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Titre:

Recherche de sources de résistance à la trachéomycose du caféier *Coffea canephora* Pierre, due à *Fusarium xylarioides* Steyaert en Ouganda

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DEDICATION

Dedicated to my family and parents

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ACRONYMS

AFLP	Amplified fragment Length Polymorphism
Anon.	Anonymous
ASIC	International Association of Coffee Science
CABI	Centre for Agriculture and Biosciences International
CORI	Coffee Research Institute (Uganda)
CORNET	Coffee Research Network (Africa)
CWD	Coffee wilt disease
DNA	Deoxyribonucliec acid
DRC	Democratic Republic of Congo
FAO	Food and Agricultural Organization
Ha	Hectares
ICO	International Coffee Organization
RAPD	Random Amplified Polymorphic DNA
KARI	Kawanda Agricultural Research Institute (Uganda)
Kg	Kilogram(s)
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries (Uganda°
Mt	Mountain
ng	Nan grams
PCR	Polymerase Chain Reaction
RFLP	Restricted Fragment Length Polymorphism
SNP	Single nucleotide Polymorphism
SSR	Simple Sequence Repeats
UCDA	Uganda Coffee Development Authority
µl	Micro litre

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La trachéomycose du caféier, due à *Fusarium xylarioides*, représente une menace pour la production de café en Afrique depuis la première apparition de cette maladie sur *Coffea liberica* var. *dewevrei* (appelé *excelsa*) en République Centre Africaine (1927). Cette maladie vasculaire transmise principalement par blessure des parties aériennes provoque la mort de l'arbre dans un laps de temps de 1 à 24 mois après l'apparition des premiers symptômes. Progressivement la maladie est apparue sur *C. canephora* en Côte d'Ivoire (1947), République du Congo (1949), Guinée (1958). En 1957, elle était signalée sur *C. arabica* en Ethiopie. La mise en oeuvre à grande échelle de campagnes d'arrachage et la diffusion de variétés résistantes de *C. canephora* Pierre a permis d'éradiquer la maladie en Afrique Centrale et en Afrique de l'ouest vers la fin des années 50. Cependant au début des années 80, la maladie a ré-émergé en RDC et s'est étendue progressivement à l'Ouganda en 1993, puis à la Tanzanie (2000). En Ouganda la maladie, s'est très rapidement étendue à l'ensemble des zones de production et en 2002, la trachéomycose affectait 90% des plantations avec un pourcentage moyen de caféiers morts de l'ordre de 44%. Aucun traitement phytosanitaire ne permet de contrôler la maladie, aucune variété commerciale résistante est disponible et il est déconseillé de replanter dans un sol infecté pendant plusieurs années.

Face à cette situation, les études réalisées en Ouganda dans le cadre d'un projet financé par l'Union Européenne, ont eu pour objectif d'analyser le développement spatio-temporel de la maladie, d'identifier des sources de résistance dans les caféiers cultivés et les populations de caféiers sauvages et de caractériser la résistance en vue de proposer des stratégies de lutte fondées sur la résistance génétique à la maladie.

Dans ce document de thèse, après une présentation générale de cette trachéomycose du caféier sur une base bibliographique, les résultats obtenus sont présentés en 4 chapitres, rédigés sous forme d'articles (les deux premiers sont soumis pour publication).

1- La dynamique spatiale de la maladie est décrite par des régressions non linéaires et le modèle logistique s'ajuste bien a cette dynamique. L'évolution spatiale de la maladie est analysée a l'aide des outils de la géostatistique. Les semi-variogrammes, réalisés à plusieurs dates, entre avril 2001 et mars 2006, indiquent un début d'épidémie en foyers, c'est à dire qu'il existe des corrélations spatiales à l'échelle des parcelles étudiées. Ces foyers sont visibles sur les cartes de répartition des symptômes. Des modélisations par « krigeage » permettent de représenter ces foyers aux différentes dates d'observation et de visualiser leur extension.

La taille des foyers augmente et ils fusionnent pour former un ensemble continu de caféiers malades, ponctué de groupes de caféiers résistants. A partir d'arbres malades, la dispersion peut se faire dans toutes les directions et infecter les arbres voisins jusqu'à une distance d'environ 10 m.. Pour contrôler efficacement la maladie, en particulier dans le cas d'arrachage, il est nécessaire d'extraire les plus proches voisins du caféier atteint dans un rayon d'au moins dix mètres. Cette opération d'arrachage est économiquement envisageable si le taux d'attaque de la parcelle est inférieur à 10%.

2- L'analyse de la diversité génétique de *C. canephora* en Ouganda, a porté sur différentes populations de caféiers sauvages issues de prospections dans les forêts primaires de Kibale et Itwara, une population de caféiers féraux des îles Kalangala et des populations de caféiers cultivés localement, comparés aux groupes génétiques décrits pour cette espèce (Dussert, 2003). L'étude à l'aide des marqueurs microsatellites a permis de séparer trois groupes de populations. Les populations de caféiers sauvages d'Ouganda constituent un nouveau groupe génétique, différent des groupes connus à ce jour. La diversité génétique de ces populations sauvages met en évidence une source nouvelle de gènes susceptibles d'être exploités dans les futurs programmes d'amélioration des variétés commerciales existantes, surtout si elles recèlent des gènes de résistance à la trachéomycose. Face à la menace de la trachéomycose et la découverte de caféiers spontanés malades, la conservation effective de ces ressources génétiques « *in situ* » n'est pas assurée et devrait être complétée par la mise en collection conservatoire « *ex-situ* »dans différents centres de conservation nationaux ou internationaux.

3- L'étude de la résistance a été effectuée sur des clones installés au champ et par inoculations artificielles en conditions contrôlées de jeunes caféiers issus de fécondations libres. Les observations au champ ont mis en évidence différents niveaux de sensibilité avec des périodes de latence très variables (1 à 24 mois) ce qui suggère la mise en place de différents mécanismes de défense quantitatif et des interactions avec les conditions environnementales. Les inoculations artificielles présentent une bonne corrélation avec la résistance observée au champ et sont utilisées pour une évaluation précoce de la résistance. Différentes espèces de caféiers étant sensibles au *Fusarium xylarioides*, une étude de la spécificité d'hôte des isolats en collection a été effectuée. Il s'avère que l'isolat CAB003 isolé sur *C. canephora* provoque la mort des jeunes plants de *C. canephora,*.ainsi que l'isolat DSMZ62457 isolé sur *C. liberica* var. *dewevrei*, mais avec une agressivité plus faible. En revanche l'isolat CAB003 (isolat canephora) n'est pas létal vis-à-vis de l'espèce. L'isolat CAB007 isolé sur *C. arabica* n'est pas létal sur *C. canephora*. Ceci indique que l'isolat CAB003 est spécifique de l'espèce *C. canephora*, alors que DSMZ62457 isolé sur *C. dewevrei* est non hôte spécifique. Si ces résultats étaient confirmés, ils impliqueraient, que *C. liberica* var. *dewevrei* pourrait être une source de gènes de résistance à

introgresser dans l'espèce *C. canephora*, et l'espèce *C. canephora* une source de gènes à introgresser pour contrôler la résistance dans l'espèce *C. arabica*. Enfin, ces études ont permis d'identifier deux génotypes de caféiers *C. canephora* (J1/1 et Q3/4) totalement résistants à la trachéomycose qui vont être placés dans différents contextes agroécologiques.

