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Coffee wilt disease

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1 Introduction

During the 20th century, a devastating disease called Coffee Wilt Disease (CWD) spread across Africa, reducing yields, destroying millions of coffee trees and costing millions of USD in lost earnings for producers (Flood, 2009). The disease has also contributed to a decline in the revenues of several African nations. CWD reached epidemic proportions twice in the 20th century – through the 1930s, 1940s, 1950s – and was responsible for the failure of the commercialisation of excelsa coffee. However, following the systematic introduction of sanitation methods (uprooting and burning affected bushes *in situ*) together with comprehensive breeding programmes in affected African countries, CWD appeared to decline in incidence and was considered a minor disease (Flood, 2009). Yet the disease re-emerged initially as a series of sporadic outbreaks on robusta coffee in remote areas of Central Africa during the 1970s. Later, incidence increased to epidemic proportions in the last decades of the 20th century and into the 21st century (Flood, 2009), affecting robusta coffee in DRC, Uganda and Tanzania. In addition, the disease has become an increasingly important constraint to production of arabica coffee in Ethiopia (Girma et al., 2001, 2009a).

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CWD, also called Tracheomycosis, is a vascular wilt disease. Initially, several fungi were implicated as the causal agent, but in 1939, Steyaert (1948) isolated a species of *Fusarium* called *F. xylarioides*. In 1956, Saccas reported that the pathogen produced perithecia on the bark of affected trees. The perithecia represent the sexual stage *Gibberella xylarioides* (Heim and Saccas, 1950). In Ethiopia, symptoms similar to those of CWD in West and Central Africa were documented on *Coffea arabica* for the first time in the Keffa province by Stewart (1957) and later by Lejeune (1958). Based on comparative studies of isolates from arabica taken from different origins and different *Coffea* species, the disease was confirmed to be caused by *F. xylarioides* (Kranz and Mogk, 1973). Unlike many other coffee diseases, CWD kills trees. The pathogen evolved in Africa and the disease remains, to date, confined to the African continent. There is an unconfirmed report of the disease in the Americas – in El Salvador (Abrego, 1965) – but is likely a mis-identification (CABI, 2020). There is only one report of the disease in South Africa and Swaziland (Anon., 1989), which may indicate that *F. xylarioides* is not common in Southern Africa, but further surveys are needed to confirm absence or presence and extent of distribution. It is also highly likely that there has been considerable confusion with other *Fusarium* species (CABI, 2020).

This chapter discusses the history, impact and spread of this disease as well as its symptoms, host range and management. The chapter also provides a summary of the outputs of the Regional Coffee Wilt Programme (RCWP), which examined many different aspects of CWD and its management following its re-emergence as a significant threat to sustainable coffee production. Future research trends include investigating host specificity in the pathogen as well as underlying resistance mechanisms in the host and the role of alternative hosts as a means to reduce field inoculum. The ecology of the fungus also needs further investigation to allow for a greater focus on more agroecological management practices.

2 History and impact

CWD was first observed in 1927 in a plantation of *Coffee excelsa*, near Bangui in the Central African Republic (CAR), then known as Oubangui-Chari (Figueres, 1940). The disease developed slowly but by 1942 it had become a serious problem throughout the country and, by 1945, had destroyed most of the country's excelsa plantations (Saccas, 1951). It is generally considered that CWD was chiefly responsible for the permanent collapse of excelsa as a commercial crop. It is likely that initially, the pathogen was present in wild coffee species and had co-evolved with these (possibly with limited symptoms) but with the planting of large areas of excelsa and its apparent greater susceptibility, then disease symptoms became more apparent. Once the

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pathogen had become established in the excelsa crop, it subsequently spread rapidly through plantations due to agricultural practices. Later Saccas (1956) also found the disease on *C. canephora* and *C. neo-Arnoldiana* in parts of CAR; all species of *Coffea* including wild relatives are susceptible to the pathogen to some degree. In 1939, Steyaert (1948) discovered it on excelsa in plantations in DRC (Democratic Republic of Congo, formerly Zaire and previously the Belgian Congo) close to the border with Sudan at Aba (Fraselle, 1950). Initially, it was considered a minor problem but, as with CAR, it later reached epidemic proportions (Fraselle et al., 1953) when it spread to robusta. Subsequently, it was found to have extended to Haut-Uélé, North Kasaï and Katanga (Kalonji-Mbuyi et al., 2009). Between 1938 and 1945 the disease became established on excelsa in Cameroon, causing complete destruction of plantations in the east of that country. Then in 1947, it was discovered on *C. canephora* in Côte d'Ivoire and major losses occurred there into the1950s, with more than 50% of the coffee-producing areas being destroyed in both Côte d'Ivoire and then DRC too. The Kouillou line of robusta was completed destroyed in Cote d'Ivoire (Flood, 2009). In 1958, the disease was reported in Guinea and spread quickly to most of the coffee areas, causing coffee production to fall by nearly 50% (Chiarappa, 1969).

