JASSA

Journal of Applied Science in Southern Africa
The Journal of the University of Zimbabwe

Volume 3, Numbers 1 and 2, 1997 **ISSN 1019–7788**

CONTENTS

JASSA

Journal of Applied Science in Southern Africa
The Journal of the University of Zimbabwe

Vol. 3, Nos. 1 & 2, 1997
ISSN 1019-7788

CONTENTS

Pathogenecity of *fusarium lateritium* var. *longum* as causal agent of collar rot of coffee in Zimbabwe

E. Masenda[1] and N. Mtetwa[2].

[1] Plant Protection Research Institute, Box CY550, Causeway, Harare.
[2] Coffee Research Institute, P.O. Box 61, Chipinge.

The pathogenecity of the fungus *Fusarium lateritium* Nees. var. *longum* Wollenw. causal agent of collar rot of coffee was studied in greenhouse experiments at Coffee Research Station from 1994 to 1997. Artificial inoculation of coffee bushes with the fungus through wounds at the base of the stem resulted in the development of typical collar rot symptoms encountered in the field, but no infection took place through root wounds or undamaged stems. There was no evidence that cotyledon infection of seedlings at the cotyledon stage resulting from seedborne inoculum developed into collar rot of bushes, as such seedlings died shortly after developing symptoms. However, artificial infection of cotyledons of seedlings from the 8th leaf stage onwards sometimes led to mainstem infection which resulted in collar rot of the young seedlings.

Keywords: *Fusarium lateritium* var. *longum*; *Coffea arabica* var. SL28; Fusarium bark disease; Collar rot.

Introduction

Fusarium Bark Disease (FBD) of coffee caused by the fungus *Fusarium lateritium Nees. var.* longum *Wollenw. is a very serious disease in the Eastern Highlands of Zimbabwe, and is suspected to have been introduced from Malawi through the importation of coffee seedlings.*

It is a wound pathogen which attacks all damaged, above ground parts of coffee causing a wide range of symptoms such as seedling blight, collar rot, branch blight, sucker blight, berry blight and scaly bark on old bushes (Siddiqui, 1963).

Collar rot, which is characterised by a girdling of the stem base, usually occurs on young coffee bushes before or in their early years of production. Leaves of affected bushes first turn yellow, a then the bush wilts and dies. Because it results in the death of the entire bush, it is by far the most serious manifestation of the fungus.

Infection takes place through wounds caused by insects, hoes or various agents (Siddiqui, 1968), and the main source of inoculum are infected berries. Conidia are either washed down the stem from infected berries or they are carried from tree to tree by wind driven rain or insects. A lesion develops at the infection site and it gradually extends to encircle the stem, resulting in a girdled appearance afterwhich the bush starts showing signs of stress such as yellowing of leaves and wilting.

Sometimes girdling occurs well up the stem and this results in the wilting of distal parts. In such cases, cutting the stem below the canker saves the bush.

On green stems and seedlings, *F. lateritium* var. *longum* is very aggressive and takes 2–3 weeks before a lesion appears and death follows 4–6 weeks later. On woody stems, symptoms take much longer before they become apparent, about 3–4 months or more depending on the thickness of the stem.

Cotyledon infection is characterised by lesions occupying matching positions on either side of the cotyledons of young seedlings, at the cotyledon and butterfly stages and it is a direct result of seed-borne inoculum. For a long time, it was widely believed that collar rot was a direct result of this type of seed-borne infection, which remains latent or slowly develops to become collar rot in young bushes (Engelbrecht, 1985). This, however had never been experimentally verified.

There also has been controversy on the identity of the causal fungus with some authors still believing the causal fungus to be *Fusarium xylarioides* Steyaert which occurs elsewhere in Africa and causes vascular wilts (Clowes and Hill, 1981; Ndimande, 1985; Wrigley, 1988).

When isolates of the fungus were send to various institutions for identification, Booth (IMI entries 247428/259245) identified the fungus as *F. xylarioides* but W.F.O. Marasas and Scott (RSA) identified it as *F. lateritium* var. *longum* according to the 1932 description of a similar fungus from Tanzania by Wollenweber. They did not find any curved female conidia characteristic in *F. xylarioides* (Booth,1971), and from laboratory and field observations, the fungus affected mainly the bark and there was no indication that it was vascular. Consequently the name *Fusarium lateritium* var. *longum* was accepted.

On Potato Dextrose Agar (PDA), cultures of the pathogen start α bright orange-pink and become bluish with age as sclerotia develop. Macroconidia of the fungus are long (92,5 um),straight with a beaked apical cell and pedicellate base, and there are very few microconidia and chlamydospores produced in culture (Engelbrecht, 1985).