4- L'héritabilité de la résistance à la trachéomycose a été calculée à 50-60% de mortalité, (i) sur les descendances pleins frères dans un demi diallèle au champ, en présence d'un inoculum naturel d'une part, (ii) sur des boutures et des descendances issues de fécondations libres, inoculées artificiellement en conditions contrôlées. L'héritabilité au sens large observée dans les différents essais analysés est moyenne (0.3). La résistance des génotypes J1/1 et Q3/4 se retrouve dans leurs descendances. Il sera donc possible d'intégrer ces 2 génotypes avec les origines spontanées de Kibale et Itwara dans des programmes d'amélioration pour la création de variétés tolérantes. La culture de ces variétés devra être associée à des techniques culturales adaptées pour réduire la dissémination du champignon.

General introduction

I.I Background

Coffee is an extremely important beverage and commodity of commerce with nearly 7 millions tons of green beans produced every year in about 80 tropical countries (FAO, 2006). About 50 of the coffee producing countries are exporters and some are smaller producers, producing for local markets (ICO, 2006). Although exceeded by cereals in tonnage, in terms of commercial value, coffee follows closely after petroleum, contributing 1 per cent of total value of world exports and imports. Coffee is a critically important element in the economy of most of the exporting countries, where it is an important earner of strong currencies, contributing variably to a solid basis for promotion of economic development, particularly since producing countries are less industrialised. In most of these countries production lies mainly in the hands of a large number of small holders, thus it provides employment to the participating families. Coffee is produced from mainly two *Coffea* species; *Coffea arabica* L and *Coffea canephora* Pierre ex A. Froehner. *C. arabica*, which grows well in highlands, produces arabica coffee (Davis, et al, 2006). It is the only allotetraploid *Coffea* species with a chromosome number of 2n = 4x = 44. This species is a predominantly inbreeding and self fertile species. Arabica coffee contributes about 65% of global coffee production (ICO, 2006). *C. canephora* Pierre, which is adapted for cultivation in low to medium altitudes, is grown for Robusta coffee (Davis, et al, 2006), which contributes about 35% of global coffee production (ICO, 2006). *C. canephora* is a diploid with a chromosome number of 2n = 2x = 22. It is predominantly out breeding and self sterile.

I.II Coffee growing in Uganda

Both *C. canephora* and *C. arabica* are cultivated in Uganda. *C. canephora* is indigenous (Maitland, 1926) and was grown by natives before the beginning of current commercial coffee cultivation but *C. arabica* coffee, which was first introduced by the colonial Government in 1900 (Thomas, 1940), was initially multiplied and distributed to farmers for commercial cultivation in preference to *C. canephora* because of its superior quality and its high price. However due to leaf rust and poor adaptation to lower altitudes, which constitute the larger proportion of coffee growing areas in Uganda, *C. arabica* cultivation shifted to the highlands and to date it is mainly cultivated in areas above 1300 meters above sea level. Main arabica coffee producing areas in

Uganda include Mbale, Sironko, Bududa, Manafwa and Kapchorwa districts on the slopes of Mt Elgon, Kabarole and Kasese districts on the slopes of Mt Rwenzori, in Nebbi district of the west Nile region and Kisoro and Kabale in highlands of south western Uganda (Figure I.111). Total acreage of *C. arabica* in Uganda is estimated to be 30,000 hectares, producing about 30,000 metric tonnes of exported green coffee beans per year (ICO, 2006).

C. canephora is indigenous to Uganda, where is endemic to indigenous forests of Kibale, Itwara, Bwamba and Zoka and perhaps also in forests in Central Uganda near Lake Victoria (Maitland, 1926; Thomas, 1940). Local inhabitants cultivated this crop long before the first European, Speke, visited Uganda in 1863 (Maitland, 1926; Leakey, 1970). Masaka and Ssese Islands in Lake Victoria are the main centres of these early cultivations. Natives on Ssese Islands obtained open pollinated seeds from Kasai forest in Mukono district on the mainland for planting gardens of a few coffee trees near homesteads (Thomas, 1940). These trees had spreading orthotropic branches (Nganda type). Thomas (1940) reports that Nganda plant materials were taken from Ssese Islands to start coffee gardens in Masaka. Before *C. canephora* was developed as a commercial crop in Uganda, it was offered to visitors for chewing and as gifts during traditional ceremonies such as marriages (Thomas, 1940). It was also used for rituals such as strengthening friendships or brotherhood. The devastation of *C. arabica* coffee plantations in Asia and also in Uganda by leaf rust (*Hemileia vastatrix)* changed the world focus on *C. canephora* which in turn stimulated Ugandan Government to develop the Robusta coffee industry (Maitland, 1926).

Serious work on Robusta coffee started in 1916, when a range of *C. canephora* genotypes from different sources were tested for liquor and tree No. 9 of Toro selection was identified to be superior (Maitland, 1926). Tree No. 9 was selected from a farm in Toro region which currently constitutes Kyenjojo, Kamwenge and Kabarole districts. But the exact location of this source farm and the source of its planting materials are not known. Toro region is a home to the current Itwara and Kibale forests, where *C. canephora* grows as part of the under storey vegetation, and we can hypothesized that these forests are the original source of planting material used on the farm, where tree No. 9 was selected, considering that by then majority of the farmers used volunteer seedlings from the wild to establish gardens.

Seedlings from tree No. 9 were raised to form seed gardens, from which seed was distributed to farmers (Thomas, 1947). Beside quality, Tree No. 9, which is of the “erecta” type

(erect tree architecture) and its descendants were also selected for bold beans and high yields. From 1920 to 1932 large quantities of seeds from these selections were distributed to farmers. However these erect genotypes did not adapt well in most areas and especially in Ssese Islands where the soils are highly leached. Consequently, between 1932 and 1934, genotypes with spreading tree architecture and small leaves (nganda) were selected from farms on the Ssese Islands and mainland near Lake Victoria to provide seeds for new plantings (Thomas, 1947). From 1934 up to early 1970s seeds from these types were distributed to farmers, as the industry progressed steadily (But *et al*, 1970), stimulated by the high prices coffee received in the 1950's (Thomas, 1947). From 1980's, farmers were supplied with rooted cuttings of 8 clones selected for high yields (2-3 metric tons of clean coffee per hectare per year), resistance to leaf rust and red blister diseases and good bean and cup qualities. Three of these clones (1s/2, 1s/3 and 1s/6) are open pollinated descendants of Toro tree No. 9 identified in the 1920s and the other 5 clones (223/32, 236/26, 257/53, 258/24 and 259/56) are Nganda types selected among descendants from farms in different parts on the mainland around L. Victoria (Leakey, 1970).

