Cultural Characteristics and Pathogenicity of *Gibberella xylarioides* Isolates in Coffee

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Abstract

The cultural variability in *Gibberella xylarioides (Fusarium xylarioides),* the cause of coffee wilt disease (tracheomycosis), was studied under standard conditions using 36 isolates collected from Arabica coffee trees in south-western Ethiopia. The isolates ranged from appressed and sparse to raised and dense in apical colony growth, but most isolates (80%) had the intermediate (slightly raised and slightly dense) colony type. The fungus isolates had generally white mycelial color although they diffused varied pigments into the media after 10 days of incubation. The typical pigments produced by *G. xylarioides* were grayish white, purplish white, light bluish and light violets. The mean radial growth rate was 3.1±0.3 cm after 7 days of incubation. The isolates varied in growth rate and those isolates from Bebeka were slower type. Inoculating seedlings of nine coffee cultivars in the greenhouse tested the pathogenic variability of four representative isolates. Highly significant difference among coffee cultivars, and among the fungus isolates; and a significant cultivar x isolate interaction, was observed both in percent dead seedlings and in length of incubation periods. Thus, the results confirmed the existence of variations both in resistance level of the host and in aggressiveness of the pathogen population. Although the resistance is predominantly horizontal, the significant cultivar-by-isolate interaction (differential effect) indicated the presence of some vertical resistance in Arabica coffee genotypes. The variation among *G. xylarioides* isolates in cultural characteristics, such as pigmentation and growth rate, coupled with variation in pathogenicity (virulence) suggests that the fungus may occur as multiple strains.

Introduction

Gibberella xylarioides Heim & Saccas, a teleomorphic state of *Fusarium xylarioides* Steyeart, is a specific coffee pathogen causing an extensive necrosis of the vascular tissue leading to wilting and death of infected plants. The syndrome is a typical vascular disease commonly called tracheomycosis or sometimes known as carbuncullariosis. The fungus was earlier reported to be a well-known pathogen of other *Coffea* species in West and Central Africa. *Coffea excelsa* (Excelsa coffee) plantations were seriously attacked in the Central African Republic and Cameroon; and a number of *C. canephora* (Robusta coffee) varieties were decimated in Ivory Coast and in Zaire by this disease in the 1950s (Booth 1971, Wrigley 1988, Coste 1992). The disease was observed again in Zaire in the early 1980s (Flood 1996) and noticed for the first time in Uganda in 1993, it is now causing economic losses to Robusta coffee in both countries (Flood and Brayford 1997, Lukwago and Birikunzira 1997).

In Ethiopia, the occurrence of *Gibberella xylarioides* on *C. arabica* was established in the early 1970s by Kranz and Mogk (1973) after isolating and identifying the fungus from dying coffee trees. Survey works conducted so far in the major coffee growing areas have demonstrated that vascular wilt is becoming the main factor of coffee tree death in the country (Van der Graaff and Pieters 1978, Eshetu 1995). In spite of the important damage it causes to coffee production in the country, there has been no detailed investigation carried out on the disease particularly on the biology of the pathogen. The existence of resistant varieties has been demonstrated in *C. excelsa* and *C. canephora* (Booth 1971) reducing coffee wilt disease successfully to a minor problem in Zaire and Ivory Coast during the 1950s. This resistance had, however, remained effective only until 1986 in Zaire and the reappearance of the disease has been perhaps attributed, above all, to a new and aggressive strain of the fungus (Flood 1996, Flood and Brayford 1997). Van der Graaff and Pieters (1978), and Pieters and Van der Graaff (1980) have also reported varietal differences in susceptibility to the disease in Arabica coffee collections at Jimma. Nevertheless, from the experience in Zaire, development and use of resistant varieties require exploration and a thorough understanding of the variability of the pathogen well in advance of selecting resistant lines.

Thus, the main objective of this work was to study the variation in cultural characteristics and pathogenicity of *G. xylarioides (F. xylarioides)* isolates collected from Arabica coffee trees in south-western Ethiopia.

