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Resistance to Gibberella xylarioides in Coffea arabica: evaluation of screening methods and evidence for the horizontal nature of the resistance

R. PIETERS and N. A. VAN DER GRAAFF[1]

Institute of Agricultural Research, Jimma Research Station, Ethiopia

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Abstract

Two testing methods, a seedling test and a conidium germination test, for measuring resistance levels to the vascular wilt caused by *Gibberella xylarioides* Heim & Saccas in *Coffea arabica* L. are described. The tests were evaluated by comparing their results with field scores of death rates in a coffee collection at Jimma Research Station in Ethiopia. A highly significant correlation was found between the results of the two tests and between the tests and the field score.

The quantitative results of the tests mentioned and the results of additional germination tests, in which six host genotypes were tested with four isolates of *G. xylarioides*, support the conclusion that the resistance is of a horizontal nature.

Introduction

Vascular wilt of arabica coffee caused by *Gibberella xylarioides* Heim & Saccas, the conidal stage of which is *Fusarium xylarioides* Steyaert, is a major cause of death of *Coffea arabica* trees in Ethiopia. Symptoms of the disease were reported for the first time on *Coffea excelsa* (Guillemat, 1946) in the Central African Empire (Oubangui Chari). Heim & Saccas (1950) isolated the fungus *G. xylarioides* from diseased material from that area. In Ethiopia, symptoms on arabica coffee similar to those caused by *G. xylarioides* were reported by Lejeune (1958). His observations were later confirmed by Kranz and Mogk (1973), who isolated *G. xylarioides* from diseased arabica coffee from Ethiopia.

Vascular wilt caused by *G. xylarioides* is an endemic disease in all coffee growing areas in Ethiopia. The disease is of no economic importance under traditional low-management growing conditions, but when coffee is grown under modern cultural practices, it often reaches epidemic proportions.

Van der Graaff and Pieters (1978) reported that coffee lines differ in resistance to *G. xylarioides* and that a wide range of resistance levels to the disease is present in the genetically mixed Ethiopian coffee population.

In Ethiopia coffee lines with high levels of resistance to coffee berry disease, *Colletotrichum coffeanum* Noack, are being selected (Van der Graaff, 1978, 1979). Before they are released to farmers it is essential to screen these lines for resistance to *G.*

[1] Formerly FAO Associate Expert Coffee Pathologist and Coffee Pathologist respectively.

xylarioides. With this objective a germination test and a seedling test were developed. In this paper these tests are described and evaluated against field observations. The nature of the resistance is also discussed.

Materials and methods

Field observations. These have been described in an earlier study (Van der Graaff and Pieters, 1978). They were made in the 'French Collection' at the Jimma Research Station. This collection, planted in 1968, includes Ethiopian and some introduced coffee lines. Individual lines consist of a number of plots, each one of eight trees distributed in rows of four. Death of trees in the collection has been recorded since 1973. Practically all trees that died showed infection with *G. xylarioides*. Records collected up to December 1976 were used for the present investigation. For calculation purposes it was assumed that trees dying before 1973 also died from the same cause. The mean percentage of death of each line for all plots was transformed to logits (field score).

Inoculum. *G. xylarioides* was isolated from dead coffee trees and maintained on 1% potato sucrose agar at room temperature (19–23 °C). To restore pathogenicity, isolates were grown on sterilized coffee twigs for one week. Conidia were washed off the twigs and rinsed three times by centrifuging and resuspending in distilled water. Conidia concentrations were measured with a haemocytometer.

Seedling test. From each coffee line to be tested 50 seedlings in the cotyledon stage were planted in a box containing heat sterilized sand. Boxes were kept closed for 14 days to ensure the high relative humidity needed for proper establishment. Each line was replicated three times. Inoculations were made by dipping a knife in a conidial suspension of *G. xylarioides* (2×10^6 conidia/ml) and subsequently nicking the stems of the seedlings. The number of dead seedlings per box was recorded at fourteen day intervals. Death rates (expressed as percentage mortality) were angularly transformed. To enable comparison between inoculation dates, a correction factor was applied to the data. To obtain this factor, observed death rates were grouped according to date of inoculation and subsequently the mean death rate of each inoculation date was determined. The inoculation date with the highest mean death rate was used as a standard, the correction factor being the difference between the standard and the mean death rate for that date. The correction factor for a particular inoculation date was added to all the individual death rates of that inoculation date. Incubation periods defined as number of days between inoculation and death of the first seedling, were determined in the same test. As corrections for differences between dates of inoculation were difficult to apply, only results from one inoculation date are presented.