3 Host range

The pathogen attacks all wild and cultivated species of *Coffea*. *Fusarium xylarioides* has also been reported to be pathogenic to cotton seedlings (IAC 20 cultivar) under laboratory conditions, less so under glasshouse conditions (Pizzinatto and Menten, 1991). Interestingly, it has been detected in the banana cultivar Kayinja syn 'pisang awak' in Uganda (Serani et al., 2007), which is often intercropped with coffee for the production of banana juice and banana beer (Rutherford et al., 2009). Serani et al. (2007) raised the possibility of solanaceous weeds being reservoirs of infection in coffee fields following the report of the pathogen being isolated from rotting tomatoes from fruit markets in Nigeria (Onesirosan and Fatunla, 1976). The report of pathogenicity to cotton also raises the possibility of other *Malvaceae* being alternative hosts.

4 Symptoms

The pathogen invades the coffee host and colonises the vascular system. Colonization of the vascular system induces host responses which disrupt water conduction, and this is manifested as wilting and desiccation of the leaves which turn inwards (Fig. 1). This is followed by defoliation and dieback of the affected branches (Fraselle, 1950). See also Fig. 2. Coffee berries ripen prematurely (Fig. 3). Swelling of the trunk can be seen as well as a series of vertical or spiralling cracks in the bark (Fig. 4). Small blackish-brown perithecia

Figure 1 Initial symptoms on infected tree – leaves turn inwards © Julie Flood, CABI.

Figure 2 Defoliation and die-back follow © Noah Phiri, CABI.

(sexual stage) may be seen in these cracks. Internally, a diagnostic blue-black coloration of the wood is seen (Flood, 1997). See also Fig. 5. Symptoms appear at any stage of crop growth and the rate of symptom development is variable. Symptoms may be pronounced on one part of the tree, a likely consequence of initial infection occurring in one of the main stems but the symptoms gradually extend to the whole plant. Juvenile plants may be killed in a few weeks from the initial observation of symptoms while mature trees may take 3-15 months to die.

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Figure 3 Premature ripening of coffee berries © Julie Flood, CABI.

Figure 4 Swelling at the base of the trunk with tiny, black perithecia (sexual stage) in the cracks © Julie Flood, CABI.

5 Ecology of the fungus

The pathogen is considered to be a soil-inhabiting fungus, but its life cycle and epidemiology are not fully understood. The fungus can be readily isolated from stems, twigs and roots of diseased coffee plants, but isolation from the soil has proven difficult. This may suggest that the fungus is unable to compete well with other soil-borne microbes and remains within infected plant debris in soil or, potentially, within alternative hosts. Host infection, colonization and symptom development are thought to be similar to other vascular wilt pathogens (Rutherford et al., 2009). Initial entry is considered to be primarily

Figure 5 Blue-black staining under the bark of an infected tree © CABI.

through roots and lower stem, facilitated by the presence of wound sites as a consequence of management practices (slashing) or from livestock grazing in the coffee fields. Once inside the plant, the fungus colonises the vascular system and surrounding tissues. The observed blue-black staining in the wood is probably due to changes in phenolic metabolism and may constitute a host response (Beckman, 1987)

Perithecia are commonly observed on the wood at the base of infected coffee in the field and are primarily seen as sexual reproductive structures but may also be a means of survival in the absence of chlamydospores (Flood and Brayford, 1997). Ascospores (sexual spores) are produced within the perithecia. Profuse production of conidia (macro and micro conidia) was noticed on mycelium at the base of stems (Fraselle, 1950). All these spore types could disseminate the pathogen and cause infection-conidial suspensions, have been routinely used to infect plants for experimentation. A putative life cycle of the CWD pathogen has been proposed by H. Maraite based on earlier drawings (Rutherford et al., 2009), but more study of the life cycle is needed.

Studies on the spatial and temporal development of CWD (Rutherford and Flood, 2005; Musoli et al., 2008) revealed that plants from a range of susceptible clones showed increased infection of less than 3% to 45% over a 2 year period, with an average of six plants developing external symptoms each month. These studies indicated that initial foci were randomly distributed and these foci enlarged over time but in no particular direction. Infection of adjacent trees was observed, suggesting root-to-root contact or short-distance dispersal of fungal material (such as via rain splash) as being involved in disease

spread, together with on-farm practices such as through wounds made at the base of the bushes during weeding. Transmission did not appear to be linked to wind direction. Musoli et al. (2008) further reported that in the early stages of plot infection, the effective range of influence of an infected tree was up to two trees away, but this rose to three trees at the end of a 6-year observation. This would suggest that once a tree exhibits disease, all trees within a three-tree radius (10m) should be grubbed out and destroyed.

6 Historical management recommendations

When CWD first emerged as a significant constraint to coffee production in the mid-20th century, management options considered were a combination of sanitation and breeding for resistance, although some chemical and biological options were also investigated.

Cultural practices included frequent inspection of the crop, along with uprooting and burning of infected material (*in situ*). Removal of coffee bushes ahead of the infection (to reduce spread between plantations) was considered effective in Cote d'Ivoire; gaps of a few hundred metres were considered enough to confine the disease (Deassus, 1954).