However, between 1970 and 1990, coffee activities in Uganda declined drastically due to poor marketing. During this period, there were no new plantings and many farmers uprooted their coffee trees, both *C. canephora* and *C. arabica*. In 1991 Government liberalized coffee marketing, which lead to improved farm gate prices. This stimulated farmers to care for their gardens and to establish new plantings, which quickly revamped coffee production and exports in the country. Continuous care of the gardens of both *C. canephora* and *C. arabica*, plus production from new plants, lead to continued increase in production, culminating in record high of about 280,000 tons (4.7 million 60kg bags) exported in 1997 (Musoli *et al*., 2001a; ICO, 2006).

Because of farmers' interest to plant coffee as a result of improved farm gate prices, demand for planting materials of the 8 elite clones was high. This demand outstretched available nursery facilities for producing rooted cuttings country wide. Consequently in 1996 Government decided to distribute to farmers a mixture of open pollinated seeds of 6 out of the 8 clones from isolated seed gardens , as a short term measure, as the facilities for rooted cuttings were being upgraded. By then planting materials of only 6 out of the 8 clones were recommended because two clones (236/26 and 259/56), were dropped from commercial list in 1988 because of their susceptibility to leaf rust caused by *Hemilia vastatrix.*. The seedlings were recommended for

farmers in areas with relatively low rainfall, which are considered to be marginal for the crop. Seedlings were recommended after it was established that their yield after 5 cropping years were similar to those of their clonal parents (Kibirige *et al.*, 1993).

Currently *C. canephora* in Uganda is cultivated at altitudes ranging from 800 meters above sea level in the rift valley in Bundibugyo district to 1600 meters above sea level in Bushenyi district. *C. canephora* acreage in Uganda is estimated at 270,000 hectares. And by 1997, Robusta coffee exports had reached 280,000 tons (4.7 million 60kg bags). However, coffee wilt disease (CWD), which emerged in the country in 1993, has progressively affected *C. canephora* cultivation, drastically reducing production, hence exports from 280,000 tons (4.7 million 60kg bags) exported in 1997 to about 142,000 tons(2.4 million 60kg bags) exported in 2005 (Figure I.I).

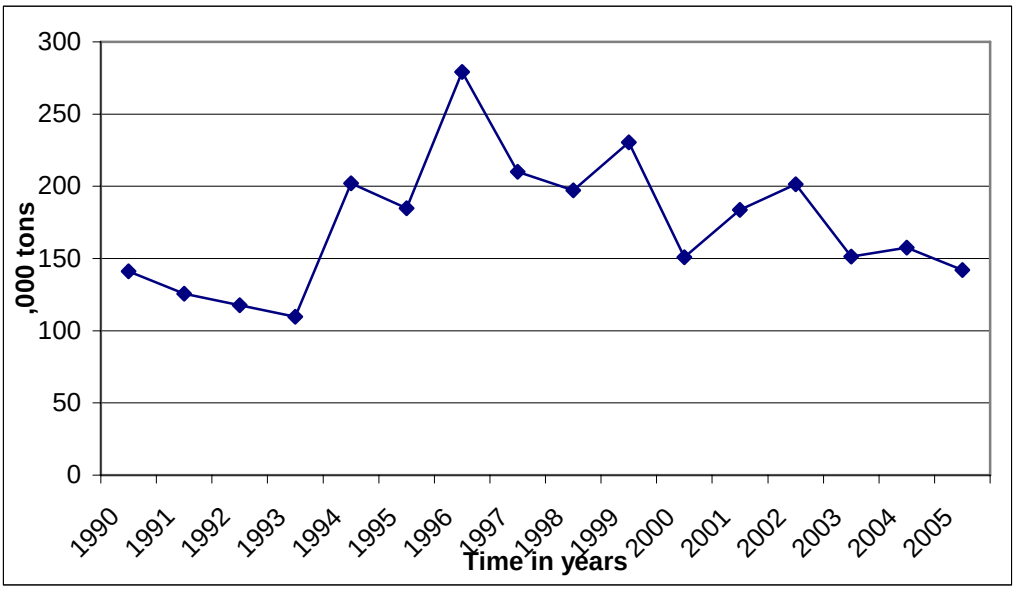


Figure I.I: Exports of green coffee beans from Ugandan

I.III Coffee wilt disease

Coffee wilt is a vascular disease caused by *Fusarium xylarioides* Steyaert, which is the conidial stage of *Gibberella xylarioides* Hem. and Saccas. CWD can be very destructive to coffee trees (Figure I.II), leading to total loss of investment. It was first reported in 1927 on *Coffea liberica* var. *dewevrei* (formerly *C. liberica* type excelsa, Davis et al., 2006) plantations in the Central African Republic (CAR) (Figueres, 1940). It progressively destroyed this crop during 1930s to 1950s in other Central African countries i.e. Cameroon (Guillemat, 1946; Fraselle, 1950; Saccas, 1951; Muller, 1997). During the same period, it destroyed *C. canephora* in Ivory Coast, causing serious

losses and disappearance of some local varieties (Delassus, 1954). In contrast several varieties of *C. canephora* imported from DRC between 1914 and 1933 exhibited some level of field resistance, which was later confirmed by artificial inoculation (Meiffren, 1961). Meiffren (1961) also reported apparent differences for the same materials planted in different areas of the region, i.e. certain *Coffea liberica* and *C. canephora* varieties showing resistance in Ivory Coast were completely susceptible in CAR, suggesting the resistance was either being influenced by environmental conditions or there were different physiological races of the pathogen in different localities of this region. Fraselle (1950) reported CWD attack on *C. canephora* at Yangambi in the DRC in 1948 and subsequently the disease became a serious problem in many parts of the country. In 1957 similar symptoms were reported on *C. arabica* in Ethiopia (Lejeune, 1958) and later Kranz & Mogk (1973) confirmed that the disease on *C. arabica* was also caused by *F. xylarioides*. Pieters and Van der Graaff (1980) reported that CWD was endemic in all coffee growing areas of Ethiopia. Van der Graaff and Pieters (1978) reported resistance among *C. arabica* lines in fields, which was later confirmed after artificial inoculations (Pieters and Van der Graaff, 1980).

