rot as the infected trees easily toppled down and the dark rhizomorphs of *Armillaria mellea* were detected in the roots. Also few coffee trees exhibited neither symptom of infection nor signs of the casual agents rather seem physiological dieback.

**Seedling inoculation tests of forest coffee accessions:**

There were significant (p<0.01) differences among the tested coffee accessions collected in the forest sites in

Table 2: Reactions of randomly collected forest coffee accessions to *Gibberella xylarioides* 'Y-21' isolate in seedling inoculation test under greenhouse conditions at Jimma Research Centre (set II experiment)

Forest coffee accessions*	Mean seedling death (%)	Incubation period (mean No. of days)
HA1	10.9 <sup>a</sup>	80.0 <sup>ab</sup>
HA2	0.0 <sup>f</sup>	- <sup>++</sup>
HA3	90.0 <sup>e</sup>	118.0 <sup>e</sup>
HA4	90.0 <sup>e</sup>	110.0 <sup>e</sup>
HA5	31.7 <sup>c</sup>	94.7 <sup>bc</sup>
HA6	0.0 <sup>f</sup>	-
HA7	21.8 <sup>d</sup>	84.0 <sup>ab</sup>
HA8	0.0 <sup>f</sup>	-
BO1	90.0 <sup>e</sup>	118.0 <sup>d</sup>
BO2	90.0 <sup>e</sup>	118.0 <sup>d</sup>
BO3	30.2 <sup>c</sup>	84.7
BO4	20.6 <sup>d</sup>	82.7 <sup>b</sup>
YA1	90.0 <sup>d</sup>	05.3 <sup>a</sup>
YA2	90.0 <sup>e</sup>	100.0 <sup>e</sup>
SN-5 <sup>1</sup>	66.6 <sup>b</sup>	99.3 <sup>ab</sup>
Catimor J-21 <sup>2</sup>	22.1 <sup>d</sup>	94.7 <sup>bc</sup>
Mean	47.1	80.6
LSD Value (p<0.01)	5.8	9.1
CV (%)	7.4	6.7

<sup>1</sup>Susceptible and <sup>2</sup>Resistant (Girma *et al.* 2009b), \*Coffee accessions coded as HA1-HA8, BO1-BO4 and YA1-YA2 were respectively collected from Harenna, Bonga and Yayu forest coffee sites ++ and 0.0 indicates no external symptom was observed on these accessions and thus no incubation period was detected, respectively. Means with the same letter(s) are not significantly (p<0.01) different from each other

seedling inoculation experiments. In set I experiment that consisted of 60 coffee accessions originated from the forest, seedlings of almost all of the Harenna accessions (P111-P135) showed the lowest infection by *Gibberella xylarioides* isolate 'Gx2' with less than 2% seedlings death that was not significantly (p<0.05) different from the two resistant checks 'Catimor J-19' and 'Catimor J-21' (Table 1). Among the tested 15 coffee accessions P111, P114, P125, P131, P132 and P134 have remarkably exhibited no wilting symptom. On the contrary, most coffee accessions obtained from Bonga (P211-P235), Berhane-Kontir (P311-P335) and Yayu (P411-P435) showed significantly (p<0.05) higher seedling deaths of about 80 percent as compared to the standard susceptible control 'SN-5' (Table 1).

In set II experiment, coffee accessions randomly collected in the fields of three forest areas and inoculated with the isolate from Yayu forest 'Y-21' showed highly significant (p<0.01) differences both in percent seedling death (wilt) and incubation period. Similar to the result of set I experiment, half of the Harenna coffee accessions HA1, HA2, HA6 and HA8 had very low seedling deaths ranging from 0 to 10 percent with longer incubation periods. While significantly (p<0.01) higher seedling death of 90 percent was recorded on coffee accessions from Bonga (BO1, BO2), Yayu (YA1, YA2) and Harenna (HA3, HA4) than the susceptible check 'SN-5' (Table 2). The results of both sets of inoculation experiments

implied that most coffee trees in the forest except those from Harenna site are highly susceptible to coffee wilt pathogen infection.

## DISCUSSION

Coffee wilt is a systemic vascular disease caused by a fungal pathogen commonly referred by its teleomorphic nomenclature *Gibberella xylarioides* Heim and Saccus. The disease attacks all commercial *Coffea* spp. including *Coffea arabica* and *Coffea canephora* at any growth stage. Although, it is currently restricted to coffee producing countries in East and Central Africa, the disease can be a potential threat to the world coffee production (Rutherford, 2006; Girma *et al.*, 2009b). It is one of the major factors constraining coffee production with rapid prevalence in plantation, garden and semiforest coffee production systems in Ethiopia (Girma *et al.*, 2009a).

In addition, the present study evidenced that coffee wilt disease is causing significant losses to coffee trees in the forest coffee systems inhabiting invaluable gene pools of *Coffea arabica*. The highest mean incidence of 27.1% was recorded at Harenna forest coffee in the southeast followed by Berhane-Kontir with 24.9% in southwest Ethiopia during the dry season of 2008. In the following season, it was more pronounced at Berhane-Kontir (29.2%) albeit similar trend was observed in Harenna forest. Some three years back, the mean percent coffee tree death estimated by Arega (2006) was about 2.4% at Berhane-Kontir while the highest was 17% at Yayu coffee areas. The overall comparison in CWD progress over the years implicates that the disease pressure is rapidly increasing in the forest coffee systems across all sites, although, slight increment of about two to five percent was recorded during the subsequent wet season. A remarkable increase in CWD severity of about 11.5% was estimated over a 6-month period in nine districts of Gedeo and Sidama zones of Ethiopia (CAB International, 2003; Girma *et al.*, 2009a) which are largely characterized by producing world renowned fine Arabica coffee ('Yirgacheffe' and 'Sidamo' speciality) in the garden coffee production system.