Several authors reported varietal differences in resistance to the pathogen, and suggested the use of resistant varieties as a means of control (Fraselle, 1950; Bouriquet, 1959; Porteres, 1959). Cultivars of *Coffea canephora* (notably robusta) then formed the basis of many of the West African breeding programmes in the mid-20th century. Resistance mechanisms were unknown but preliminary studies indicated that caffeine, thought to inhibit the pathogen, was detected in higher concentrations in tissues of *C. canephora* than in *C. liberica* (Rabechault, 1954) and a higher content of chlorogenic acid in the wood of resistant material (variety Robusta) was also reported (Bouriquet, 1959). Similarly, van der Graaff and Pieters (1978) reported that coffee lines of *Coffea arabica* in Ethiopia also differed widely in resistance to *F. xylarioides* and considered that these differences provided an excellent opportunity to control the disease with resistant varieties. Pieters and van der Graaff (1980) reported two methods of screening for resistance – a seedling test which involved wounding seedlings with a knife dipped in spore suspension and a conidial germination test conducted directly on the bark of the tree. Both tests correlated significantly with field scores and with each other, and provided the basis for a screening programme to be used in a more extensive programme involving selection for resistance to Coffee Berry Disease (*Colletotrichum kahawae*) and *F. xylarioides*. van der Graaff and Pieters (1978) suggested that resistance in *C. arabica* was likely qualitative in nature with no evidence of vertical resistance. However, Zadoks and van Leur (1983) re-analysed the data from Pieters and van der Graaff (1980) and demonstrated a significant cultivar-isolate interaction indicating that vertical

resistance was involved. Girma et al. (2009b) further corroborated the work of Zadoks and van Leur (1983). The nature of the resistance has considerable implications for breeding, with a more qualitative resistance being likely to be more sustainable while vertical resistance (involvement of major genes) could be more easily overcome by evolution of more virulent races of the pathogen.

Chemical control was also investigated and spraying the soil surface with 2.5% copper (II) sulphate, was advocated as an effective control measure (Saccas, 1956), while Gaudy (1956) reported that spraying the affected coffee bushes with copper oxychloride was effective in controlling the disease. However, the immense costs of using this approach as well as its environmental impact meant that the chemical approach was rarely used.

With regard to biocontrol, Rabechault (1954) isolated four actinomycetes, one bacterium and *Corticium*, *Marasmius* and *Trichoderma* spp., all of which were inhibitory to the pathogen, but this avenue of research does not seem to have been much pursued and there are no biological control agents currently available. Consequently, sanitation and screening/breeding for resistance were prioritised for the management of the disease. An international conference held in 1956 made recommendations for a management programme for CWD to include (1) systematic sanitation over vast areas where affected coffee plants were uprooted and destroyed; (2) where possible, relocation of coffee production to new locations; and (3) replanting with more resistant germplasm. Implementation of these recommendations reduced the impact of CWD. However, it should be noted that much of this early work in the mid-20th century involved a plantation model of coffee production where large-scale, systematic sanitation (over several hundred metres ahead of the disease front) was possible. Decisions were made by relatively few people and there was an ample supply of labour.

7 Re-emergence

Successful implementation of the recommendations above appeared to reduce the impact of the disease and literature produced during the 1970s and 1980s referred to CWD as being a minor disease, of little importance to arabica and robusta coffee production (Flood, 2009). Yet in the remote areas of DRC, the disease continued to be observed around the abandoned plantations in the north-east of the country (Kalonji-Mbuyi et al., 2009). As early as the 1970s, farmers in Aketi (76 km from Isiro) had observed the disease in abandoned plantations and during a survey conducted from 1974 to1975, a number INERA (Institut National pour l'Etude et la Recherche Agronomique) fields around Yangambi were also reported as affected. Reports continued throughout the 1980s around the town of Isiro, where abandoned plantations appeared to be the focus for the disease.

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In 1995, CABI's plant health diagnostic laboratory isolated *F.xylarioides* from samples of robusta coffee from the Beni and Rutchuru areas in eastern DRC. The International Coffee Organization (ICO) facilitated contact between Office Zairois du Café (OZACAF) and CABI in March 1996 and requested that a plant pathologist be sent to conduct an independent assessment of the situation in country; this was the start of my own personal involvement with CWD.

I travelled to the North East of DRC in July 1996 and conducted surveys of plantations and smallholder farms as well as interviewing farmers about the disease they called 'coffee AIDS'. Growers wanted information on the management of the problem. Unfortunately, the only recommendations available was the same as in the 1950s – sanitation coupled with long-term breeding programmes. On some plantations, sanitation was being conducted, but the disease was spreading faster than the workers could undertake the work (Fig. 6). On one plantation I visited, 80% of the production area had already been destroyed. For smallholders, uprooting of infected coffee bushes *in situ* using just hand tools was impossible (Flood, 1996).

I undertook a similar visit to Uganda in 1997 as similar symptoms had been seen on coffee in Uganda. From 1994, samples from the districts in Western Uganda had been received by the Coffee Research Institute (CORI) and the pathogen isolated (Hakiza et al., 2009). Several surveys had already been undertaken in Uganda and the pathogen confirmed. The disease was also confirmed in a CORI experimental plot at Kituza in Mukono (Hakiza et al., 2009). After 1993, the incidence and severity of CWD continued to rise in Uganda and yields began to decline. In the worse affected districts. 40-50% of coffee fields

Figure 6 Affected plantation in Zaire in 1996. Gaps in the foreground indicate losses © Julie Flood, CABI.

were completely destroyed or abandoned. An estimate by the Ugandan Coffee Development Authority (UCDA) in 1999 indicated that 14.5 million robusta coffee trees (4.8% of the coffee crop) were destroyed country-wide. Yield loss at a district level indicated that CWD caused 77% loss in yield of robusta coffee at the national level (Hakiza et al., 2009)

Initially, in Uganda, there was some success with sanitation at the experimental farm but the approach proved unsustainable due to the high labour cost and the spread of inoculum from coffee in the adjacent forest introducing fresh inoculum. Initially, only robusta coffee was affected in Uganda, but Rutherford et al. (2009) also reported that the disease had been observed on excelsa coffee held at a coffee germplasm conservation site; arabica coffee was free of symptoms in Uganda.