Figure I.II: (a) Healthy *C. canephora* tree and (b) *C. canephora* tree killed by CWD

As this disease continued to threaten the coffee industry throughout Africa, it was decided in 1956 to implement systematic elimination of all affected plants over large areas and to search for resistance both in wild and cultivated varieties (Anon. 1998). Following this initiative,

C. canephora resistant varieties identified in the DRC were used for replanting within the DRC and Ivory Coast (Saccas, 1956; Meiffren, 1961). In Cameroon the disease was eliminated by rigorous systematic uprooting (Muller, 1997) of the *C. dewevri* plantations. These strategies proved to be successful as the disease had declined drastically by the end of the 1950s and it eventually disappeared from Cameroon and Ivory Coast and probably DRC and CAR. The disease remained affecting *C. arabica* in Ethiopia and it is doubtful if any of these strategies were applied. In 1986 new large scale outbreaks of CWD were reported *on C. canephora* in the north-east of DRC (Flood & Brayford, 1997), from where it spread rapidly to affect this crop in Uganda (1993) and north-west Tanzania (1997).

I.1V Magnitude of current coffee wilt disease problem

Coffee wilt disease was reported for the first time in Uganda in 1993, in areas bordering the Democratic Republic of Congo (Flood and Brayford, 1997). The disease symptoms as described previously (Fraselle, 1950; Saccas, 1951) were observed in Uganda on affected *C. canephora* trees in the field. It was observed at CORI that Coffee wilt disease kills coffee trees within a short period. The time lapse between first symptoms to death of affected trees was noticed to vary between genotypes. The period was noticed to be relatively shorter for more susceptible varieties.

Surveys carried out in Uganda in 2002 found coffee wilt disease in all *C. canephora* growing areas and on more than 90% of farms, where it had destroyed 44.5% of the crop nationwide (CORI, 2003; CORNET, 2003; Oduor *et al*, 2005) (Table I.I and Figure I.II). Similar surveys found this disease on many *C. canephora* farms in Kagera (Robusta coffee producing) region of Tanzania near Lake Victoria and also on *C. arabica* in Ethiopia, on (Table 1.I). In the Democratic Republic of Congo wilt was found causing large losses of *C. canephora* trees especially in north-eastern part of the country (Table I.I). Follow up surveys conducted in 2003, indicated that the disease was on the increase (CORNET, 2003; Oduor *et al*., 2005). During the survey, the disease was not found in Cameroon and Ivory Cost. Given the rate at which CWD spread from DRC to Tanzania through Uganda, there is fear that this disease might cross to other coffee producing countries within Africa and to other continents. In Uganda CWD is also destroying *C. canephora* gene pools available at research stations (Figure I.IV) and it was observed on wild *C. canephor*a trees in Kibale and Itwara forests. Thus with rapid spread of this

disease from tree to tree, field to field, district to district and across countries, the overall economic and social effects are alarming with looming disaster that has to be urgently addressed.

Table 1: Coffee wilt disease incidence in countries surveyed during surveys coordinated by CORNET

Country	% infected farms	Mean % tree infection	Infected Coffea species	Country distribution
Uganda	90.3	44.5	*C. canephora*	All Robusta areas
Tanzania	2.2	0.7	*C. canephora*	Kagera region
DRC	26.5	17.8	*C. canephora*	North Kivu & Oriental provinces
Ethiopia	27.9	3.0	*C. arabica*	
Rwanda	None	None		
Cameroon	None	None		
Ivory Coast	None	None		

Table was modified from Oduor *et al*., 2003.

I.V Probable factors responsible for coffee wilt disease re-emergence

The cause of re-emergence of coffee wilt disease as a serious problem in DRC and its subsequent spread to Uganda and Tanzania was not known. There were speculations about different factors that could have individually or in interaction led to the re-emergence of this disease.

i) Either there was spontaneous emergence of a new and more aggressive strain of *F. xylarioides*, which overcame resistance of cultivated *C. canephora* populations in DRC and eventually spread to Uganda and Tanzania.

ii) Or *F. xylarioides* strain which affected *C. canephora* and *C. dewevri* spp. in West Africa and Cameroon was/were different from the strain affecting *C. canephora* in DRC, Uganda and Tanzania and that the West African and Cameroonian strain(s) could have survived on non-coffee host, especially on species in genera related to *Coffea*, and slowly accumulated inoculum as it spread through this secondary host(s). And through this secondary host(s), it spread up to DRC thus causing re-emergence of CWD on *C. canephora* in this country and

its eventual spread to Uganda and Tanzania.

iii) Or CWD was never eradicated in DRC and a breakdown of management practices, previously put in place by the DRC government for controlling it such as reduced care of coffee fields, abandonment of cultural control practices and use of susceptible planting materials, just like abandonment of any other coffee improvement practices, led to massive increase of inoculum in abandoned fields. Thus causing massive infections of coffee trees, leading to the disease re-surging.

iv) Or *F. xylarioides* could have stayed in endemic state on wild *C. canephora* plants and it re-emerged on farms through human activities.

