10
CWD Survival and Transmission: Field studies of CWD infection

10.1 Main Findings

- Coffee wilt disease (CWD) transmission through wounding of coffee with an infected machete was studied in the field and screen house. Some evidence of transmission in the field was seen, but further tests are needed, along with improvement in experimental procedure to develop a standard technique that can reliably deliver a dose of infective inoculum.
- The CWD transmission from infected wood to adjacent uninfected seedlings was confirmed in screen house trials, suggesting that leaving infected wood near uninfected trees in the field is a significant infection pathway.
- The CWD transmission from infected soil to healthy seedlings potted in that soil was also confirmed, with infectivity lasting at least 3 months and declining subsequently.
- Further fieldwork is required to assess the extent of the infectious period of the soil under a range of ambient conditions, but from this study a period of at least 1 year fallow before planting is advisable to avoid reinfection.
- The study of soil infectivity has been hampered by difficulties in isolating the disease from soil samples. Soil particles or extracts spread on culture plates invariably develop a range of other infections but not CWD. Therefore, further work is needed to develop a quick and reliable way to assess the presence of the disease in soil samples. Such a technique would be especially important to regularly monitor the health of soils before replanting and soils being used to multiply seedling materials for nationwide dissemination.
- Preliminary results of screening insects as possible CWD vectors could find no evidence that they carry the disease, although they were found to be carrying spores of other Fusarium diseases.
- Results to test whether other crop and weed plants might harbour CWD all proved negative, strongly suggesting that no common plant growing in the vicinity of coffee in Uganda harbours CWD.

10.2 Introduction

Our knowledge of CWD in the field is limited – how does it spread? How long do spores remain viable in the soil? If we had a good understanding of this, it would help to develop better ways to prevent and control the disease.

A range of activities were undertaken by CAB International and Coffee Research Institute (CORI) at Kituuza, Uganda to gain a better understanding about the survival

Phiri N. and Baker, P.S. (2009) *Coffee Wilt in Africa Final Technical Report*. CAB International.

and transmission of CWD under field conditions, and to identify possible sources of infection. These were undertaken at the Mayuge on-farm disease-mapping site and at CORI, and involved laboratory, screen house and field studies both on-station and on-farm. They included small-scale trials to study the possible transmission of CWD by machete, survival in (and infection from) soil and coffee wood pieces, an investigation of the occurrence of the disease on a number of plant species and the possibility of its occurrence on, and hence transmission by, insects commonly associated with coffee.

All trials were designed to provide at least preliminary data within a few months but which could continue well beyond the life of the current project. The trials were also designed to utilize or replicate, as far as possible, on-farm conditions and usual farm management practices, and to determine if changes in management practice may be of benefit in reducing the CWD transmission and spread. Four trials were established as described below.

10.3 Transmission and Spread of CWD Through the Use of a Machete

The machete and hoe are the two implements most commonly used by farmers and are used for a wide range of activities including pruning, weeding and digging. They may, therefore, play an important role in pathogen transmission and disease spread. A study was undertaken at CORI in a coffee field to investigate transmission of disease inoculum on a machete and subsequent development of CWD symptoms on coffee plants.

10.3.1 Experimental methods

Block 38 at CORI was selected, which had both mature CWD-affected coffee trees and trees considered to be susceptible to the disease but on which no external wilt symptoms had developed. Twelve trees showing no external symptoms of CWD were selected for wounding at one of three points: the stem base (ground level), midway along a primary stem and midway along a length of fresh green growth at the top of a stem. For each tree, only one wound was made and only at one of the three points.

Six of the trees were wounded with a sterile machete, two for each wound position, by wounding the bark to a depth of approximately 5 mm with the blade. The blade was cleansed prior to wounding each tree by swabbing with 70% (vol./vol.) alcohol and allowing to air dry.

The other six trees were wounded with an infected machete: a piece of stem, approximately 1 m long and exhibiting clear discoloration of the wood due to CWD throughout its length, was cut from a tree affected by CWD (grade 5) for use as the source of inoculum. These six trees were wounded as described above but, for each tree, by first making several cuts through the bark of the infected stem piece to infect the machete.

Trees were checked weekly for the development of external symptoms of CWD, scored according to a visible scale as described in Table 10.1, and recorded with the date on which these were first observed.

Table 10.1: Grading and associated external symptoms applied for assessing severity of coffee wilt disease (CWD) on coffee trees.