I confirmed the presence of the pathogen (Flood, 1997) as reported by the national scientists and recommended regional collaboration as a means of managing this disease. A proposal was developed as a fully integrated programme of activities addressing different aspects of the disease and its management. Five interrelated and interdependent projects were developed, each dealing with specific components. The collective name of these projects was the Regional Coffee Wilt Programme (RCWP).

8 Case study: Regional Coffee Wilt Programme (RCWP)

The Regional Coffee Wilt Programme (RCWP) (2000–2007) was co-ordinated by CABI, the co-ordination being funded by the CFC.

Activities of the RCWP included biological and socio-economic surveys in project participating countries (DRC, Uganda, Ethiopia, Tanzania, Rwanda, Cote d'Ivoire and Cameroon) to ascertain the extent of the disease and its impact on livelihoods. These surveys were funded by the EU through the Association for Strengthening Agricultural Research in Eastern and Central Africa/African Coffee Research Network. The RCWP also funded research on durable resistance (through EU-INCO-DEV), epidemiology and pathogen variation (funded by UK Department for International Development (Crop Protection Programme) and EU INCO-DEV). There was considerable focus too on extensionist and farmer training and the raising awareness of the disease to facilitate surveillance and limit spread. This component was also funded by the CFC. For further details of the interrelations between the projects see Phiri and Baker (2009).

Summaries of the findings are outlined in the following sections.

8.1 Biological surveys in country

Major findings from the surveys undertaken, indicated that CWD was present in four of the participating countries – in DRC, Uganda, Tanzania on robusta coffee and in Ethiopia on arabica coffee. The disease appeared to be absent from

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other surveyed countries (Rwanda, Cote d'Ivoire and Cameroon). In Ethiopia, several surveys undertaken prior to the RCWP had indicated the presence of CWD in the major coffee-growing regions (Girma, 1997). From the surveys undertaken under RCWP, it seemed that in Ethiopia, CWD was affected by the farming system with lower rates of infection in forest and semi-forest coffee but much higher rates in garden and plantation coffee (Phiri and Baker, 2009). These observations could be linked to the degree of intervention, with greater opportunity for the spread of the pathogen through agronomic practices. Phiri and Baker (2009) reported that the incidence of CWD under plantation conditions ranged from 77% in Chira to 10.8% in Gomma with a mean of 31%+/− 18% so there was considerable variation in effect. These authors also commented that the strains affecting arabica coffee in Ethiopia may be less aggressive than the strains attacking robusta; Ethiopia had continued to increase national production (Phiri and Baker, 2009). Nevertheless, Girma et al. (2009a) reported many coffee-producing areas had over 50% of infected trees on-farm. For example, the disease was particularly high in garden coffee in the quality coffee production zones of Sidama and Gedeo (with an incidence of over 90% and severity of 25%).

In DRC, the surveys revealed that the disease was present in North Kivu, Oriental and Equateur provinces but absent in provinces of Bandundu, Bas Congo, Kasai Occidental, Kasai Orientale and South Kivu (Kalonji-Mbuyi et al., 2009). The highest incidence of the disease was in Uganda, where 90% of farms surveyed had CWD and an average severity of 45%. In Tanzania, CWD was only detected in the Kagera region that borders Uganda, but the incidence and severity were increasing in that region (Kilambo et al., 2009).

8.2 Socio-economic surveys in country

Socio economic surveys were also undertaken under the RCWP, and the results suggested that in many of the participating countries, coffee was in a poor state with poor soil fertility and old tree stock (>59% over 15 years old). None of the farm characteristics, agronomic practices or environmental characters had a consistent effect on CWD occurrence across Uganda, Ethiopia and Tanzania. However, many farmers weeded using hand tools – machete and hoes – which can wound the trees, aiding the invasion of the pathogen. Interestingly, Tanzanian farmers conducted more mulching and intercropping together with less pruning and slashing (all practices believed to lessen the spread and impact of the disease). This may have contributed to the overall lower incidence of CWD seen in Tanzania (Phiri and Baker, 2009), but the outbreak was confined to one area in that country. In contrast, Ugandan farmers used lower levels of fertilization and highest level of pruning and did little weed slashing, but still the incidence was high in the country. Farmers' awareness of CWD varied widely

from country to country, with 90% of farmers being aware of CWD in Uganda but only 17% in Ethiopia and 15% in Tanzania.