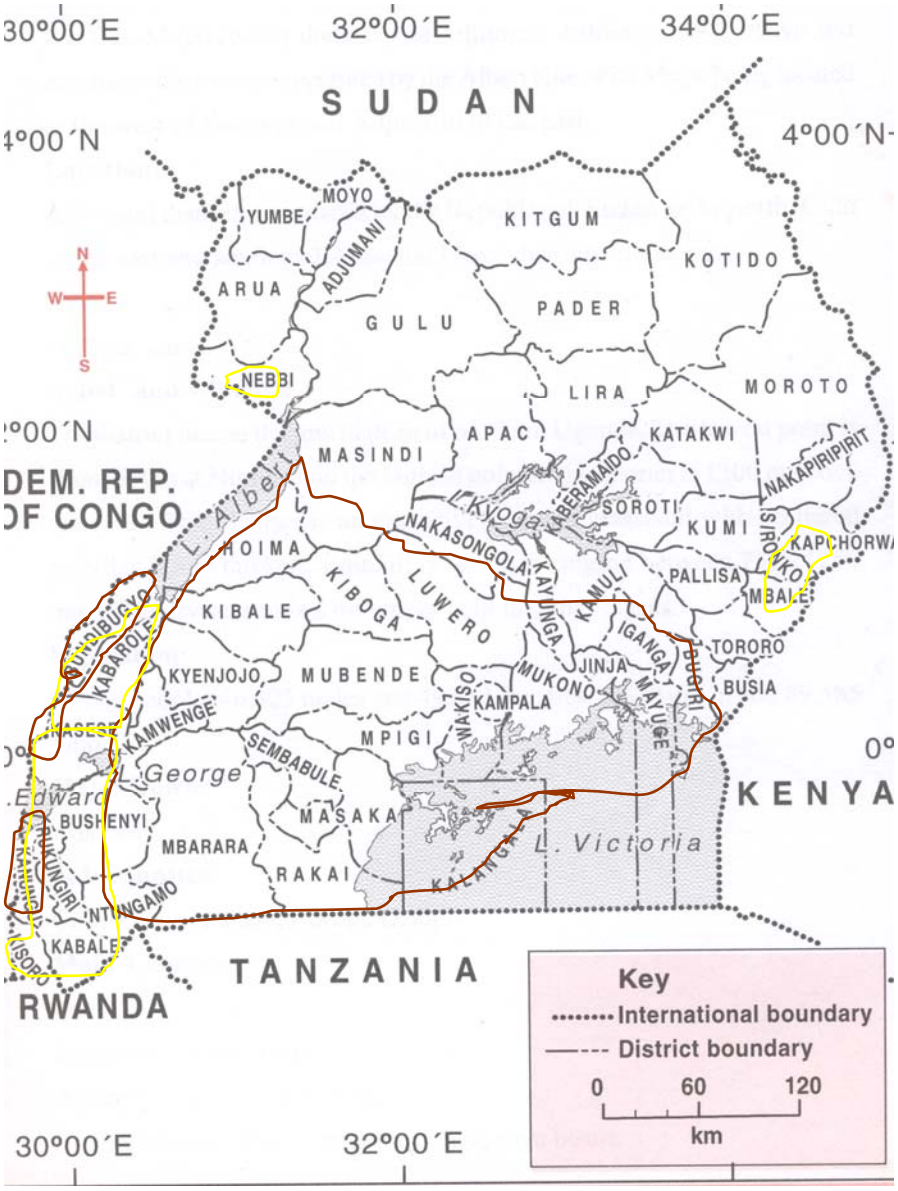
In another scenario it was acknowledged that:

i) The disease was now affecting coffee in Uganda and Tanzania, a region beyond the Congo zone, with different environment, which could influence its pathogenecity and aggressiveness, as indicated by Meiffren (1961).

ii) Genetic compositions of *C. canephora* in Uganda and Tanzania may not be necessarily the same as that in the DRC, Cameroon and Ivory Coast, since *C. canephora* is known to be very diverse (Montagnon, 1992, Dussert *et al*, 2003) and they would probably respond differently to the disease.

iii) In Uganda CWD was reported only on *C. canephora*, even in areas where the *C. canephora* and *C. arabica* are growing side by side. Screen house tests conducted on young seedlings of open pollinated seeds of some *C. canephora* and *C. arabica* in the germplasm collections at the Coffee Research Institute using field isolates of *Fusarium xylarioides* obtained from diseased *C. canephora* trees revealed high mortality among *C. canephora* seedlings and total resistance among *C. arabica* varieties (Musoli *et al*, 2001b), confirming field reports. In Ethiopia, however, the pathogen continued to affect *C. arabica* (Girma and Hindorf, 2001). This seemed to suggest that the disease strain affecting *C. arabic* in Ethiopia was different from strain(s) affecting *C. canephora* in Uganda

All these scenarios need to be investigated, since they can interact to influence the success and effectiveness of CWD control measures that would be put in place. These studies investigated those factors that should lead to and interact with control measures based on durable host genetic resistance.



Predominantly Robusta growing zone

Predominantly Arabica growing zone

Figure I.III: Map of Uganda showing coffee growing districts

Figure I.IV: Aerial photograph of CORI showing *C. canephora* germplasm conservation fields (A, B and C) devastated by coffee wilt disease. Note the gaps left by coffee trees killed by the disease

I.VI Justification for this research

In Uganda, it has become absolutely necessary to control coffee wilt disease. Phytosanitory control measures, which were emphasized in the beginning, were found ineffective to prevent the disease spread (Wetala *et al*., 2000) and they are difficult to implement. Chemical control is considered ineffective, since it had failed in previous attempts in Central and West African countries (Muller, 1997). Moreover currently there are no commercial varieties that are resistant against this disease to be used in re-planting. As a result, developing wilt resistant cultivars has become the main aim of *C. canephora* improvement programme. Therefore, this study was carried out to generate prerequisite knowledge required for designing and implementing an effective breeding strategy that would generate *C. canephora* cultivars with durable resistance to CWD and that are adapted to wide *C. canephora* agro-ecological conditions found in Uganda.

I.VII Research goal and main objectives

The goal for this study was to contribute effectively to the control of coffee wilt disease in Uganda through host genetic resistance. The main objectives of the study were to generate knowledge on:

1 Field spatial and temporal development of coffee wilt disease
2 Genetic diversity of *Coffea canephora*
3 Host resistance to coffee wilt disease in local and exotic populations of *C. canephora.*

Each of these broad objectives aimed at answering one or more specific questions. The aim of the specific objectives was to improve our knowledge on main biological factors of CWD and its host that are likely to influence the success of breeding wilt resistant commercial varieties as highlighted below.

Field spatial and temporal developments of coffee wilt disease in C. canephora

Before this study was carried out, information on the spatial and temporal field spread of coffee wilt disease under Ugandan conditions was lacking. Information on spatial and temporal spread of this disease in a multi host field is important for understanding effects of different hosts genotypes on the disease spread, hence role of host resistance in controlling the disease epidemics. Information from this study is also vital for designing a phytosanitory control strategy that can be implemented in tandem with host resistance.

Genetic diversity of Ugandan Coffea canephora

Before this study was carried out, 5 global *C. canephora* diversity groups, Guinean and Congolese B, C, SG1 and SG2, (Dussert, 2003) were known (Figure I.V). These groups were described basing on diversity among *C. canephora* populations in Central Africa (Congo region, Cameroon) and West Africa (Ivory Coast, Guinea), areas where coffee wilt disease existed in the past. Genetic diversity among Ugandan (East Africa) *C canephora* genotypes was not known and the genetic relationship between Ugandan *C. canephora* and the known global *C. canephora* diversity groups was also not known. Information on genetic variability and diversity of the variability among *C. canephora* is vital prerequisite to sourcing CWD resistance genes and valuable genes for improving other agronomic traits.