Severity grade	Description
0	Tree missing at the start of mapping (due to previous death by CWD or other reasons)
1	No CWD symptoms apparent
2	Leaves curling inwards
3	Dieback and defoliation of 1-24% of tree
4	Dieback and defoliation of 25-50% of tree
5	Dieback and defoliation of 51-75% of tree
6	Dieback and defoliation of 76-100% of tree, or tree dead
D	Tree dead and cut back to stump by farmer

10.3.2 Results

One of the trees wounded with a contaminated machete on the fresh green growth at the top of a stem developed symptoms of CWD above the wound point. The symptoms were first observed 49 days after wounding, and were classed as severe (grade 5). *Fusarium xylarioides* was successfully isolated from a piece of the stem showing disease symptoms. A second main stem on the same plant, which had not been wounded, also developed CWD symptoms 4 weeks later and by the end of the project, the entire plant had died. *F. xylarioides* was also successfully isolated from its stem.

A second plant, wounded midway along a primary stem with a contaminated machete, also developed CWD symptoms 223 days later. However, these symptoms developed on both of the two remaining untreated stems on this plant (1 to 3 weeks after the first) and did not develop on the stem that was wounded. Hence, the infection may have been unrelated to the initial wound.

Symptoms of CWD were not observed on any of the trees wounded with a cleansed machete.

Although it may be considered unusual for symptoms to develop so rapidly[1] on one of the two trees, these observations nevertheless suggest that wounding healthy trees with a machete previously used to cut or prune infected trees can lead to transmission of *F. xylarioides* and subsequent development of CWD.

Subsequently, some more controlled screen house wounding experiments on seedlings were carried out, but failed to induce infection, probably due to a fault in inoculum preparation. Further screen house and field experiments are needed with many more

[1] On wounding a mature tree with a cleansed machete and subsequently applying a conidiospore suspension (1×10^6 spores per millilitre) of *F. xylarioides* to the wound site, CWD symptoms are usually observed within 14–21 days and the tree killed after approximately 220 days (S. Olal, personal communication).

replicates to assess the probability of a disease-contaminated machete infecting a tree with one or more wounds. Importantly too, perhaps, the effect of using an infected hoe to disturb superficial roots near coffee trees should also be studied.

10.4 CWD Survival in Coffee Wood Pieces

Farmers consider coffee wood to be an excellent source of firewood. As such, old or damaged trees, including those that have been killed by CWD, are usually cut down and physically dragged across the farm to an area adjacent to the home. Here they are cut into short lengths and stored for gradual use as firewood. This practice may have obvious implications for the transfer of CWD inoculum to disease-free areas of the farm, partly through fallen wood pieces and *F. xylarioides* survival structures remaining on the ground, and for the establishment of a new inoculum source in the form of a wood stack near the home. Farmers also use diseased coffee stems as stakes for beans and other crops, another potentially significant way to infect soil.

A trial was established in the screen house at CORI to investigate the survival of *F. xylarioides* in coffee wood pieces and the role of wood pieces as a source of CWD. Sufficient soil to fill 125 cylindrical pots (8 cm diameter, 16 cm in height) was collected from a field site at CORI, where coffee had not been grown, and steam sterilized. Trees exhibiting severe (grade 5, Table 10.1) symptoms of CWD were identified from an affected site at CORI as a potential source of inoculum and stem pieces, 2 to 3 cm in diameter and exhibiting clear discoloration of the wood due to CWD throughout their length, were removed. Stem pieces were cut into 5 cm lengths until 144 wood pieces were available.

On establishment of the trial, all 125 pots were filled with sterilized soil. Ten pots were each planted with a single 6–8-month-old coffee seedling of *Coffea canephora* (variety Is/6), known to be highly susceptible to CWD. A single wood piece was placed horizontally on the surface of the soil of five of the pots planted with seedlings and at a distance of approximately 2 cm from the seedling to ensure that no contact was made with the seedling stem. The remaining five pots were intended as an untreated control throughout the course of the trial, on which CWD was not expected to develop.

Wood pieces were also placed in a similar manner in the remaining 115 pots without seedlings. At approximate 1-month intervals, a further five pots with a wood piece were each planted with a seedling. The remaining wood pieces were stored under cover in a CWD-free area and fungal isolations made from a single piece of stored wood at each stage at which seedlings were newly planted to check for the presence of *F. xylarioides*.