The studies reported in this communication are aimed at demonstrating the pathogenecity of *F. lateritium* var. *longum* as causal agent for collar rot on coffee in Zimbabwe, and establishing the relationship between cotyledon infections in seedlings and collar rot of young coffee bushes in the field. The time it took for collar rot symptoms to appear on young coffee bushes after infection by *F. lateritium* var. *longum* was also determined.

Materials and methods

Inoculum of *F. Lateritium* var. *longum* was prepared using a single spore isolate obtained from the diseased stem of a coffee plant 3–4 weeks earlier. Single spore isolates were prepared by scraping lightly the sporulating part of the diseased tissue into sterile deionised water in a test tube. Five serial dilutions were made and the resulting suspension was streaked onto 2% water agar. After 24 hours, with the aid of a stereo microscope, and under a laminar flow cabinet, individual germinating spores were picked out by needle and placed on Potato Dextrose Agar (PDA) and incubated at room temperature. After 10 days, cultures resulting from the single spore isolates were examined, and contaminated cultures or those not conforming to the description of the fungus were discarded. Subcultures prepared from a single spore isolate and incubated for over 10 days at room temperature were used in the experiments. An aqueous conidial suspension of 4 x 10^6 conidia/ml was prepared using sterile deionised water. Soil was sterilised using a soil steriliser. The greenhouse used had 25°C and 75% humidity and the humid chamber inside the same greenhouse had 95% humidity.

In all the experiments, seedlings of the susceptible variety SL28 in individual pots were used. A complete randomised design with five or ten replicates was used. Disease assessment which was done bi-weekly done by checking for a lesion on the site of inoculation. Lesion size was not considered since this was not a comparative study but only sought to demonstrate the pathogenecity of the fungus. Results were strictly observational and no statistical analysis was done.

Pathogenecity of F. lateritium *var.* longum *on roots of seedlings at butterfly stage*

Coffee seedlings at the butterfly stage (8 weeks after germination) and ready for transplanting into individual pots were used. They were removed from the germinating trays and their roots washed with sterile deionised water. Since these roots already had wounds and breaks due to the uprooting, no further wounding was done. Each seedling was dipped in a conidial suspension or in sterile water and then planted in a polythene bag filled with sterile or non-sterilise soil. Five seedlings per treatment were used. There were four treatments altogether. Treatments 1 and 2 had roots of seedlings dipped in a conidial suspension of *F.lateritium* var. *longum* and planted in sterilised and non-sterilised

soil respectively. Treatments 3 and 4 had roots dipped in sterile deionised water and planted in sterilised and non-sterilised soil respectively.

Disease assessments were done by examining roots of uprooted seedlings for lesions through destructive sampling.

The plants were placed in a greenhouse and observed for a year.

Pathogenecity of* F.lateritium *var.* longum *on tap roots of 2 year old coffee seedlings.

Two year old potted coffee seedlings were used. An opening on the lower part of the bag was made and soil was eased out carefully until the tap root was visible. Incision wounds were made on the tap root a few centimetres from the tip. Inoculation was done by brushing a conidial suspension of *F.lateritium* var. *longum* (4×10^6 conidia/ml), on the wound. There were 2 treatments, treatment 1 was *F.Lateritium* var. *longum* inoculated on tap root wound and treatment 2 was sterile water brushed on tap root wound. All plants were uprooted after 3 months for observation through destructive sampling. Five plants per treatment were used.

Pathogenecity of* F.lateritium *var.* longum *on stem collars of 2 year old seedlings.

Two year old coffee seedlings from the nursery were used. Incision wounds on stem, at soil level or 5 cm above soil level were made using sterile scalpel blades. Inoculation was done by brushing a conidial suspension of *F.lateritium* var. *longum* (4×10^6 conidia/ml) on the wounds.

The experiment consisted of 6 treatments:

F. lateritium var. *longum* conidial suspension brushed on wounds at soil level (treatment 1).

F. lateritium var. *longum* conidial suspension brushed on wounds 5 cm above soil level (treatment 2).

F. lateritium var. *longum* brushed on undamaged stems (treatment 3).

Sterile deionised water brushed on wounds at soil level (treatment 4).

Sterile deoinised water brushed on wounds 5cm above soil level (treatment 5).

Sterile deionised water brushed on undamaged stems (treatment 6).

Five plants per treatment were used. The plants were placed in the humid chamber for 3 days, then removed and placed in the greenhouse where they were observed for a year. Disease assessment was by checking for a lesion and girdling at the site of inoculation .