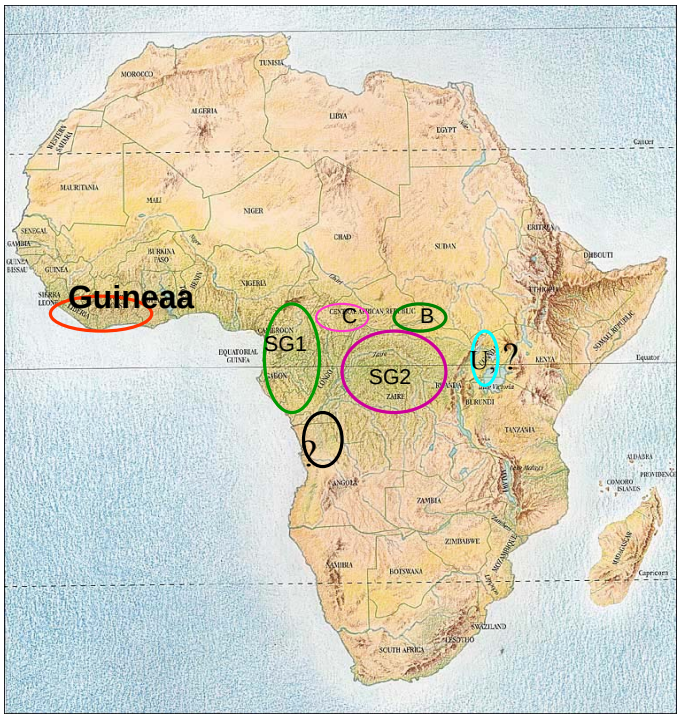


Figure I.V.- Geographical distribution of *C. canephora* Pierre diversity groups status in 2003. Guinean group is located in Ivory Coast and Guinea. Congolese groups B, C, SG1 and SG2 are located in Central African Republic, Cameroon and the Congo basin. The Ugandan population (U) is previously unknown.

Host resistance to CWD

Before this study was carried out resistance to coffee wilt disease in *C. canephora* was not known and information on genetic control of the resistance was lucking. Information on type of resistance and inheritance of the resistance is needed for designing and implementing an effective breeding programme to develop CWD resistant varieties.

Resistance of Ugandan *C. canephora* genotypes to *F. Xylarioides* strains affecting *C. arabica* and *C. liberica var. dewevrei* was also not known prior to this study. This information is necessary for designing breeding programmes to develop resistance which can be used across the entire African region. It is also important for designing effective germplasm exchange and quarantine strategies.

From field reports and results of screen tests on *C. arabica* using *F. xylarioides* isolate from *C. canephora* trees, it was established that *F. xylarioides* isolate affecting *C. canephora* does not affect *C. arabica*. However effect of this isolate on other diploid *Coffea* species, particularly *C. dewevri* was not known prior to this study. This information is also important for designing effective germplasm exchange and quarantine strategies and for considerations in interspecific hybridisation in respect to resistance against coffee wilt disease and associated agronomic and quality traits.

I.VIII Thesis outline

Analysis of disease data on *C. canephora* genotypes studied in experiments conducted in the field, screen house and controlled climatic chambers was used to investigate aspects relating to CWD resistance and interactions between the disease and host, stated in main objectives 1 and 3. Analysis of molecular data from interaction of host DNA with microsetallite markers was used to investigate aspects relating to the genetic diversity of *C. canephora* (host), to achieve the main objective 2. The rationale, methodologies and findings of the various investigations carried out are presented in different chapters of this thesis. Chapters 1 and 2 constituted journal articles which were submitted for review and publication. Chapter 4 also constituted a journal articles which is still in preparation.

Chapter 1: Spatial and temporal analysis of Coffee Wilt Disease caused by Fusarium xylarioides in C. canephora

Data collected for 5 years from a field experiment with known genotypes was used to analyze spatial and temporal field spread of coffee wilt disease. This study provided information on the epidemic of the disease (development of new infections, pattern of spread and rate of mortality of infected trees overtime). Genotypes which withstood field infections were considered resistant and they illustrated the importance of controlling this disease using genetic resistance. This study also provided information on effect of infected trees on neighbouring healthy trees. The chapter constituted a journal article, which was submitted to the European Journal of Plant Pathology for review and publication.

Chapter 2: A new genetic diversity group from Uganda within Coffea canephora Pierre

DNA data from Ugandan *C. canephora* genotypes of wild and cultivated origins was analyzed in comparison to genotypes from known *C. canephora* diversity groups using microsetallite markers. This study provided information on genetic diversity and structure of wild and cultivated Ugandan *C. canephora* and location of Ugandan *C. canephora* genotypes in the global *C. canephora* diversity. The chapter constituted a journal article, which was submitted to Genetics journal for review and publication.

Chapter 3: Assessment of resistance to coffee wilt disease caused by Fusarium. xylarioides in Coffea canephora

Studies in this chapter were conducted in three parts:

Part 1: Assessment of resistance to CWD

Analysis of disease data on *C. canephora* in the field and artificial inoculations (screen house & climatic chambers) was used to generate information on resistance of *C. canephora* to coffee wilt disease. Resistance in the field and in artificial inoculations was compared and different traits measured for quantifying resistance were compared. Information generated in these studies is useful in screening germplasm for resistance against coffee wilt disease.

Part II: Analysis of host specificity to F. xylarioides isolates.

Analysis of disease data on *C. canephora* in controlled climatic chambers was used to generate

information on response of *C. canephora* to *F. xylarioides* isolates causing coffee wilt disease in *C. arabica* and *C. liberica* var. *dewevrei.* This information is important in selecting resistant varieties for use in different agro-ecological areas infested with different *F. xylarioides* isolates.

Analysis of disease data on *C. liberica* var. *dewevrei* in controlled climatic chambers was used to generate information on response of this species to *F. xylarioides* isolates causing coffee wilt disease in *C. canephora*. This information is a prerequisite for interspecific hybridisation to introgress CWD resistance from one species to the other.

Part III: Assessment of Ugandan C. canephora populations for resistance to coffee wilt disease

Analysis of disease data on *C. canephora* under artificial inoculations was used to assess the level of CWD resistance among wild, feral and cultivated populations. This information is valuable for sourcing resistance among diversity sub-groups within Ugandan *C. canephora* population.

Chapter 4 Inheritance of resistance to coffee wilt disease (Fusarium xylarioides Steyaert) in Coffea canephora Pierre

Analysis of disease data on *C. canephora* clones and half diallel, and half sib progenies plus part of the analysis in Chapter 3 generated information on resistance in this species to coffee wilt disease and inheritance of the resistance. This information is important for selecting parents and breeding strategies to develop varieties with effective and durable resistance. Through this study and studies reported in Chapter 3, resistant *C. canephora* clones, which can be multiplied for on-farm adoption studies and also used in the breeding programme, were identified. This chapter constitutes a journal article, which is still in preparation for submission to the New Phytologist Journal.

Chapter 5: Discussion and conclusion

Finally results reported in chapters 1-4 where discussed in light of available literature on wilts in different pathosystems, and practical application to on-going research and future breeding activities to develop wilt resistant commercial varieties of *C. canephora* for revamping the coffee industry in Uganda.