Seedlings were maintained in the screen house at CORI, where daily temperatures rise to a maximum of between 22 and 27 °C during the day and fall to between 15 and 18 °C during the night. Relative humidity ranged from 65 to 70%. Plants were watered twice weekly and checked at 2–3-day intervals for the development of external symptoms of CWD. Where symptoms developed, the date on which these were first observed was recorded and fungal isolations made on to tap water agar (TWA) medium to confirm the presence of *F. xylarioides*.

On seedling death, fungal isolations were made from leaves, stems and roots of a few randomly selected plants to check for the presence of *F. xylarioides* in plant tissues.

If no external symptoms of CWD developed on any seedlings after 6 months, one seedling per treatment would be removed and fungal isolations made to determine whether *F. xylarioides* was present in the plant tissues.

10.4.1 Results

None of the coffee seedlings planted in the absence of a wood piece on initiation of the trial subsequently developed symptoms of CWD. However, symptoms were observed on five seedlings in pots containing wood pieces:

- One seedling from the batch planted on the day that wood pieces were placed;
- Two seedlings planted at 33 days after wood placement;
- Two seedlings at 64 days after wood pieces were placed.

Symptoms were first observed on these plants at 95, 68, 76, 67 and 69 days, respectively, after the wood pieces were placed in pots. Formation of perithecia was also observed on several of these wood pieces. *F. xylarioides* was readily isolated from stored wood pieces at monthly intervals for up to 6 months after the collection of the wood from the field.[2]

These findings confirm that wood pieces obtained from the CWD-infected coffee trees can act as a source of infection for healthy coffee plants, and that seedlings planted in soil bearing wood pieces are at risk of infection for at least 64 days after the first contact between wood and soil, at least where seedlings and wood are in close proximity. However, from this trial, it is unclear whether seedlings became infected directly from the wood piece or indirectly through the soil.

10.5 Survival Potential of *F. xylarioides* in Soil

Circumstantial evidence (e.g. the use of susceptible seedlings as bait plants) strongly suggests that the CWD pathogen can survive in soil and act as an infection source to coffee plants for many months. This would appear to be true both for naturally infested field soil as well as soil artificially inoculated with the pathogen. In an example of the latter, soil obtained from a disease-free site, artificially inoculated with *F. xylarioides* and stored in sealed containers under laboratory conditions retained the capacity to cause CWD on planted, susceptible seedlings for many months (G. Hakiza, personal communication). However, the fungus retained the ability to infect seedlings for a longer period where the soil had been sterilized prior to the inoculation in comparison to soil that was not sterilized.

Information on the ability of *F. xylarioides* to remain viable in soil and retain the ability to infect coffee again has implications with respect to management of CWD. Where coffee plants affected by CWD are removed and destroyed by farmers, for example, it would help to clarify how long land must remain fallow or under an alternative crop before susceptible coffee may be replanted.

A trial was therefore established in the screen house at CORI to evaluate the survival or inoculum potential of *F. xylarioides* in field soil over time. Soil was obtained from

[2] CORI staff report that *F. xylarioides* has previously been isolated directly from standing coffee trees for a similar period following the death of trees due to CWD.

three field sites, Blocks 17 and 36 at CORI and a forest site (where wild coffee types were growing). CWD had been prevalent on coffee trees at all three sites for several years at high incidence and severity. A single coffee tree exhibiting severe symptoms of CWD was selected at each site and uprooted and removed. On uprooting, and at approximate monthly intervals following uprooting, soil from the hole was used to fill 20 cylindrical pots (8 cm diameter × 16 cm height). Each pot was planted with four 6–8-month-old *C. canephora* coffee seedlings. Seedlings were maintained and assessed as described for the wood piece trial above; additionally, both the date on which symptoms were first observed and the date on which plant death occurred were recorded (the period between the two to be used as a measure of infectivity). In addition, and on each occasion that seedlings were planted, a few grams of soil from each site was sprinkled on to the TWA medium and checked for growth of *F. xylarioides*.

10.5.1 Results

Nine of the coffee seedlings planted in soil obtained from the three CWD-affected sites developed clear external symptoms of CWD (Table 10.2). All seedlings were subsequently killed by the disease. These seedlings were among those planted in soil obtained immediately after tree uprooting and also at 47 and 95 days after uprooting.

Although this represents a relatively small proportion of seedlings planted, development of disease on seedlings planted in August showed that the soil had remained infective for 95 days or approximately 3 months. Furthermore, this was the case for soil obtained from all three sites. Although based on a small number of plants, a gradual increase was also observed in the time taken for disease symptoms to first appear on seedlings through the consecutive plantings, rising from 49–55 days to 150 days. CWD symptoms would be expected to develop on susceptible seedlings after approximately 50 days, when they are planted in sterile soil artificially inoculated with *F. xylarioides* (S. Olal, personal communication). This indicates that the level of infectivity of the

Table 10.2: Development of coffee wilt disease (CWD) on seedlings planted in soil obtained from the CWD-affected sites, Blocks 17 and 36 and a forest area.