Materials and Methods

Cultural and morphological characteristics

The macroscopic and microscopic features of representative *G. xylarioides* isolates, selected from a large collections obtained from 12 fields in different localities of south-western Ethiopia, were considered to determine ranges of cultural and morphological variation.

Cultural appearances

Thirty six monoconidial isolates of the fungus, each replicated twice, were plated on potato sucrose agar (PSA) amended with antibiotics. The cultures were incubated in a growth chamber at 25 $\pm$ 2 °C in 10 - 12 hr dark/fluorescent light cycle (Booth 1971). The cultural features included aerial mycelial growth that was rated as raised, slightly raised and appressed; and colony density scored as dense, slightly dense and sparse (Nelson *et al.* 1983). Radial colony growth rates of each isolate was estimated from colony diameter measurement (cm) taken at two perpendicular planes on reverse side of the plate. The growing mycelial color on obverse side and type of pigment diffused into the medium, on the reverse side of each plate, was described in reference to standard color chart (Kornerup and Wanscher 1967). These qualitative and quantitative parameters were recorded at 4, 7, 10, and 14 days interval of incubation periods.

Morphological characteristics

Ten isolates were used to study shape and size of conidia. The culture of each isolate, grown in plates for 10 days as described before, was flooded with 10 ml of sterile distilled water and then rubbed gently from the agar surface to free the conidia. The spore suspension was uniformly stirred and then filtered into another sterile beaker through double layers of cheese clothes. The characteristic shapes of the fungus conidia (macro- and microconidia) were described and frequency of various shapes of the population was determined. The size (length and width) of conidia were also measured (µm) with ocular micrometer fitted into 10x eye pieces and 40x objective of binocular compound microscope. Average and ranges of sizes of 150 conidia were calculated per isolate.

Pathogenic variations

The pathogenic variability in the fungus population was studied by inoculating four representative *G. xylarioides* isolates on seedlings of nine Arabica coffee cultivars observed to possess various levels of resistance to the wilt disease under field conditions (Table 1). The four typical isolates, namely, Gx12, Gx26, Gx31 and Gx43 were selected from Bebeka, Teppi, Jimma and Gera, respectively; each representing the most frequent (95 %) isolates group in a locality.

Seedlings of each coffee cultivar were raised in heat sterilized sandy soil filled in disinfected plastic boxes (25 cm x 18 cm x 7 cm). Sterile water was applied as needed to maintain optimum moisture for seed germination, emergence and growth of seedlings in the greenhouse. When the seedlings were at two fully expanded cotyledon stage (about 75 days old), the inoculum of each of the selected isolate was reproduced, from the preserved stock culture on sterile sand, on sterilized coffee twigs by adapting methods of Pieters and Van der Graaff (1980) with minor modification. After 10 days, inoculum suspension was prepared by thoroughly rinsing-off the twigs having good colony growth with distilled sterile water in a disinfected beaker. The spore concentrations of each isolate was determined using haemocytometer and then adjusted to about 2.3×10^6 per milliliter.

Table 1. Performance of Arabica coffee cultivars selected for studying host-pathogen interaction with *G. xylarioides* isolates.

Cultivar	Performance	Reaction *	Reference**
74165	CBD resistant	HR	1
7440	CBD resistant	MR	1
74304	CBD susceptible	S	1
F-17	High yielder	HR	2
F-61	High yielder	MR	2
SN-5	High yielder	S	2
35/85	stress tolerant	HR	2
24/85	stress tolerant	MR	2
61/85	stress tolerant	S	2

**Reaction to coffee wilt disease under field conditions; HR = highly resistant, MR = moderately resistant, S = susceptible.*
***References for the reaction*
1. Van der Graaff 1979, Pieters and Van der Graaff 1980. 2 Girma Adugna 1997.

The coffee seedlings were inoculated with viable conidia of each isolate following Pieters and Van der Graaff (1980) stem nicking procedures. A sterile scalpel was first immersed into the suspension and then the stem of each seedling was nicked at about 2-cm height in such a way that a drop (a milliliter) of the conidial suspension could have remained in the notch. Seedlings (30 per box) of all cultivars were independently treated with the four isolates and a sterile water as a check. In order to create more humid conditions for infection, all treated plants were immediately covered with transparent plastic sheet (2 m x 10 m) and maintained in a cool place for 10 days. They were then transferred and arranged on greenhouse benches. The experiment was laid out in a split-plot design with 9 x 5 factorial treatment combinations of coffee cultivars as main plot and the four isolates and a control as subplot factor in two blocks.