Farmers found it difficult to understand the nature of the disease and this was reflected in their use of diseased coffee wood – often dragging it through their stands of living trees, for use as firewood or selling it as firewood to others. Many used diseased stems as supports for other crops (tomatoes and beans) as well as using it for fencing, or for house and animal shed construction. These actions undoubtedly contributed to the spread of the disease. Initially, farmers tried to up-root and burn the affected bushes (47%) or conducted pruning (26%), although this was not practiced in Ethiopia. Cutting down the trees and burning the above ground materials had also been attempted (12%) whilst some farmers tried removing the bark of infected trees (7%). The use of various "concoctions" such as ash, cow urine had also been attempted. Yet by the time of the surveys, 80% of farmers interviewed had given up trying to manage CWD because they considered the activities to be ineffective. Some farmers (6%) also indicated that management was too labour intensive – they could not undertake measures themselves and hiring labour was too expensive. Farmers surveyed obtained management information from extension staff (37%) or from other farmers (22%), with 19% receiving information from the radio, newspapers, pamphlets and direct contact with researchers. As farmers could not undertake management options, many farmers adopted coping strategies (Phiri and Baker, 2009) which included diversification of crop and activities such as brewing and trade or opening new coffee fields, etc. Many farmers in Uganda had abandoned their fields when the disease was spreading quickly in the country (early 1990s–early 2000s), while in Ethiopia, farmers replanted in a new locality (77%) or replaced with new coffee bushes (22%). There was less diversification in Ethiopia, which again may suggest a less aggressive strain or there was a greater possibility to relocate their coffee production to new areas.

Phiri and Baker (2009) concluded it was difficult to summarise across the countries, but they suggested a combination of education and motivation lead some farmers to make sensible agronomic choices. For details of the history, spread, incidence and management of CWD in each participating country, see chapters in Flood (2009).

8.3 Studies of the pathogen

Also as part of the RCWP, studies of pathogen variability were undertaken.

The presence of a sexual stage (*Gibberella xylarioides*) implies some degrees of variability, but relatively little was known about this. With the emergence of the pathogen as a major constraint to coffee in Africa, coupled with the development of molecular methodologies, there was an opportunity

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to examine the pathogen. A broad range of primarily PCR-based approaches, microsatellite loci, AFLPs, RAPDs, ISSRs, nuclear housekeeping genes, IGS and ITS regions were used. Several hundred isolates of the pathogen were examined (Rutherford et al., 2009) and although overall variability within the pathogen appeared to be limited, two major distinct populations were detected (Adugna et al., 2005; Tshilenge-Djim et al., 2004). The first were those isolates obtained from robusta coffee affected in DRC, Uganda and Tanzania (since the re-emergence of CWD), while the second group of isolates was obtained from arabica coffee in Ethiopia. Genetical variability among isolates recovered historically from robusta and excelsa coffee from CAR, Guinea and Côte d'Ivoire indicated they were all different to the two populations responsible for the disease in the 1990s (Rutherford et al., 2009). Yet, isolates from robusta coffee in the late 20th-century epidemic were identical to those in DRC in 1960, which suggested that CWD had never been completely eradicated in DRC after the mid-20th-century outbreak and the disease has spread from DRC to neighbouring countries (Lepoint, 2006; Phiri and Baker, 2009). Unfortunately, the original isolate from Steyaert (1948) is lost, so we can never know how that links to the later isolates.

Mating trials between the isolates from the different hosts (arabica, robusta and excelsa) suggested three biological species. A fourth group was suggested as a group of incompatible strains. Lepoint et al. (2005) and Lepoint (2006) proposed that there could be four different species, namely, *Gibberella abyssiniae* (responsible for the Ethiopian outbreaks *on arabica); G. congoensis* (responsible for epidemic in DRC, Uganda and Tanzania on robusta); *Fusarium quineensis*, an asexual form responsible for the mid-century outbreaks in Guinea, Côte d'Ivoire and possibly CAR; and *Gibberella xylarioides*, originally described as a disease on *Coffee excelsa*. Investigating the host-pathogen interactions, Girma et al. (2009b) reported host specialization with isolates from robusta causing disease on robusta but not on arabica and vice versa. This linked to previous field reports of the disease affecting robusta in Uganda but not arabica in that country (Flood, 1997). Yet one strain (DSMZ62457) caused some mortality in seedlings of robusta, arabica and excelsa, and Girma et al. (2009b) suggested that further work was required to confirm host specificity.

8.4 Host variation and screening for resistance

Significant variations were detected between wild and cultivated populations of robusta coffee in Uganda. Ugandan genotypes were sufficiently different from the Congolese and Guinean types to represent a genetically distinct diversity within the *C canephora* genome. It was suggested (Phiri and Baker, 2009) that these valuable resources need to be planted at secure conservation locations as a matter of urgency because of problems with CWD and with rapid

deforestation. Implementation of *in situ* conservation would also help sensitise local communities to ensure farmers multiply, conserve and utilise the plants in their own fields to maintain genetic diversity (Musoli et al., 2006).