CHAPTER 1

Spatial and temporal analysis of Coffee Wilt Disease caused by *Fusarium xylarioides* Steyaert in *Coffea canephora*

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Abstract

Coffee wilt disease caused by *Fusarium xylarioides* Steyaert is endemic in some African countries, affecting commercially important coffee species, causing serious economic losses. Currently there is an emergency to control this disease. Coffee wilt disease development in naturally infected *Coffea canephora* fields at the Coffee Research Institute in Uganda was assessed from April 2001 to March 2006 to generate information about temporal and spatial spread of the disease, required in an effective disease management strategy. Disease progress curves showing temporal disease development and maps of diseased trees were generated from the disease data. Semi-variance analysis and kriging were performed on the data to show the spatial-temporal structure of disease. Host influence on the spatial-temporal structure was deduced through distribution pattern of

diseased and healthy trees and analysis of variance. Results show that the disease epidemic progresses gradually overtime. The disease was found to spread irregularly from initial infections to healthy neighbour trees, leading to aggregated patterns. An infected tree can infect up to three healthy trees away, in any direction. Disease foci form and grow with time, coalescing to one continuous stretch, only punctuated in spots planted with resistant hosts. There were varying levels of susceptibility among host genotypes, exhibiting varying rates and levels of disease development. The implications of these findings to the control of this disease are discussed.

Introduction

Coffee production worldwide is about 7 millions tons of green beans per year (FAO, 2006). After oil, it is the second biggest commodity on the international markets, earning more than 9,000 million dollars per year. Arabica coffee produced from *Coffea arabica* L and Robusta coffee produced *Coffea canephora* Pierre, are the main commercial coffee types. Robusta coffee constitutes 35% of global coffee production (ICO, 2006).

Coffee wilt disease (CWD) is a vascular disease caused by *Fusarium xylarioides* Steyaert, which is the conidial stage of *Gibberella xylarioides* Hem. & Saccas. Fraselle (1950) and Saccas (1951) give the description of the disease symptoms on coffee trees in the field. Although Saccas (1956) reported *F. xylarioides* to attack coffee bushes through wounds, infection right from the soil through the root system without wounding is also possible (G Hakiza, CORI, Uganda, personal communication). Infection is possible right from the cotyledon stage up to very advanced host age. The incubation period in the host is not known but it is suspected to range from days in young plants to about 6 months in mature plants, before invasion of vascular tissues to cause wilting (Saccas, 1956; Muller, 1997). Diseased trees die within one to 15 months (P. Musoli, personal observations).

Coffee wilt disease was first reported in 1927 on *C. liberica* spp. in the Central African Republic (CAR) (Figueres, 1940). The disease subsequently destroyed this crop during 1930s to 1950s in Cameroon (Guillemat, 1946; Fraselle, 1950; Saccas, 1951; Muller, 1997). During the same period, it destroyed *C. canephora* in Ivory Coast (Delassus, 1954). Fraselle (1950) reported CWD on *C. canephora* at Yangambi in the DRC in 1948 and subsequently the disease became a serious problem in certain parts of this country. Lejeune (1958) reported similar disease symptoms on *C. arabica* in Ethiopia and later Kranz and Mogk (1973) confirmed that the disease

on *C. arabica* was also caused by *F. xylarioides.* Pieters and Van der Graaff (1978) reported that this disease was endemic in all coffee growing parts of Ethiopia.

Coffee wilt disease was controlled in Central and West African countries by combined use of resistant varieties and cultural practices (Muller, 1997). But in the 1980s, new outbreaks were reported on *C. canephora* in the DRC (Flood and Brayford, 1997), from where it has spread affecting the same species in Uganda and Tanzania.

In Uganda, coffee is the most important cash crop, both in terms of employment and value of production, and is a major source of foreign currency. However, CWD, which emerged in 1993 (Flood and Brayford, 1997), has rapidly devastated the Robusta coffee crop, disrupting national economy and decreasing the incomes of coffee producers (Lukwago and Birikunzira, 1997). Surveys carried out in 2002 found the disease in all *C. canephora* growing areas and on over 90% of the farms in Uganda. The disease has already destroyed over 44% of the crop nationwide (Oduor et al., 2005). Similar surveys found this disease on many farms in the DRC, Ethiopia and Tanzania. Currently CWD is a major threat mainly to the Robusta coffee industry in Africa.

Information on the epidemiology of CWD is scanty. Knowledge on the epidemiology of this disease is required for designing and effective implementation of control strategies. The scarcity of epidemiological information partly accounts for the failure to control CWD in Uganda using phytosanitory measures (Wetala et al., 2000).

Spatial and temporal analyses have been applied in the field of plant pathology at a variety of scales, from plot to agricultural regions, to analyze interactions between pathogens, hosts and the environment in relation to plant disease epidemics (Chellemi et al., 1988). Information generated from spatial and temporal analyses is valuable for understanding mechanisms that bring about the patterns and for developing strategies to manage the diseases (Ristaino and Gumpertz, 2000). Different Geo-statistical tools have increasingly become important in spatial and temporal analyses (Ristaino and Gumpertz, 2000). Semi variance (variogram) analysis and kriging are among the Geo-statistical tools widely used in the analysis of spatial-temporal dynamics of different plant diseases (Rekah et al., 1999; Van de Lande and Zadoks, 1999; Jaime-Garcia et al., 2001).

In this study the spatial-temporal pattern of CWD in a naturally infected field of mixed *C. canephora* genotypes was analyzed using a combination of classical and Geo-statistical analyses. The study used semi variance (variograms) analysis to relate the spatial and temporal spread of coffee wilt disease among the coffee trees. The disease probability maps derived by kriging were

related to the spatial and temporal pattern of diseased trees in the field. The study addressed 3 specific questions: 1) How does the coffee wilt disease epidemics develop in time? 2) How does the coffee wilt disease pattern change in space and time? And 3) How does host heterogeneity affect the disease epidemics and the spatial pattern? Findings to these questions can be used in designing and implementing coffee wilt control strategies.

Materials and methods

Studies were carried out in Uganda on a *C. canephora* field experiment at CORI, Kituza. The experimental field was planted in October 1997. The trial was initially designed to evaluate varieties for yield, growth habits, quality and resistance to coffee leaf rust (*Hemileia vastatrix*) and red blister disease (*Cercospora coffeicola*), since at that time CWD was not a serious problem in the country and it had not yet reached Kituza. However as the wilt disease started and continued to affect coffee trees in this field, it became necessary to understand its spread.

The experimental field covers an area of 0.50 ha planted with 20 *C. canephora* clones. A total of 16 out of 20 clones were single tree selections among progenies of specific crosses and the remaining four were commercial clones; 1s/2, 1s/3, 223/32 and 257/53. All the clones were selected for agronomic traits (yield and cup quality) other than resistance to CWD.