Data collection and analysis

The number of healthy and wilting seedlings (based on external symptom) was identified and recorded fortnightly for 210 days starting from 30 days after inoculation. Incubation periods, and percentage of dead seedlings were computed from the cumulative number of wilted seedlings 195 days after inoculation over the total number of originally inoculated seedlings and transformed to angular values (arcsine $\sqrt{\%}$). MSTAT-C microcomputer statistical package was used to analyze the data.

Results

Cultural appearances

The culture of 36 isolates were categorized into three classes based on the nature of colony growth: appressed (flat) and sparse, slightly raised and slightly dense (intermediate), and raised and

dense; but 80 percent of the isolates belonged to intermediate type. The average colony growth rates of the fungus isolates were 1.6, 3.1, 4.4, and 6.0 cm after 4, 7, 10, and 14 days of incubation period, respectively (Table 2). The isolates obtained from Bebeka showed slower growth rate than the isolates from other localities.

The mycelial color of the isolates generally belonged to the white color group (white to grayish white), and on the reverse side, the fungus produced varied pigments in the agar. Grayish white (with or without bluish spots around the center), purplish white, light violet and light bluish colors were commonly observed after 10 days of plating (Table 3). The color of young colony (4 days) on both sides of the plate is white. The fungus begins to diffuse some pigments into the agar after 7 days and typical colors of the fungus develop between 10 and 14 days.

Table 2. Radial growth rate (means and standard deviations in centimeter) of *G. xylarioides* isolates grown on PSA in 10-12 hr light/dark cycle at 25 ± 2 °C at 4 incubation periods.

Localities	Incubation periods (days)*			
	4	7	10	14
Bebeka	1.3 ± 0.1	2.7 ± 0.2	4.0 ± 0.3	5.6 ± 0.6
Teppi	1.6 ± 0.2	3.2 ± 0.1	4.4 ± 0.1	6.0 ± 0.1
Jimma	1.6 ± 0.1	3.3 ± 0.2	4.6 ± 0.3	6.1 ± 0.4
Gera	1.7 ± 0.1	3.3 ± 0.3	4.7 ± 0.4	6.3 ± 0.6
Mean	1.6 ± 0.1	3.1 ± 0.3	4.4 ± 0.3	6.0 ± 0.3

** NS = not significant*

Table 3. Colony colors produced by *G. xylarioides* isolates after 10 days of incubation period on PSA in 10 - 12 hr light/dark cycle at 25 ± 2 °C.

Color	Color	Proportions (%)	
Notation*	(Pigmentation)	Obverse side (mycelia)	reverse side (diffusates)
A 1	White	20. 0	-
B 1	Grayish white	80. 0	10. 3
2 A 2	Pale	-	3. 3
14 A 2	Purplish white	-	23 7
17 A 5	Light violet	-	36. 7
21 A 2	Bluish white	-	10. 3
21 A 5	Light blue	-	16.7

** Color notation according to Kornerup and Wanscher 1967.*

Shape and size of conidia

The *Fusarium* state (imperfect state) of *G. xylarioides* has two types of conidia, macroconidia and microconidia, which are normally variable in shape and size even in a culture originated from a single conidia. Some of the macroconidia are distinctively curved while others are falcate or fusoid and all with hooked ends. In this case the curved and falcate-shaped macroconidia dominated the conidia population of the isolates (Table 4).

The macroconidial dimension ranged from 15.6 to 27.4 μm in length and 3.3 to 3.7 μm in width. The microconidia were smaller and rather variable in shape. They were cylindrical, curved, allentoid, u - and comma - shaped but the curved and allentoid ones were the most frequent (Table 4). The average microconidial dimension was 7.2 x 2.6 μm.

Table 4. Frequency of conidial shapes of *G. xylarioides* isolates from coffee trees.