In addition to conservation of valuable genetic resources, screening robusta coffee for resistance was also undertaken in Uganda, DRC and Tanzania. Various methods were evaluated in Uganda to establish a reliable, cost-effective method of evaluating coffee germplasm to CWD. Two methods were trialled: the root dip method, where coffee roots were dipped in a spore suspension of the pathogen for 20 minutes, and, the stem nicking method, involving scraping the stem of coffee seedlings with a scalpel (with inoculum). Standard concentrations and exposure times established that spore concentrations as low as 13 spores per ml were sufficient to cause seedling death (Phiri and Baker, 2009). Wild robusta from the Kalangala and Itwara forests showed a high level of resistance to CWD. In Uganda, there was a considerable variation in the susceptibility of field-grown clones to the disease, while in Tanzania, all commercial clones that had been previously released were susceptible to CWD (using similar screening methods). In Tanzania, the Maruka germplasm collection seemed to have high levels of resistance, but more field work is needed to confirm this. Several genotypes showed resistance in screening trials in DRC, with mortality rates lower than in Uganda and Tanzania. Studies in Uganda highlighted that CWD resistance was heritable, but its transmission from parent to progeny is only about 33%, suggesting that commercial CWD-resistant robusta varieties should be cloned. In Uganda, a major breeding programme involving screening for resistance as well as agronomic traits was initiated. Seven candidate clones were released to farmers in 2010 with the intention of further evaluating these clones under farmer conditions as well as agronomic traits such as cup quality and yield.

Arabica coffee was screened in Ethiopia and the stem nicking method at the cotyledon stage was found to be the most effective way to screen for resistance (Girma et al., 2009b). In trials, Catimor lines J19 and 7440 were resistant, but Caturra Rojo and cultivar 24./85 were more susceptible (Girma et al., 2009a). Having tested arabica cultivars from several countries including Kenya, Girma et al. (2009b) concluded arabica strains of CWD would pose a serious threat to *C arabica* in other countries.

Host-pathogen interaction studies in Uganda (Musoli et al., 2009) indicated that although there was host specialisation, it was not clear cut; one isolate (CAB007) collected from Arabica in Ethiopia induced some initial symptoms on *C. canephora*, but it was not fatal. This may be due to the inoculation method used. Further, several isolates from *C. arabica* and *C. canephora* induced symptoms in *C. liberica*, and Musoli et al. (2009) suggested that further studies are required to confirm host specificity.

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8.5 Transmission pathways and management

Transmission from contaminated wood to adjacent un-infected seedlings was confirmed in screen house trials (Phiri and Baker, 2009). This confirmed that leaving infected wood near uninfected trees in the field is a significant pathway and underlines the concern about use of contaminated wood for fences, firewood and stakes. CWD transmission from contaminated soil to healthy seedlings was also confirmed. Infectivity in soil lasted at least 3 months but then declined. Consequently, a fallow period could be a useful management strategy. A fallow of 1 year was advised before replanting to avoid re-infection. Despite proving that contaminated soil could be an inoculum source, it is difficult to isolate the pathogen from soil, which would be the critical test to assess the pathogen's presence. The pathogen was not isolated from insect pests of coffee (including coffee berry borer) or from bees or termites on affected farms (Rutherford and Flood, 2005) A wide-scale survey of 6 coffee growing districts in Uganda (with a high incidence of CWD) revealed that of 270 plant samples (105 species of crops and weeds) the pathogen was not recovered including from roots (Kangire et al., 2002). Yet Serani et al. (2007) reported isolation of the pathogen from banana roots, and these isolates induced symptoms in inoculated coffee plants. This raises concerns about the use of bananas as an intercrop and a replacement crop for coffee. Artificial inoculation of a wide range of crops and weeds (from coffee fields) with the pathogen is needed. There was evidence that wounds made with a machete previously used to cut infected coffee stems could transmit the pathogen to uninfected trees, but Phiri and Baker (2009) advised further work using more replication and more comparison of the positioning of, for example, wounds near the tree base and pruning wounds in branches in order to have some definite conclusions.

8.6 Environmental influence on infection

With regard to favourable environmental conditions, Musoli et al. (2008) reported no apparent effect of temperature on CWD development but temperatures do not fluctuate markedly in Uganda (Phiri and Baker, 2009). Some negative correlation between the number of coffee plants showing symptoms for the first time and severity of rainfall at least in first 18 month of assessment and the number of symptomatic plants increased during periods of low rainfall that followed periods of high rainfall. This pattern could be linked to death of roots during the wet season, allowing more infection to occur while symptoms become more apparent in the dry season when vascular occlusion (pathogenic activity) and drought combine to ensure insufficient water is being distributed internally. It is likely that with extended dry seasons

predicted in future climatic scenarios that CWD could have a more severe impact.

8.7 Agronomic treatments of CWD

A major part of the RCWP was the initiation of on-farm participatory trials in Ethiopia, DRC, Tanzania and Uganda. Through preliminary workshops, farmers were consulted about the treatments to be applied and some of their suggestions were incorporated. The trials were then evaluated over a number of years. Treatments included mulching and herbicides to reduce slashing, use of fungicide stem paints to be applied to the base of trees, fungicides sprays and applications of ash. On farm trials and on station trials were set up. Workshops were held to discuss the results and any promising options were incorporated into Farmer Field School curricula. Copper-based fungicides used as stem paint applications and herbicide sprays showed promise but were too expensive for many producers. Interestingly, although effective in DRC and Uganda, stem paints were not so effective in Ethiopia. Stems were painted with copper oxychloride, which is contact fungicide but *F xylarioides* is a systemic pathogen so the reduction in incidence could be linked to the indirect effect for example of sealing of wounds (Phiri and Baker, 2009) or an effect on the pathogen's development under the bark or production of perithecia at the tree base. Reducing the use of machetes, that is, reducing the chances of wounding trees had a big effect on reducing CWD incidence. As herbicides were expensive for farmers, slashing with machetes was advocated together with hand weeding around the base of the trees to reduce the incidence of wounds at the base of the trees. This approach was very effective especially in Ethiopia. The application of mulches to the base of the trees was also beneficial in Ethiopia and could have been due to mulches being a weed suppressant but also may have influenced the nutrient status of the plants.