Germination test. Primary lateral branches of approximately the same age and size were selected and cut in node and internode parts. Node parts were discarded. Bark and cambium were partly removed from ten internode parts per coffee line, exposing a strip of secondary wood on which a drop of a *G. xylarioides* conidial suspension (2×10^4 conidia/ml) was placed. The internode parts were incubated in germination boxes at 25 °C and 100% r.h. for 10 h, after which the conidia were stripped off with transparent adhesive tape and stained in a 1% methyl violet solution. The germination rate of one hundred conidia per internode part was determined. If germination rates near 100% occurred in an experiment the percentage of conidia with a germ tube longer than the conidium was used. A high correlation ($P < 0.01$) was found between results of both methods. Percentages death rates were transformed to logits. The ten internode parts were considered to be replications. Two types of experiments were made: one in which individual lines were compared by inoculating them with one isolate of *G. xylarioides*, and a second one in which the pathogenicity of four isolates was determined on six coffee lines ranging in field score from resistant to susceptible.

Results

Field observations were analysed in detail in an earlier publication (Van der Graaff and Pieters, 1978) when significant differences between lines were found. The highest correlation between the field scores and death rates in the seedling test occurred six months after seedling inoculation. Consequently these death rates were used for further analyses.

In the seedling tests highly significant differences ($P < 0.01$) between death rates from individual coffee lines were present. The frequency distribution of death rates did not differ significantly from the normal distribution. Highly significant differences ($P < 0.01$) were also found between the incubation periods in the seedling tests.

Table 1. Results of field observations, germination tests and seedling tests. Test results and least significant differences for $P = 0.05$ were taken from larger experiments.

Line	Field observation[1]	Germination test[2]	Seedling test	
			death rate[3]	incubation period[4]
F24	0 (-4.59)	-1.84	9.16	211
F18	3 (-3.17)	-1.96	14.46	128
F5	15 (-1.65)	-1.39	13.95	120
SN10	18 (-1.45)	-1.25	30.98	140
SN4	19 (-1.38)	-1.19	26.24	90
F54	25 (-1.04)	-0.87	23.02	145
SN9	29 (-0.84)	-0.60	23.37	139
F9	59 (0.40)	-0.33	35.47	86
LSD		0.48	22	29

[1] Mean percentage dead trees in collection in the period 1968–1976. In parentheses, logit of (mean percentage + 1).
[2] Percentage germination in logits.
[3] Death rates six months after inoculation, in angular transformation.
[4] Number of days between inoculation date and appearance of the first dead seedling.

Tabel 1. Resultaten van veldwaarnemingen, kiemingstoetsen en zaailingtoetsen. Toetsresultaten en kleinste significante verschillen zijn overgenomen uit omvangrijkere experimenten.

Table 2. Correlation between test results and field scores and among test results.

	Field score	Incubation periods	Death rates
Germination test	0.80 (11)	0.67 (12)	0.71 (10)
Death rates after 6 months	0.74 (10)	0.65 (23)	
Incubation periods	0.73 (12)		

In parentheses, degrees of freedom.
All correlations are significant at $P < 0.01$.

Tabel 2. Correlatiecoëfficiënten tussen de toetsresultaten en de veldscores en tussen de resultaten van de toetsen.

In germination tests in which coffee lines were compared by inoculating them with one isolate, highly significant differences ($P < 0.01$) between lines were observed. Results of field observations (Van der Graaff and Pieters, 1978), seedling tests and germination tests for a number of coffee lines are presented in Table 1. Data for both tests are from larger experiments in which coffee lines with an unknown field score were also tested. Correlation coefficients between results of individual tests and between results of tests and field scores are given in Table 2. As can be observed from the table,

Table 3. Results of a germination test in which the pathogenicities of four isolates of *G. xylarioides* were tested against six individual coffee lines.

Lines	Isolates				Mean
	a	b	c	d	
F20	-2.33	-1.77	-1.91	-0.55	-1.64
F30	-2.58	-2.20	-0.72	-0.67	-1.54
S947	-2.13	-1.60	0.05	-0.40	-1.02
F54	-2.18	-1.24	-0.78	0.13	-1.01
F21	-1.47	-0.97	-0.25	0.27	-0.60
S288	-0.67	-1.09	-0.70	0.83	-0.40
Mean	-1.89	-1.48	-0.72	-0.07	

Source of variation	Degrees of freedom	F	LSD (5%)	
Treatments	23	4.84**	Within table	1.17
Isolates	3	22.78**	Isolates	0.47
Lines	5	5.54**	Lines	0.58
Interactions	15	1.02		
Error	216			

Tabel 3. Resultaten van een kiemingstoets waarin de pathogeniteit van vier isolaten van G. xylarioides getoets werd tegen zes koffielijnen.

results from both tests were highly significantly correlated with the field score and with each other.

In the germination tests in which the pathogenicities of four isolates of *G. xylarioides* were tested against six coffee lines, highly significant differences among isolates and among coffee lines were present, but no significant interaction could be demonstrated between isolates and coffee lines (Table 3).