Macroconidia		Microconidia	
Shape	Proportions (%)	Shape	Proportions (%)
Curved	41.5	Cylindrical	10.1
Falcate	46.3	Curved	43.4
Fusoid	12.2	Allentoid	33.3
		Comma - shaped	10.
		U - shaped	2.9

Pathogenic variability study (cultivars vs. isolates interaction)

The four representative *G. xylarioides* isolates, namely, Gx12 (IMI 375906), Gx26 (IMI 375907), Gx31 (IMI 375908) and Gx43 (IMI 375909), were found to be pathogenic on seedlings of the 9 coffee cultivars in the greenhouse but with certain variation in external symptom expressions.

Coffee cultivar 61/85, 24/85 and F-17 showed significantly ($p < 0.05$) higher disease levels with 62.6, 60.5 and 51.4 mean percent death, respectively (Table 5). Similarly, significantly ($p < 0.05$) shorter incubation period of about 30 days was recorded for seedlings of the cultivars 61/85 and 24/85 (Table 6). On the other hand, significantly ($p < 0.05$) lower percent seedling death of 28.8, 24.3, and 12.0 was observed on cultivars 35/85, 74165 and 7440, respectively (Table 5), and with longer incubation periods of 84, 108, and 112 mean number of days, respectively (Table 6). *G. xylarioides* isolates, Gx26, Gx43, and Gx31 caused higher seedling deaths with 58.2, 53.4 and 52.2 mean percentages, respectively, than isolate Gx12 (Bebeka isolate) with 0.0 % (Table 5).

Table 5. Percent seedling death of 9 Arabica coffee cultivars (transformed to angular values) inoculated with 4 *G. xylarioides* isolates in the greenhouse at Jimma, 1997.

Coffee	*Gibberella xylarioides* isolates *				
Cultivars	Gx12	Gx26	Gx31	Gx43	Mean
74165	0.00 j	40.52 e - i	33.93 f- i	22.59 g- j	24.26 E
7440	0.00 j	17.12 h - j	11.61 ij	19.28 h - j	12.01 F
74304	0.00 j	64.55 a - f	48.82 b - h	38.03 f - i	37.85 CD
F-17	0.00 j	77.79 a - c	52.60 a - g	75.05 a - d	51.36 AB
F-61	0.00 j	54.75 a - g	57.10 a - f	70.80 a - e	45.66 BC
SN-5	0.00 j	70.80 a - e	62.37 a - f	46.80 c - h	44.99 BC
35/85	0.00 j	43.81 d - h	35.25 f - i	35.97 f - i	28.76 DE
24/85	0.00 j	73.82 a - d	82.97 a	85.19 a	60.49 A
61/85	0.00 j	80.37 ab	85.10 a	85.10 a	62.64 A
Mean	0.00 N	58.17 M	52.18 M	53.42 M	

** Gx12, Gx26, Gx31, and Gx43, were G. xylarioides isolates obtained from Bebeka, Teppi, Jimma and Gera, respectively. Means followed with the same letter(s) are not significantly (P < 0.05) different from each other according to Duncun's Multiple Range Test (DMRT). LSD values for the cultivars, the isolates and the interactions comparisons were 10.8, 9.2, and 27.6, respectively.*

Table 6. Incubation periods (number of days) of *G. xylarioides* isolates on seedlings of 9 Arabica coffee cultivars in the greenhouse at Jimma, 1997.

Coffee	*Gibberella xylarioides* isolates *				
Cultivars	Gx12	Gx26	Gx31	Gx43	Mean
74165	0.0 h **	126 a - c	154 ab	154 ab	108.5 A
7440	0.0 h	133 a- c	168 a	147 ab	112.0 A
74304	0.0 h	35 gh	112 a - e	119 a – d	66.5 CD
F-17	0.0 h	84 c - g	98 b - f	63 d - g	61.2 CD
F-61	0.0 h	56 e - h	77 c - g	84 c - g	54.2 D
SN-5	0.0 h	98 b - f	77 c - g	105 b – f	70.0 C
35/85	0.0 h	105 b - f	133 a - c	98 b - f	84.0 B
24/85	0.0 h	49 f - h	49 f - h	28 gh	31.5 E
61/85	0.0 h	56 e - h	35 gh	28 gh	29.7 E
Mean	0.0 R	82.4 Q	100.3 P	91.8 PQ	