8.8 Recommendations to reduce CWD

From the results of the trials and investigations, recommendations stressing an integrated management approach were made (Phiri and Baker, 2009). This approach included regular monitoring to detect infections early and management options that included regular cleaning of tools following use on trees with symptoms of CWD (using household disinfectant or heating); minimising inadvertent wounding made whilst slashing weeds; uprooting and burning of infected trees *in situ*; removal of coffee tree stumps; leaving a fallow period of at least 3 months but, preferably, planting an intercrop and leaving for a year before replanting coffee; replanting with more resistant coffee material (if available); discouraging the use of diseased firewood and if essential (due to fire wood shortage) cutting the short lengths *in situ and* placing them in a

sack rather than dragging the diseased wood through areas with unaffected trees.

Apart from on-farm activities, regional and national distribution of coffee material may constitute a means of transmission, so it was recommended that distribution systems of coffee materials from nurseries are investigated further. Quarantine was also strongly advocated by Phiri and Baker (2009). Monitoring and surveillance needs to be maintained to reduce the possible spread to other coffee-growing areas of Africa and to other coffee-growing areas worldwide.

Phiri and Baker (2009) stressed that the re-emergence of CWD in Central Africa should be an example of what happens when there is little preparedness for disease epidemics and when governments do not act quickly enough to contain the disease and reduce its impact. There should be lessons learned from this disease – the need for improved preparedness in order to achieve greater sustainability in coffee production in Africa. Chemical control is difficult for this pathogen, but painting cut stems and the base of infected plants with copper fungicides did seem to help reduce incidence, and for valuable germplasm (e.g. in germplasm collections), benomyl applied as root drenching (once every month) may be effective (Phiri and Baker, 2009). Nevertheless, wide-scale use of fungicides is not advocated due to cost to smallholders and to environmental concerns. One of the major concerns about the destructive ability of this pathogen was that it attacks all *Coffea* species including wild relatives, which normally could be a likely source of resistance to be used in breeding programmes, so conservation of germplasm is essential. Breeding for resistance remains an ideal strategy for managing this disease especially for smallholders. In-country breeding programmes in Ethiopia, Uganda and Tanzania were ongoing at the time of the report (Phiri and Baker, 2009), but this is likely to be a long-term approach which will need significant investment. It is also important that countries share any resistant germplasm.

This integrated approach would use a combination of cultural, mechanical, physical, chemical and biological control in a systematic way. However, for this approach to be effective, a greater awareness of the impact of CWD was needed. Consequently, a major effort (through the RCWP) was made to train a range of stakeholders in CWD-affected countries and also to raise awareness of the threat of CWD in Africa and beyond. Over 2500 extensionists were trained as trainers and facilitators under the RCWP. In addition to the technical training of CWD identification and management, FFS members learned better husbandry practices for coffee which often resulted in improved productivity. It is estimated that more than 1 million farmers were trained by extensionists between 2002 and 2007, including a substantial number of women farmers.

An estimated total of 487 700 items of information concerning CWD were produced under the RCWP and were disseminated to coffee-growing regions of Ethiopia, Uganda, Tanzania, DRC and Rwanda. Printed materials

were produced in 13 languages and over 250 radio broadcasts were made in 5 countries as well as television programmes reaching tens of millions or people (Negussie et al., 2009).

9 Future trends in research

Although the RCWP had a significant impact on understanding more about the pathogen, the disease, its spread and management, there remain some interesting avenues for future research. This includes a greater understanding of the proposed host specificity. Buddie et al. (2015) confirmed the complexities of the CWD pathogen already highlighted by previous researchers (Lepoint et al., 2005; Lepoint, 2006; Girma et al., 2009b; Rutherford et al., 2009) regarding the presence of multiple taxa (or biological species) within *G. xylarioides sensu lato*. Buddie et al. (2015) emphasized the significant and consistent differences observed in IGS amplicon size between C. *arabica* and *C. canephora* strains, which would be of considerable importance to plant pathologists and epidemiologists monitoring development and spread of CWD as well as for breeders trying to derive resistant germplasm, and for national and local authorities providing management advice and technical support to farmers. Buddie et al. (2015) further tested the species concepts in this complex using additional markers and the extent of similarity across all *G. xylarioides* strains examined, and implied that the population is largely homogenous but with clear variation, allowing discrimination of strains from *C. arabica*, C. *canephora* or from *C. excelsa*. These conclusions were supported by the largely nuclear ISSR analysis, and may suggest recent divergence of the two established host-specific groups, but this does need further study.