The spatial distribution of coffee wilt epidemics was found to decrease from the more disturbed peripheral parts towards the centers of the intact (undisturbed) forest coffee systems except in the fields at Harenna and Berhane-Kontir sites. The disease occurrence dropped from 15.4 to 0.0 and from 19.6 to 0.0% across the fields (from field 1 to field 3) at Bonga and Yayu forests, respectively (Fig. 1). These differences in progression over seasons (years) and spaces could be ascribed primarily to human factors (degree of human exploitation

of the forest coffee) in addition to the variations in coffee populations and may be to the fungus strains. The coffee trees at Berhane-Kontir and Harenna forests, where CWD incidences were high and epidemics were fairly uniform throughout the fields, are almost transformed to semiforest type as farmers have been intensively exploiting the so called 'wild' coffee. It was observed that, in order to harvest good yield and maximize income, the dense tree stands of upper forest strata have been thinned out, bushes and shrubs were removed and then transplanted with self-raised or naturally regenerated coffee seedlings from year to year. Feyera (2006) evidenced that the difference between managed (semiforest) and undisturbed forest was minimal as the number of plant species declined by 50% and the only dominant stand was coffee in Yayu, Harenna and Berhane-Kontir areas. The author found that grazing animals, frequent weeding (2 to 4 times per year) and clearing all the herbaceous vegetation were the common activities from year to year (Feyera, 2006). These activities are known to facilitate dissemination of the fungus ascospores and conidia within and across the fields and create wounds to the host thereby favoring coffee wilt development and prevalence (Girma *et al.*, 2001; Girma *et al.*, 2009a; Phiri *et al.*, 2009; Rutherford *et al.*, 2009).

On the other hand, besides the non human impacts in the undisturbed forests, the low incidence might be due to the suppression of the fungal pathogen by the natural communities of bacteria and fungal species. Mekete *et al.* (2008) recorded a large number of endophytic bacteria (201) and fungi (128) including non-pathogenic *Fusarium* and *Trichoderma spp.* in forest coffee agroecologies of Ethiopia. Besides, the antagonistic potential of 21 bacterial isolates that significantly inhibited the mycelial spread of *Gibberella xylarioides in vitro* was reported by Muleta *et al.* (2007).

The results of greenhouse inoculation experiments proved that there is important diversity in coffee populations (within and among the forest sites) in reaction to *G. xylarioides* infection. However, there is a tendency towards occurrence of higher frequency of susceptibility reactions except in Harenna coffee populations that consistently revealed higher level of resistance in both sets of experiments, even despite the fact that relatively more CWD incidence recorded in the fields. Arega (2006) also reported similar findings that 50% (five out of ten) of Harenna coffee collections showed less than 15% seedling deaths, as opposed to coffee accessions collected in Bonga (16), Berhane-Kontir (20) and Yayu (20) forest areas which showed more than 85%

average infections. The inconsistent response of Harenna coffee populations in the field and the greenhouse conditions could perhaps be the difference in aggressiveness of the pathogen isolates used in the seedling inoculation tests, as they were originated from southwest Ethiopia. This in turn warrants studying host pathogen interactions among coffee accessions and the pathogen strains representing forest coffee systems, as variations in aggressiveness among the pathogen populations was already reported by a number of workers (Adugna *et al.*, 2005; Girma *et al.*, 2009b; Rutherford *et al.*, 2009).

In conclusion, the present assessment supported by detailed diagnosis coupled with frequent isolation and identification of the causal pathogen *Gibberella xylarioides*, coffee wilt disease is proved to be prevalent in the forest coffee systems in southwest and southeast Ethiopia. The disease incidence, although varying from field to field and from one forest coffee site to the other, has been increasing spatially and temporally thereby becoming one of the major factors threatening forest coffee genetic resources. It is decimating those coffee trees that perhaps possessing resistances to other diseases, insect pests and nematodes; with good yield and quality attributes like low caffeine content. Thus, the forest coffee conservation strategies should take the disease into account and apply the recommended principles and practices of CWD management (Girma *et al.*, 2009a; Negussie *et al.*, 2009; Phiri *et al.*, 2009). Development of CWD resistant Arabica coffee varieties through large-scale collection and screening against the pathogen would be inevitable, although it seems that most coffee trees exhibit susceptibility in the forest coffee populations.

*Gibberella xylarioides* is isolated and identified from infected coffee wood samples collected in the intact and undisturbed forest sites. This finding provides additional insight that the Arabica isolates of the pathogen is independently descended/evolved population on its hosts. However, this requires further survey and many more isolate collections (exploring in other intact coffee forests) with subsequent characterization and analysis of the population structure employing the recent molecular approaches hand-in-hand with host-pathogen interactions.

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