Period between tree removal and collection of soil or planting of seedlings (days)	Source of soil	Number of seedlings with CWD symptoms	Period between planting to observation of symptoms (days)	Period between first symptoms to seedling death (days)
0	Block 36	2	55[a]	15[b]
	Block 17	1	55	39
	Forest	3	49[b]	10[b]
47	Forest	1	105	22
95	Block 36	1	150	15
	Forest	1	150	13
		Total = 9	Mean = 94	Mean = 19

[a] Mean of 2 plants.
[b] Mean of 3 plants.

field soil to newly planted coffee declines over time following the removal of diseased trees. Little difference was observed in the time taken from initial symptom development to seedling death (on average 19 days), with the exception of those seedlings planted in soil from Block 17 (39 days).

Attempts to isolate *F. xylarioides* from soil by sprinkling on to TWA and by dilution plating proved unsuccessful throughout the project – many other fungi were identified by this method, including several species of Fusarium, but not *F. xylarioides*. Successful development of a DNA-based diagnostic technique, while it would not differentiate between living and non-living fungal material, would constitute a major step forward in this respect. Further research is required to investigate why recovery of *F. xylarioides* from soil is problematic and to develop an effective approach to isolation.

10.5.2 A caveat

In the trials initiated through the project, and as described above, only a small proportion of coffee trees and seedlings planted in *F. xylarioides* infested soil, artificially inoculated with the pathogen or treated with contaminated implements or materials, developed symptoms of CWD prior to the termination of the project. In some instances, this may simply be due to the relatively low inoculum levels involved, or to the time required for symptoms to develop and become clearly visible. However, it is also possible that the low infection levels were related to the onset of an unusually dry period coinciding with when the majority of the trials were initiated, in May 2004. Average daily rainfall during May 2004 was only 2 mm, as opposed to 3.8 and 5.3 mm in May 2002 and May 2003, respectively. It is therefore recommended that the experiments are repeated to coincide with a more rainy and humid period.

10.6 Occurrence of *F. xylarioides* on Insects Associated with Coffee

To help determine if vectors could be involved in transmission of CWD, a range of insect pests, bees and other pollinators and termites were collected from coffee fields at CORI and at Kawanda Agricultural Research Institute (KARI), Uganda, where CWD is prevalent on *C. canephora*. Insect pests and coffee fauna were also collected at field sites in Mukono, Luwero, Mpigi, Bushenyi, Kanungu and Kamwenge districts of Uganda.

Fungi present on the surface of the insects were investigated by washing these insects in sterile distilled water, removing the insect and transferring the washing from each insect on to a TWA plate amended with 150 mg/l streptomycin sulfate. Washed insects were dried carefully by placing on sterile, absorbent paper, aseptically cut into several pieces and also placed on to TWA, one per plate, amended with 150 mg/l streptomycin sulfate. Plates were incubated for 3 days at 25 °C and monitored for growth of fungal organisms. Emerging fungal colonies were subcultured on to the synthetic nutrient agar (SNA) and potato-dextrose agar (PDA) and identified to genus or species level.

10.6.1 Results

F. xylarioides was not isolated from any of the insects obtained from the CWD-affected sites. Other Fusaria were recovered, including *F. oxysporum* from coffee berry borer and scale insects, *F. solani* from termites, bees, leaf skeletonizers and attendant ants, *F. stilboides* from beetles and *F. semitectum* from fruit flies and beetles. A *Curvularia* species was isolated from coffee berry moths.

10.7 Occurrence of *F. xylarioides* on Other Plants

It was previously reported that *F. xylarioides* was recovered from surface-sterilized roots of the banana cultivar 'Kayinja' (syn. 'Pisang Awak') obtained from two farms, in Mpigi and Kibale districts of Uganda, where banana was intercropped with coffee (Serani, 2000). Kayinja and other crops are frequently grown alongside coffee, particularly in Central Uganda, and the finding suggests that these may provide an alternate host for the CWD pathogen and therefore have some influence on the survival of *F. xylarioides* and disease spread. They may also be of relevance with respect to CWD management where, for example, their cultivation is being considered as an intercrop or rotation crop or to help reduce pathogen inoculum levels in soil following the removal of coffee affected by CWD.