Pathogenecity of* F.lateritium *var.* longum *on cotyledons of SL28 coffee seedlings at butterfly stage

Eight week old seedlings which were at the butterfly stage were used. A conidial suspension of 4×10^6 conidia/ml was used. Inoculation was by pin and paper disc method described by Engelbrecht (1985) where a paper disc attached to a pin is dipped in the suspension then pinned to the cotyledon, such that the paper disc is in contact with the cotyledon. Ten seedlings per treatment were inoculated. After inoculation, the seedlings, which were in trays, were placed in the humid chamber for 3 days then, the pins and paper discs were removed, and the plants were left in the greenhouse for observations.

Disease assessment was done by checking for the presence of lesions with concentric zones at the site of inoculation.

The treatments were *F.lateritium* var. *longum* inoculated on cotyledons,, Sterile deionised water inoculated on cotyledons and no inoculations done on seedlings.

Pathogenecity of* F. lateritium *var.* longum *on coffee seedlings of different ages

Seedlings at the following stages of development were used:

Seedlings at cotyledon stage (SCS)

Seedlings at butterfly stage (SBS)

Seedlings at 4 leaf stage (S4L)

Seedlings at 8 leaf stage (S8L).

Seedlings at 12 leaf stage ready for field transplanting (S12L).

Ten seedlings for each stage were used.

A conidial suspension of *F.Lateritium* var. *longum* was inoculated onto the cotyledons using the pin and paper disc method.

Disease assessment was done the presence or absence of cotyledon infection

and observing whether this infection reached the mainstem of the seedling.

There were 3 treatments, treatment 1 was; *F.lateritium* var. *longum* inoculated on cotyledons; treatment 2 was; sterile deionised water inoculated on cotyledons and treatment 3 was; no inoculations done on cotyledons.

Results

Pathogenecity of F. lateritium *var.* longum *on roots of seedlings at butterfly stage*

No signs of disease were present and a check on the roots done at the end of the observation period revealed no disease symptoms (Table 1).

Table 1: Reaction of roots of coffee seedlings at the butterfly stage 12 months after inoculation with *F. lateritium* var. *longum*.

Treatments	Seedilings with root lesions
1	0
2	0
3	0

Pathogenecity of F.lateritium *var.* longum *on tap roots of 2 year old coffee seedlings*

No lesion development was observed on both the plants inoculated with the pathogen, and the control plants (Table 2).

Table 2: Pathogenecity of *F. lateritium* var. *longum* on tap roots of 2 year old coffee seedlings 3 months after inoculation.

Treatment	No. of seedlings with infected tap root
1	0
2	0

Pathogenecity of F.lateritium *var.* longum *on stem collars of 2 year old seedlings*

All plants which had wounds inoculated by *F. lateritium* var. *longum* eventually developed collar rot and died. A close examination of these plants showed that lesions had begun to grow around the wound site three weeks after inoculation. Plants with wounds 5 cm above soil level developed the typical collar rot symptoms earlier than those with wounds at soil level. Three and a half months after inoculation, all plants had girdled stems and one was dying. Plants with wounds at soil level started showing symptoms 4 months after inoculation and the first one died 6 months post inoculation (Table 3). Plants whose undamaged stems were brushed with a conidial suspension and those whose wounds were brushed with sterile deionised water did not develop collar rot.

Table 3: Pathogenecity of *F. lateritium* var. *longum* on stems of 2 year old seedlings 4 months after inoculation.

Treatment	No. of Seedings with collar rot	No. of dead seedlings
1	4	1
2	5	0
3	0	0
4	0	0
5	0	0

Table 4: No. of seedlings at butterfly stage with cotyledon infection 6 weeks after inoculation with *F. lateritium* var. *longum*.

Treatment	No. of seedlings with cotyledon infection
1	10
2	0
3	0

Pathogenecity of F.lateritium *var.* longum *on cotyledons of SL28 coffee seedlings at butterfly stage*

Three weeks after inoculation, all seedlings inoculated with *F.lateritium* var. *longum* developed cotyledon lesions which gradually spread and killed them (Table 4). Since cotyledons remain attached to seedlings until quite late (about one and half years) the trial was repeated using older seedlings.

Pathogenecity of F. lateritium *var.* longum *on coffee seedlings of different ages*

After 3 weeks, lesions started to develop on all the cotyledons which were inoculated with *F. lateritium* var. *longum*. For seedlings

at cotyledon stage, the infection spread rapidly to the mainstream resulting in death within 4 months of inoculation. Premature abscission of cotyledons, which was more pronounced on older seedlings, stopped the infection from spreading to the mainstem resulting in fewer deaths (Table 5).

Table 5: No. of coffee seedlings with cotyledon infection and mainstem infection 6 months after inoculation.

Age	No. of seedlings with cotyledon infection	No. of seedlings with mainstem infection
SCS	10	10
SBS	10	6
S4L	10	2
S8L	10	5
S12L	10	1

Discussion

Coffee plants inoculated with *F. lateritium* var. *longum* developed the typical collar rot, symptoms, proving that the fungus can cause collar rot, although this does not exclude the existence of other pathogens likely to cause similar symptoms on *Coffea arabica* in Zimbabwe, for example *Rhizoctonia solani* on nursery seedlings (Rothwell,1980).