** Gx12, Gx26, Gx31, and Gx43, were G. xylarioides isolates obtained from Bebeka, Teppi, Jimma and Gera, respectively.*

*** 0.0= indicates no incubation period (no external symptom was observed until end of the test). Means followed with the same letter(s) are not significantly (P < 0.05) different from each other according to DMRT. LSD values for the cultivars, the isolates and the interactions comparisons were 13.7, 17.5 and 55.6, respectively.*

The Teppi isolate (Gx26) has also induced wilting symptom within significantly shorter incubation period of about 82 days than Jimma isolate (Gx31) with 100 days (Table 6). On the average, the Teppi isolate was more aggressive than the others, while Bebeka isolate was the least aggressive one.

Discussion

Variations in cultural features such as colony growth rate and pigmentation were present among *G. xylarioides* isolates, and except in growth rate, variations occurred independent of their origin. The Bebeka isolates were of slow growing type and this was perhaps due to the low room temperature at Jimma (as compared to Bebeka) which might not have favored colony growth of these isolates. Nevertheless, in this study, all the cultural and morphological features of the fungus were in the domain of the taxonomic descriptions given by Booth (1971), although this finding emphasized the existence of only the female strain.

In the pathogenic variability study, there were highly significant ($P < 0.01$) differences among coffee cultivars and *G. xylarioides* isolates; and significant ($p < 0.05$) interaction between the cultivars and the isolates both in percent seedling deaths and incubation periods. According to Vanderplank (1984), the highly significant ($p < 0.01$) differences among coffee cultivars, and also among the pathogen isolates (i.e. the main effects) indicate the existence of horizontal resistance in the host, and variation in aggressiveness in the fungus population. The significant ($p < 0.05$) interaction between coffee cultivars and *G. xylarioides* isolates both in percent seedling death and in incubation periods (i.e. the differential effect), to some extent implicates vertical resistance in the host varieties and virulence in the pathogen strains.

The field performance of some cultivars generally consistent with results of the seedling tests. In this case the highly resistant cultivar 74165 and 35/85 under field conditions (Table 1) have shown similar host resistance reaction with significantly low disease levels (Table 5) and long incubation periods (Table 6) in the greenhouse. The cultivar 24/85 and F-17 that had been known to be moderately and highly resistant, respectively, in the field; have demonstrated susceptible response to the aggressive isolates of the pathogen under greenhouse conditions.

In comparing the combined cultivar vs. isolate interaction on percent seedling deaths, Teppi isolate (Gx26) induced a higher rate of death on cultivars SN-5 (70.8), 74304 (64.6) and 74165 (40.5) than the Gera isolate (Gx43) on the same cultivars (SN-5 (46.8), 74304 (38.0) and 74165 (22.6)). On the contrary, this isolate (Gx26) has caused lower seedling infection than Gx43 on cultivar F-61 (Table 5). Teppi isolate (Gx26), thus, was found to be fairly more aggressive than Gera isolate (Gx43) on SN-5, 74304, and 74165 coffee cultivars but less aggressive than Gera isolate (Gx43) on cultivar F-61. Jimma isolate (Gx31) seems to be moderately aggressive on most cultivars (74165, 74304, F-17, and SN-5). The Bebeka isolate (Gx12) became non-aggressive or weakly pathogenic to all the cultivars (Table 5).

Thus, the results of the present investigation corroborated the existence of variations both in resistance levels of coffee genotypes and in aggressiveness of the pathogen strains. Although the resistance was predominantly horizontal, the significant cultivar x isolate interaction (differential effect) presented some evidence for vertical resistance in Arabica coffee and *G. xylarioides* pathosystem. The variation among *G. xylarioides* isolates in some cultural characteristics such as growth rate and pigmentation along with the significant cultivar x isolate interaction may suggest some kind of specialization in the fungus population.

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