During a visit to the Mayuge on-farm mapping site, plants of a number of commonly cultivated bean types (white haricot beans, a local 'black bean' and beans used for drying ('K132')) as well as weed species (Table 10.3) growing within 1 m of coffee trees exhibiting moderate-to-severe external symptoms of CWD (i.e. severity grades 5–6) were uprooted. The plants were taken to the laboratories at CORI, where the adhering soil was shaken off, and the roots and lower stem were washed under the running water.

Fungal isolations were attempted from the lower stem and roots by cutting root and stem pieces, 0.5–1.0 cm in length, which were placed on to TWA medium (two to three pieces per plate) both before and after the surface sterilization. Root and stem pieces were surface sterilized by immersing in sodium hypochlorite solution (2% a.i.) for 1–2 min, rinsed two to four times in sterile distilled water and gently dried by placing on sterile tissue paper. Roots and stems were placed on separate agar plates and the TWA medium amended with 150 mg/l streptomycin sulfate to inhibit bacterial

Table 10.3: Weed species and bean types collected from Samuel Kirunda's farm for isolation of *Fusarium* spp. from lower stem and roots.

Weed species	Common local name	Common English name
Synedrella nodiflora	Makai (Lugandan)	
Solanum nigrum	Katukuma (Lugandan)	
Ageratum conyzoides L.		Goat weed
Oxygonum sinuatum	Kafumita bagenda (Lugandan)	
Euphorbia heterophylla	Kisandasanda (Lugandan)	
Senecio discifolius		
Bidens pilosa	Sere (Lugandan)	Black jack
Rottboellia cochinchinensis (Graminae)	Lajanawara (Luo)	

growth. Plates were incubated at 25 °C for up to 7 days and, where emerging fungal colonies emerged, these were picked off on agar blocks, subcultured on to fresh SNA medium and identified to species level where possible.

10.7.1 Results

Although a number of *Fusarium* species were recovered from both sterilized and non-sterilized roots of the various weed species and beans collected from the Mayuge farm site, these did not include *F. xylarioides*. No fungi were recovered from the plant stem pieces. This would indicate that, at least at the time of investigation, none of these plants were harbouring the CWD pathogen. The finding is strongly supported by a comprehensive survey undertaken across six coffee-growing districts in Uganda having a high CWD incidence (Kangire *et al*., 2002). Of 270 plant samples obtained, covering 105 species of cultivated crops and a number of weed species, *F. xylarioides* was not recovered from any plant parts, including roots. Several other Fusaria were again recovered, including *F. oxysporum* from Kayinja, a species responsible for Panama disease of banana in Uganda and elsewhere.

Of note, *F. xylarioides* isolated from cotton seed has previously been reported to be pathogenic to cotton seedlings under laboratory and (less so) in glasshouse conditions (Pizzinatto and Menten, 1991). It has also been isolated, among other known fungal pathogens, from rot of tomatoes obtained from fruit markets in Nigeria. Artificial inoculation of tomato fruits confirmed that it could cause a slow-spreading soft rot (Onesirosan and Fatunla, 1976).

Screen house studies, involving artificial plant inoculation with *F. xylarioides*, have been initiated by CORI to assess pathogenicity of the fungus to seven solanaceous crops, a *Phyllanthus* species and Kayinja, and to assess their ability to act as hosts for the pathogen. These experiments were still ongoing at the end of this study.

10.8 Conclusions

The project research has helped to clarify the mechanisms of the CWD transmission and disease spread. Early indications from a number of small-scale on-farm and on-station trials established during the project suggest that wounds made with a machete previously used to cut infected coffee wood would be sufficient to transmit the pathogen to, and induce CWD development on, healthy coffee, at least in the case of mature trees. However, further work is required with many more replicates to establish the probability of infection, especially for example with machete wounds near the soil or pruning of branches, under a wide range of field conditions. This will require a standardized infection method, which was attempted in the present study but further refinements may be needed.

Pieces of coffee wood, an important source of fuel for farmers, can also act as a source of infection to young coffee seedlings where the wood is already affected by CWD, while infested field soil can remain infective to seedlings for at least 3 months after the removal of affected coffee trees from fields. However, the infectivity of such soil, as shown by the time taken for the CWD symptoms to appear on newly planted seedlings, does appear to decline in the absence of an affected tree.