Also, the strains are heterothallic, and perithecia have been reported to occur frequently on coffee trees in the field (Lepoint et al., 2005; Hakiza et al., 2009), so the lack of genetic variation revealed in a sexually reproducing population is surprising, suggesting that other factors may be involved, which needs further research. Members of the *G. fujikuroi* complex are known to include strains with various 'spore killer' genes that, depending on mating combination, can result in perithecia containing only one genetic type of ascospore or perithecia with aborted ascospores (van der Gaag et al., 2000). If this were the mechanism for the CWD pathogen, it would have major implications for selection in, and management of, a largely clonal pathogen reliant on development and dispersal primarily through mycelial growth and conidia production. It would give coffee breeders more confidence in trying to identify or develop resistance to currently recognised forms of the pathogen. Further work is needed on this aspect. One output of the RCWP was the establishment of CWD isolate collections at CABI (UK), in CIRAD (France) and in UCL (Belgium), so further studies can be conducted.

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Another area for future research involves understanding more about the underlying resistance mechanisms in this H-P interaction, which would aid breeding programmes, but little has been published on this. Breeding programmes for cultivars of both arabica and robusta were ongoing in the first decade of the 21st century (Flood, 2009) and it would be useful to understand more about the progress of these breeding programmes including adoption by farmers of any new planting material. For a smallholder production system, the use of resistant material provides a more environmentally sustainable management option. Kilambo et al. (2012) working at the Tanzania Coffee Research Institute reported some success with the screening of 875 breeding lines of *C. canephora* (artificially inoculated with pathogen using the root dip method) and 201 lines were found to be resistant to CWD. In 2006, these 201 genotypes were planted in clonal mother garden to raise planting materials for field evaluations. Production and cup taste of the 875 lines were also assessed between 2001 and 2008. Six CWD-resistant robusta lines that were also resistant to leaf rust with productivity ranging from 0.5 kg to 3.0 kg of clean coffee per tree and cup taste described as 'clean/smooth cup of natural robusta' were selected. These lines were planted in multi-location evaluation trials in CWD hot spot areas in the Kagera region of Tanzania. Eighteen months later the varieties were considered resistant, and Kilambo et al. (2012) reported that the six breeding lines were expected to be released for commercial use. It would be helpful to have updates of progress with trials and with the proposed commercial release. The availability of such resistant material to researchers would also allow investigation of resistance mechanisms.

Understanding more about alternative hosts for this pathogen would also be useful in determining a field management strategy. The pathogen has been isolated from tomatoes (Onesirosan and Fatunla, 1976) and from cotton (Pizzinatto and Menten (1991), so potentially, members of the *Malvaceae* and *Solanaceae* found in coffee fields (weeds) could act as alternative hosts and should be tested by artificial inoculation. Similarly, the pathogen was isolated from bananas (Serani et al., 2007), indicating that *Musa* species could act as an alternative host. The reduction of alternative hosts in the coffee gardens would reduce inoculum as part of an integrated approach.

Biological control has not really been exploited for this pathogen and given the vascular nature of the pathogen could be difficult due to the specific ecological niche, that is, the vascular system to which these pathogens have adapted. However, many investigations have reported success for different forms of the species of *Fusarium oxysporum* (Alabouvette et al., 2009), including the use of non-pathogenic fusaria to compete for infection sites on the roots of host plants (to reduce inoculum prior to entry into the plant). Nevertheless, these authors also noted that, despite an increasing number of papers dealing with biological control, there are very few products available especially in the field situation and

more research into the field aspects of biological control are needed. Mulaw et al. (2013) have reported the successful antagonism of endophytic *Trichoderma* to *F. xylarioides*, but the interactions were only studied in *vitro*. More research on these interactions *in vivo* are needed including glasshouse studies, but ultimately, trials are required under conditions in the farmers' fields. We need to determine a practical approach for producers to be used as part of any ongoing integrated management of the disease. It was also suggested by Phiri et al. (2009) that a biocontrol approach could be further trailed using antagonistic microbes as sealants for wounds following rejuvenation (stumping) of coffee trees.

An alternative approach to using a specific antagonist as a putative biocontrol would be to understand more of the ecology of the pathogen in the soil with a view to increasing the diversity and activity of a wide range of antagonistic microbes so as to decrease the pathogen inoculum. Such approaches could include the use of mulches (a practical approach for the farmers) to encourage and support a more diverse microbiome. In recent years, the study of disease organisms in crops including coffee has been greatly facilitated through the development of next-generation sequencing technologies. This includes comparative genomics through the analysis of whole genome sequences but also the more in-depth analysis of microbial communities in the rhizosphere (and phyllosphere) through metagenomics and transcriptomics approaches. The latter have been particularly useful in developing our understanding of the microbiome and the role it plays in the functioning of the phytobiome. These approaches have broader implications for sustainable coffee management and the development of biological solutions to reduce losses and enhance yield.

Phiri and Baker (2009) previously stressed that the re-emergence of CWD in Central Africa was an example of what happens when there is little preparedness for disease epidemics and when governments do not act quickly enough to contain the disease and reduce its impact. Although not a future research trend as such, improved biosecurity and preparedness are needed going forward. On-going monitoring of this disease (and other coffee diseases) is required and regular surveys should be undertaken across the African continent to detect new pathogens, new strains and new antagonists as well as establishing protocols to deal with these constraints. In addition, other coffee-producing countries in Africa and beyond need to be aware of the threat of CWD. In trials, arabica cultivars from several countries including Kenya were susceptible to the arabica strain of the pathogen (Girma et al., 2009b), so raising awareness of the threat from CWD remains critical for sustainable coffee production globally.

10 Where to look for further information

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