The fact that infection took place through wounds implies that any factors causing damage to the stem base (stem borers, dusty surface beetles, hoes, sucker removal and pruning) are of paramount importance in collar rot outbreaks. In a FBD survey done in Chipinge in 1997, (Masenda & Mtetwa, unpublished) all coffee bushes affected by collar rot had old wound scars at the site of infection caused either by borers or hoes. This is also supported by reports by Engelbrecht (1985) and Siddiqui (1968) who found a strong positive correlation between wounding of bushes and the incidence of FBD.

The study demonstrated that *F. lateritium* var. *longum* cannot infect roots and no transmission is possible via this media.

It was also shown that collar rot of young coffee bushes is not a direct result of cotyledon infections affecting seedlings at the cotyledon and butterfly stages, because at these early stages, infection with *F. lateritium* var. *longum* is always fatal. Those seedlings which for some reason did not develop infection, remained unaffected for the rest of the observation period and there was no indication of latent infection. However, cotyledon infection of seedlings from the 8th leaf stage onwards sometimes resulted in collar rot. It should be pointed out that infection at this late stage in the development of the seedling was not a result of seed-borne inoculum but, due to infection of wounds.

Infection of seedlings in the nursery beds can therefore lead to the transfer of the disease to fields at transplanting. To minimise risks of transmission, the disease has to be controlled in the nursery beds and this can be achieved by the use of clean seed, removing diseased plants and the application of fungicides; measures which were also advocated by Engelbrecht (1985).

From field observations on farms in Chipinge, infected berries constituted the main source of inoculum, a fact also alluded to by Engelbrecht (1985). Field hygiene such as removing infected berries from bushes, and the stripping of bushes of all crop after harvest to avoid carry over of inoculum are therefore important control measures. In his studies, Engelbretcht (1985) also established that berry moths were responsible for a high percentage of field transmission of *F. lateritium* var. *longum* hence, the control of the moth is another way of reducing the incidence of Fusarium Bark Disease.

The fungus does not appear to remain viable in field soils for longer than two years (Engelbrecht,1985) but, this is debatable since most fields continue having a history of *Fusarium* once infected. Future studies therefore need to focus on the viability of the fungus in soil and on the identification of possible wild alternative hosts.

Varieties like S.Agaro and Geisha have been reported to be resistant to FBD (Engelbrecht,1985; Siddiqui,1980). In the FBD outbreak of 1997 in Chipinge, the least affected fields were those planted with these varieties (Masenda & Mtetwa, unpublished). More work therefore needs to be done on mechanisms governing resistance to this pathogen and the identification of resistant germplasm.

ACKNOWLEDGEMENTS

We would like to thank colleagues at the Plant Protection Research Institute and at Coffee Research Institute for all their support and assisstance.

REFERENCES

BOOTH, C. 1971 The Genus Fusarium. CMI, Surrey, UK.

ENGELBRECHT, S.L.G. 1982 Epidemiology and chemical control of Fusarium Bark Disease of Coffee in Zimbabwe. Ph.D thesis. University of Pretoria.

CLOWES, M. ST.J. AND HILL, R.H.K. 1981 Coffee Diseases. In: Coffee Handbook. Coffee Growers Association, Harare.

NDIMANDE, B.N. 1985 A review of the current knowledge on Fusarium Bark Disease of Coffee in Zimbabwe. In: Advances in Coffee Management and Technology in Zimbabwe 1980–1985 Clowes, M.St.J. and Logan, W.J.C. (eds). Coffee Growers Association, Harare.

ROTHWELL, A. 1980 A revised list of plant diseases in Zimbabwe. In: Kirkia. 1980 **12**(1): 183–190.

SIDDIQUI, M.A. AND CORBETT, D.C.M. 1963 Coffee Bark Diseases in Nyasaland. (I) Pathogenecity, Description and Identification of the causal organism. In: *Trans. British Mycol. Soc.* **46**(1): 9–101.

SIDDIQUI, M.A. AND CORBETT, D.C.M. 1968 Coffee Bark Diseases in Malawi. (II) Properties of the causal organism and conditions favouring the disease. In: *Trans. British Mycol.Soc.* **51**(1): 129–135.

SIDDIQUI, M.A. 1980 The selection of arabica coffee for Fusarium Bark Disease resistance at Bvumbwe. In: Kenya Coffee. **45**: 55–59.

WRIGGLEY, G. 1988 Coffee — Tropical Agriculture series, Longman Scientific and Technical, Singapore.