Despite speculation that insects may play a role in the transmission of *F. xylarioides*, the fungus was not found on the body parts of a range of insects commonly found on coffee farms and associated with coffee itself, including pests, bees and termites. Nor was the fungus found on a wide range of potential alternative hosts, including crops commonly cultivated alongside the coffee and weed species occurring on coffee farms. These findings, while still somewhat preliminary, do generally concur with currently held perceptions on CWD and, more importantly, provide evidence to support current recommendations being promoted in some areas with respect to the on-farm CWD management.

Based on the results of the present study as well as that of others, sanitation advice is outlined in Box 10.1.

Box 10.1

On-farm sanitation methods to reduce the spread of coffee wilt disease (CWD).

Cleansing: Carry out regular cleansing of farm implements, at least following use on trees exhibiting CWD symptoms and prior to use on trees that appear to be healthy. This may be achieved by wiping down with a household disinfectant (e.g. 'Jik'), although this is often not possible or practical for many farmers. Heating tools in a fire is an alternative.

Wounding: Efforts should also be made to minimize inadvertent wounding of trees, including through the use of implements such as machetes, spades and hoes during planting and weeding, improper pruning and the tethering of domestic animals to tree stems or sufficiently close to coffee plants to cause damage.

Tree disposal: Diseased trees should be uprooted and burned as soon as CWD symptoms are first observed and preferably burnt *in situ*. Tree stumps should also be removed and burned, and not left to rot (as is current practice) as they constitute a source of inoculum.

Fallow: Coffee should not be immediately replanted in fields where CWD has been found. A period of at least 3 months is required, and since other plants do not harbour the infection, it would be better to plant another short-cycle crop and leave a gap of 1 year before replanting. Wherever possible, a species or variety resistant to CWD should be replanted.

Firewood: Coffee wood is a valuable source of firewood for farmers, where unhealthy trees including those affected by CWD are often cut down and stored as firewood on-farm, usually adjacent to the home, for many months. Wherever possible, the use of diseased coffee for firewood should be discouraged. Where coffee wood is required for firewood on farms affected by CWD because of shortage of other wood, this should be obtained in a manner that minimizes inoculum spread – cutting into required lengths *in situ* is recommended, followed by transfer of the cut wood to a bag or sack that can be sealed or tied up before transfer of the wood to a storage site. If feasible, the wood should also be moved through an already affected part of the farm as opposed to disease-free areas. However, it is unlikely that most farmers will observe these safety practices.

Seedlings: Depending on the availability, both seeds and vegetative cuttings may be used as planting material. Although farmers and nursery operators prefer seeds, which are easier to handle, semi-soft wood cuttings from upright growing shoots are also taken from mother bushes maintained to provide such a supply. Planting materials, in the form of either vegetative cuttings or seedlings, are also distributed from private nurseries to farmers by local authorities. It is important to consider that while coffee material, including trees, stem pieces and seedlings, may not exhibit symptoms of CWD, it may still be contaminated or infected with *F. xylarioides*, and therefore act as a source of infection. Even where symptoms are present, these may not be recognizable to the inexperienced eye or may be overlooked.

Nationwide sanitation: Quite apart from farm-level activities to prevent CWD spread, it is important also to consider actions at a broader scale. Regional or national distribution of coffee material may constitute an important and very efficient means of spread for CWD (and indeed other diseases and pests), and may at least partly explain the rapid and widespread development of CWD in countries such as Uganda. It is recommended that nurseries and distribution systems be investigated further, specifically with respect to how farms from which seed[3] or cuttings may be obtained are selected, and to what extent contamination of plant material by *F. xylarioides* is possible. New coffee plantings established with distributed plants should also be monitored to determine what risk they may present in terms of the disease.

Where areas are replanted with coffee after implementing such measures, the *F. xylarioides* type originally present should be determined and coffee known to have resistance to that type planted, if possible. Replanted areas should also be closely monitored for re-emergence of the disease and recommendations revised accordingly. Care should also be taken to prevent reintroduction of CWD and spread of the disease between farms generally, by restricting or preferably preventing exchange of planting material.

[3] Coffee berries do not appear to constitute an important source of infection. CORI previously assessed 43 coffee berry samples obtained from 43 trees slightly, moderately and severely affected by CWD using standardized protocols whereby 400 berries per sample were plated on to potato-dextrose agar (PDA). *F. xylarioides* did not emerge from any of the berries. Plantlets were also raised from 50 berries from each sample. Of the plants raised from all of the berries, and after 1 year of growth, only one plant developed the symptoms of CWD.