Discussion

Experiments to detect resistance to *G. xylarioides* are only useful if the results reflect the performance of the tested coffee lines in the field.

The correlations observed between test results and field scores and also the correlation between the two tests (Table 2) justify the conclusion that results from both tests give a good indication of the resistance levels to *G. xylarioides* in the tested coffee lines.

The results obtained in the various experiments all show a quantitative expression of the resistance. In the seedling test the level of resistance expresses itself by means of the incubation period. In the germination test the resistance level influences the progress of the germination. Both a longer incubation time and retardation of conidial germination are quantitative processes which indicate horizontal resistance (Van der Plank, 1968). The lack of interaction between coffee lines and inocula, as observed in the experiment in which inocula were tested against coffee lines, is a further indication for the horizontal nature of the resistance (Van der Plank, 1968).

Under low-management growing conditions, such as those prevailing in Ethiopia, the system *G. xylarioides* – *C. arabica* is balanced, and epidemics of *G. xylarioides* do not occur. The frequency distribution of the resistance levels as observed in the seedling test (Fig. 1) can be considered to represent the expression of that balance. The levels of resistance fluctuate around the mean, which is probably the level on which selection for higher resistance is counterbalanced by other selection pressures (Van der Plank, 1975).

Since the first outbreaks and subsequent spread of Coffee Berry Disease (CBD) in Ethiopia (Mulinge, 1972), coffee culture in Ethiopia is gradually changing. New coffee

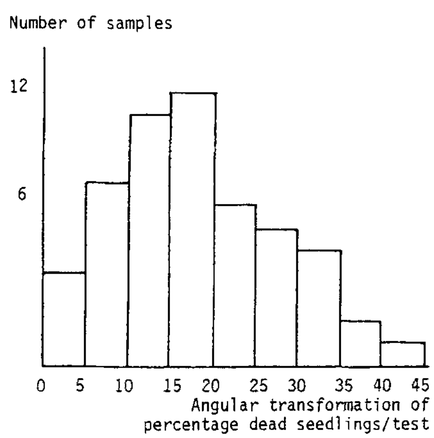

Fig. 1. Frequency distribution of mean death rates of coffee lines in the seedling test with three replications.

Fig. 1. Frequentiediagram van de gemiddelde sterfte in koffielijnen in de zaailingentoets met drie herhalingen.

lines with a high level of resistance to CBD are now being introduced. It is envisaged that modern cultural practices will be applied on these lines thus increasing the chance of infection by *G. xylarioides* through more soil disturbance. In this way the existing balance between *G. xylarioides* and *C. arabica* will be unsettled. Therefore, the use of lines combining a high level of horizontal resistance to *G. xylarioides* with CBD resistance is imperative to diminish the danger of epidemics of the vascular wilt in new plantations.

Samenvatting

Resistentie tegen Gibberella xylarioides in Coffea arabica: beoordeling van toetsmethoden en aanwijzingen voor de horizontale aard van de resistentie

Twee toetsmethoden voor de bepaling van resistentieniveau's van *C. arabica* tegen *G. xylarioides*, een kiemingstoets en een zaailingentoets, werden beoordeeld aan de hand van veldgegevens.

Significante verschillen tussen resultaten voor de getoetste koffielijnen in beide toetsen en hoge correlaties tussen de resultaten van toetsen onderling en met de veldbeoordelingen (Tabel 2) rechtvaardigen de conclusie dat de toetsen geschikt zijn voor het schatten van resistentieniveau's.

Het systeem *G. xylarioides-C. arabica* is in evenwicht onder de huidige primitieve cultuurmethoden in Ethiopië. Het in de zaailingentoets gevonden gemiddelde resistentieniveau (Fig. 1) zal voldoende zijn om het uitbreken van een epidemie te voorkomen. Op grond van de kwantitatieve aard van de resultaten van beide toetsen en de afwezigheid van interactie tussen isolaten van *G. xylarioides* en koffielijnen (Tabel 3) wordt de conclusie getrokken dat de resistentie van horizontale aard is.

De koffiecultuur zal in Ethiopië in de toekomst veranderen door invoering van koffielijnen met een hoge resistentie tegen koffiebesziekte en door het toepassen van moderne cultuurmaatregelen. Nieuwe koffielijnen zullen een resistentieniveau tegen *G. xylarioides* moeten bezitten dat hoger ligt dan het huidige gemiddelde resistentieniveau, omdat nieuwe cultuurmaatregelen de kans op infectie door deze vaatparasiet zullen vergroten.

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Addresses

R. Pieters: Direction de la Recherche Agronomique, c/o PNUD, Casier ONU, Rabat Chellah, Morocco.
N. A. van der Graaff: Plant Protection Service, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy.