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Seed transmission of *Fusarium xylarioides* in *Coffea canephora* in Uganda

G.J. Hakiza, D. T Kyetere, M. Erbaugh[1]. HWarren[2] and S. Olal
Coffee Research Institute (CORI), P.O. Box 185, Mukono (Uganda)
[1] Ohio State University, U.S.A.
[2]Virginia Tech, U.S.A.

Abstract

A study was carried out to determine whether or not *Fusarium xylarioides* (= *Gibberella xylarioides*) could be transmitted by infested seed of robusta coffee (*Coffea canephora* Pierre). Seeds were collected from symptomatic and asymptomatic plants from various robusta coffee growing districts in Uganda and from experimental fields at the Coffee Research Institute (CORI), based at Kituza in Mukono district. A total of 43 seed samples from 13 districts were collected between 2000 and 2003. To test for presence of the wilt pathogen, the seeds were separated into two groups. The first group of seeds were surface sterilised and the second group were not sterilised. Both seed categories were plated on blotter, agar plates or planted in sterile sand beds. Fungi detected on seeds plated on blotter and agar plates, from both sterilised and unsterilised seeds were *Fusarium stilboides, F. lateritium, Aspergillus ochraceous, A. niger, A. flavus, Colletotrichum gloeosporioides* and other unidentified fungi. *F. xylarioides* was not detected by either blotter or agar plate methods. Seed germination varied between 30 – 50% for seeds from wilted trees compared to 76 – 85% for seeds from healthy trees. Although plants from diseased mother trees remained stunted no wilt pathogen was recovered from them 3 years from planting. All tests were negative for the coffee wilt pathogen, indicating that *F. xylarioides* is not seed transmitted and that the rapid spread of the disease throughout the robusta districts cannot be attributed to the use of seeds for propagation.

Key words: ***Gibberella xylarioides*** robust coffee seed transmitted

Introduction

Robusta coffee (*Coffea canephora* Pierre) is of considerable economic value to Uganda as a source of foreign exchange, local revenue and source of employment for over 2 .0 million people who are involved in its cultivation, processing and marketing. The crop has been cultivated for many years without any major production constraint, on an estimated 240,000 ha of Uganda's farmland, until the appearance of the dreaded coffee wilt disease (CWD) in the early 1990's. Robusta wilt disease is a vascular wilt disease caused by *F. xylarioides* Steyaert (= *Gibberella xylarioides* Heim and Saccs) that occurs in all robusta districts of Uganda. The disease occurs only in Africa affecting robusta coffee in Uganda, Democratic republic of Congo (DRC), Tanzania and on arabica coffee in Ethiopia (Van der Graaf and Pieters, 1978; Kranz and Mogk, 1993; Flood, 1996). The disease spreads rapidly, attacks all coffee species and all affected plants are killed (Wrigley, 1988). According to the survey of 2002, wilt incidence varied between 5% in a number of districts to over 50% in the worst affected districts. In some fields, losses of trees were more than 90%. Wilt symptoms include wilting, leaf fall, sometimes chlorosis and eventually death of the plant. The external symptoms are apparent on a single branch or a single side of an infected plant. Examination of the stem at the collar after removal of the bark on the wilted side of the affected bush reveals darkening (blue black) of the vascular tissue. Yellowing of leaves may sometimes occur but not in all cases. Symptoms are well described in literature (Van der Graaf and Pieters, 1978; Pochet, 1988; Flood, 1996; Waller and Holderness, 1997).

Although actual losses in monetary terms have not been accurately quantified, it is evident that many farmers have been severely affected by loss of regular income. In addition, new plantings continue to be attacked by the wilt disease and in the previously low incidence areas; it has continued to spread.

The extent and rapid rate at which CWD has spread to new areas suggests other primary sources of inoculum than soil and infected plant parts or debris. One possible primary inoculum source could be the seed. It has been speculated that the use of seed for propagation of the crop could have contributed to the rapid spread of the disease. Seed transmission is one of the most effective ways in which plant pathogens are disseminated around the globe. Robusta coffee is highly heterogeneous and the best way to maintain desirable traits in the progeny is to propagate it by vegetative cuttings or by means of tissue culture techniques. However, the multiplication rate of cuttings is very low and the protocol for robusta coffee propagation by tissue culture techniques is still not perfected. Consequently, there is need

to use seeds for propagation. The robusta-replanting programme has been using mainly seeds. The objective of this study was therefore to establish if the disease could be transmitted through seed or not.

Materials and methods

Visual inspection

Robusta coffee seed samples were screened for the presence of F. xylarioides and the sexual form *Gibberella xylarioides.* The seed samples were obtained from naturally infected trees in farmers' fields in the districts indicated in Table 1. The seeds were harvested from trees categorised as healthy (without wilt symptoms and were presumed healthy), mildly and severely diseased/dead trees. From each sample, 400 seeds were randomly selected for dry seed examination and visually examined with the help of stereo binocular microscope. The same seed samples were subjected to seed health testing using the blotter and agar plate methods.

Blotter method

Three layers of moistened filter paper were placed in glass Petri dishes (9 cm diameter). From a random sample of 400 seeds, 10 seeds were arranged on the blotter in each Petri dish. Incubation was done at room temperature under fluorescent light, with photoperiod of 12 hours at room temperature (23 – 25 C). The seeds were incubated for 7 days, checked under a stereomicroscope and identification confirmed under compound microscope at x40. Colonies of each fungal species were recorded.

Agar plate method

Potato dextrose agar (PDA) was prepared and poured into Petri plates. Ten seeds per plate and 400 seeds per sample were sown on agar and incubated as indicated under blotter method. After 5 – 7 days incubation the plates were checked. Colonies were examined for pigmentation and sporulation and fungi identified as indicated in blotter test.

Germination test

Seeds were sown on sterile sand contained in plastic trays. The sand was kept moist until the seeds germinated 6 – 7 weeks from planting. Upon germination, seedlings were examined and categorised as normal, abnormal, infected seedlings, dead and ungerminated seeds were counted. Germination percentage of each seed sample was assessed. Ten ungerminated seeds, abnormal and dead seeds from each category (seeds from healthy, mildly diseased and severely affected trees) were examined and cultured on agar for detection of *F. xylarioides.*

Growing on test

Seedlings (50 per sample) from the germination trays were transplanted into black polythene pots (15 x 22 cm laid flat) filled with sterile loam soil and maintained under screen house for 2 – 3 years. Seedling heights were measured at 4 weekly intervals. Inspection for wilt symptoms was done at the same time. Plants (5) were sampled at random for *F. xylarioides* bioassay every six months.

Results and discussion

Visual inspection of seeds indicated more shrilled, defective, dark and smaller seeds from severely diseased trees, followed by those from mildly diseased trees and least from healthy trees. No fungal fruiting bodies such as perithecia or any other were observed in all categories of seeds.

Blotter test

F. xylarioides was not detected from all seed samples examined by the blotter method. However other fungi *F. stilboides, F. lateritium, Aspergillus ochraceous, A. niger, A. flavus, Colletotrichum gloeosporioides, Colletotrichum* species as well as other unidentified fungi were detected. Seed sources are indicated in Table 1

Agar plate method

As in the case of blotter method, the coffee wilt fungus was not detected by use of this method. Similar fungi observed in blotter test were also found. Growth of fungi was more profuse on agar. The most abundant species were *F. stilboides*, followed by *Colletotrichum gloeoesporioides* and *A. niger*.

Germination test

Seed germination in all samples tested was lowest in the case of seeds obtained from trees with wilt disease. The worst seeds were from severely diseased or dead trees. No wilt pathogen was recovered from seeds that did not germinate. Examination of the root systems of a sample of plants showed no discolouration and *F. xylarioides* was not recovered from the stem and root tissues.

From the results, coffee wilt appears to have adverse effects on coffee seed germination. The more the severe the symptoms on the trees, the lower the germination. This could be attributed to the fact that when the trees are attacked, supplies to the developing berries are considerably reduced or stopped. Consequently, only berries that are fully developed at the time of wilt attack are able to germinate.

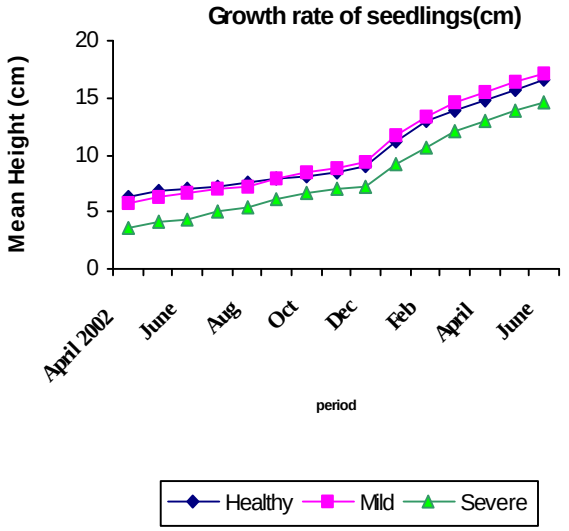
Growing on test

Seedlings being maintained in pots in the green house are still in good health, 15 months after planting. Attempts to recover the pathogen from the roots and stem of a sample of plants taken at 4 weeks interval have been unsuccessful. Growth measurements over time revealed that plants from diseased trees were stunted and had reduced growth rate as compared to seedlings from healthy trees.

Table 1. Germination of coffee seeds collected from healthy, mildly and severely diseased trees from various locations in Uganda.

Source of seed	Number of samples	Percent germination of coffee seeds from		
		Healthy	Mild	Severely diseased
Mukono	5	77.0	56.0	46.5
Mubende	3	84.2	52.5	40.3
Kibale	2	79.5	50.1	43.4
Jinja	3	80.6	61.3	39.8
Iganga	4	85.5	55.6	44.7
Mayuge	4	80.9	51.8	40.1
Mpigi	3	81.8	62.6	45.2
Wakiso	2	76.7	58.9	38.0
Bushenyi	3	79.3	54.3	36.9
Sembabule	4	81.5	63.5	43.2
Masaka	3	79.4	65.0	37.5
Kayunga	4	82.4	60.4	33.1
Rakai	3	83.1	61.4	36.4

In previous experiments carried out at CORI, seeds from wilting robusta trees consistently had poor germination. Similarly in this attempt, seed germination was much lower than seeds from healthy plants.



Means are for 20 plants for each category of seed source

Figure 1: Comparison of growth rate (height in cm) of robusta coffee seedlings from seeds of healthy, mildly diseased and severely diseased trees affected with CWD (Planted November, 2001).

Growing on test

Seedlings being maintained in pots in the green house are still in good health, 15 months after planting. Attempts to recover the pathogen from the roots and stem of a sample of plants taken at 4 weeks interval have been unsuccessful. Growth measurements over time revealed that plants from diseased trees were stunted and had reduced growth rate as compared to seedlings from healthy trees.

There was little difference in the growth rates of seedlings raised from seeds from healthy and mildly diseased trees. Seedlings from severely / dead trees however, had slower growth rate than the other two seed sources.

Conclusion

In the present investigation, seeds from coffee bushes showing wilt symptoms as well as from bushes without symptoms were tested for the presence of *Fusarium xylarioides* (*Gibberella xylarioides*) and the possible transmission of the pathogen to seedlings was investigated. The seeds were collected from 13 robusta growing districts of Uganda. In all the tests carried out, F. xylarioides was not observed or recovered from any of the seed samples. A growing - on test was conducted in the CORI screen house, for over 3 years. All seedlings did not develop wilt symptoms. The wilt pathogen was not recovered either in any of the seedlings tested periodically for the presence of the pathogen.

The results from these investigations so far suggest that seed transmission might be rare, if indeed it can occur. In practice, it is recommended that seeds for production of coffee plants should not be harvested from diseased trees. The results of this study are in support of this recommendation due to poor seed germination and it is prudent to remain cautious when dealing with coffee wilt disease.

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