The experiment was laid out in a randomised complete block design with four replicates running along contours. Each replicate was sub-divided into 20 plots of six trees arranged in straight line patterns of 3 rows x 2 columns at 3 x 3 m spacing. Each of the clones was randomly allocated and planted in a plot in each of the replicates. Clones Q/6/1 and Q/1/1 were only planted in replicates 1 & 2 due to insufficient planting materials. Their plots in replicates 3&4 remained without plants. Clones H/4/1 and R/14 were not planted in replicate 4 because of a similar reason. The experiment was surrounded by two guard lines of none experimental materials and a guard line in between replicates of similar materials.

The experiment was weeded, trained, pruned and supplied with organic and inorganic fertilizers following routine procedures for maintaining *C. canephora* gardens (MAAIF, 1995).

Data collection and analysis

Assessment of CWD took precedent to other traits, because of its severity within the field. Degree of CWD infection was measured by assessing percentage plant defoliation caused by the disease. All trees in experimental plots were assessed twice every month on a disease severity scale of 1 to 5

where 1 = no disease, 2 = 1-25% defoliation, 3 = 26-50% defoliation, 4 = 51-75% defoliation, 5 = 76-100% defoliation. Plants in level 5 were considered dead. Non-experimental coffee trees in rows and columns surrounding experimental gardens and separating replicates were not assessed.

Coffee wilt disease was first observed in the experimental field in 1999 but systematic assessment did not start until April 2001 and lasted until March 2006. From March 1999 to Match 2001, all affected trees were uprooted to minimize and, if possible, eradicate the disease.

Percent tree mortality was calculated from the disease severity data as a proportion of plants that attained level 5 on the disease severity scale. The percentage tree mortality was plotted over time to give the disease progress curve. The percentage new tree mortality per six month time period was plotted to give the disease epidemic curves.

Maps for spatial distribution of diseased (sick and or dead) coffee trees at annual intervals were generated from the disease severity data using SAS (SAS Institute Inc., Cary, NC, 1989). The maps were used to visualize the spread of this disease over time.

Isotopic semi-variance analysis was performed to determine the spread of CWD over time measured with and without host effects. All disease severity data were analyzed using semi-variance analysis performed with GS^+ (Gamma Design Software, 2004). In this analysis, semi-variances on the data for all possible pairs of trees were determined as:

$$Y(h) = {}^1/_2 N(h) \sum [z(s_i) - z(s_i+h)]^2,$$

where Y(h) is the semi variance for interval distance class h, N(h) is the total number of pairs of trees for the lag interval h (lag distance), $z(s_i)$ is the disease severity value of a coffee tree located at point s_i, and z(si+h) is the disease severity value of a coffee tree at distance h from s_i. Semi variances for each interval class were plotted against corresponding lag distances to constitute a semi-variogram. The GS^+ program provides five types of isotropic models, each of which can be described by three parameters (Figure 1.1) that relate to the spatial structure of the disease.

The three parameters are: I) Nugget variance or C_0 – this is the y-intercept of the model. It represents variance due to error, II) Sill or C_0+C – this is the model asymptote which provides an estimate of total sample (population) variance where C is the variance due to spatial structure and III) Range or A – this is the separation distance over which spatial dependence is apparent, sometimes called the effective range in order to distinguish range (A) from the model's range parameter (A_0). The software also provides three statistics to aid the interpretation of the model output: Residual sums of squares (rss) an absolute measure of how the model fits the data, the

lower the rss the better; r^2 another indication of how well the model fits the data although not as robust as the rss, and the proportion $C/(C_0+C)$ which provides a measure of the proportion of sample variance (C_0+C) that is explained by spatially structured variance C. A high ratio of C/(Co+C) implies there is a large spatial dependence. Since the analysis is performed on repeated measurements, it allows analysis of changes in the spatial dependence from the variogram parameters for the different data sets.

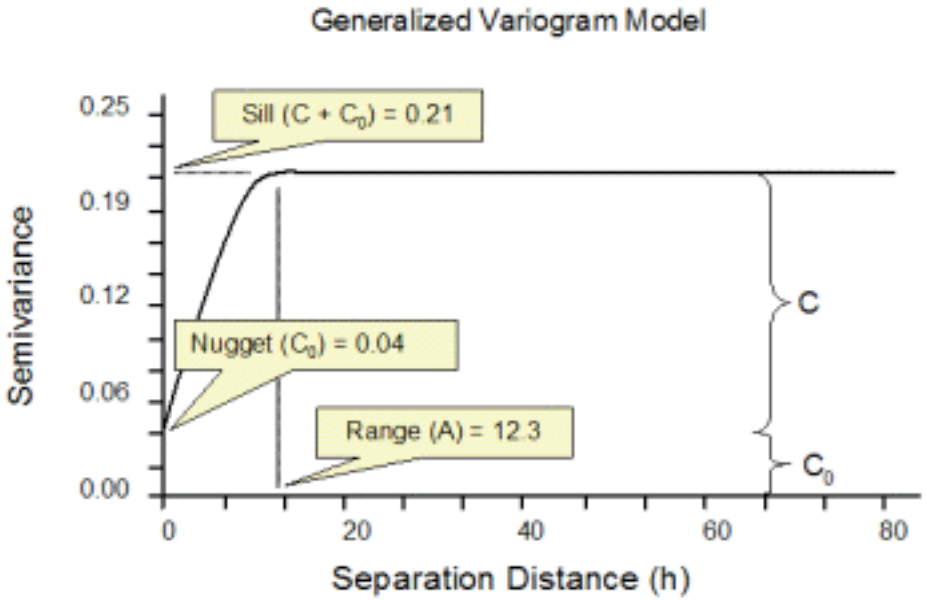


Figure 1.1. Generalized Variogram Model (GS^+ User's Guide Version 7, Gamma Design Software 2004).

For the analysis without host effects, residuals were calculated for all individual tree scores by subtracting corresponding family or clone means. The residual data was then subjected to the semi-variance auto-correlation statistics analysis and semi-variograms were derived to show the spatial spread of the disease measured without host effects.

Kriging analysis was performed on data sets used in semi-variance analyses to show spatial gradients of the disease measured with and without host effects also using $G.S^+$ software. Kriging interpolates disease data values for points not physically sampled using values from neighbouring measured points (trees) plus knowledge about the underlying spatial relationships given by variograms to produce two-dimensional maps of disease intensity. Since the analysis is performed on repeated data sets, the analysis allows visualisation of the disease gradients over time.

Results

Temporal pattern

The level of CWD, as indicated by percentage mortality, was relatively high at the beginning of the assessment (25.2%, Figure 1.2a). Percent mortality increased from 25.2% when assessment started in April 2001 to 64.5% in March 2006. The disease epidemic, as indicated by new mortalities (Figure 1.2b), was highest between April 2001 and June 2002 but subsequently decreased and levelled of over time before finally reaching 0.4%

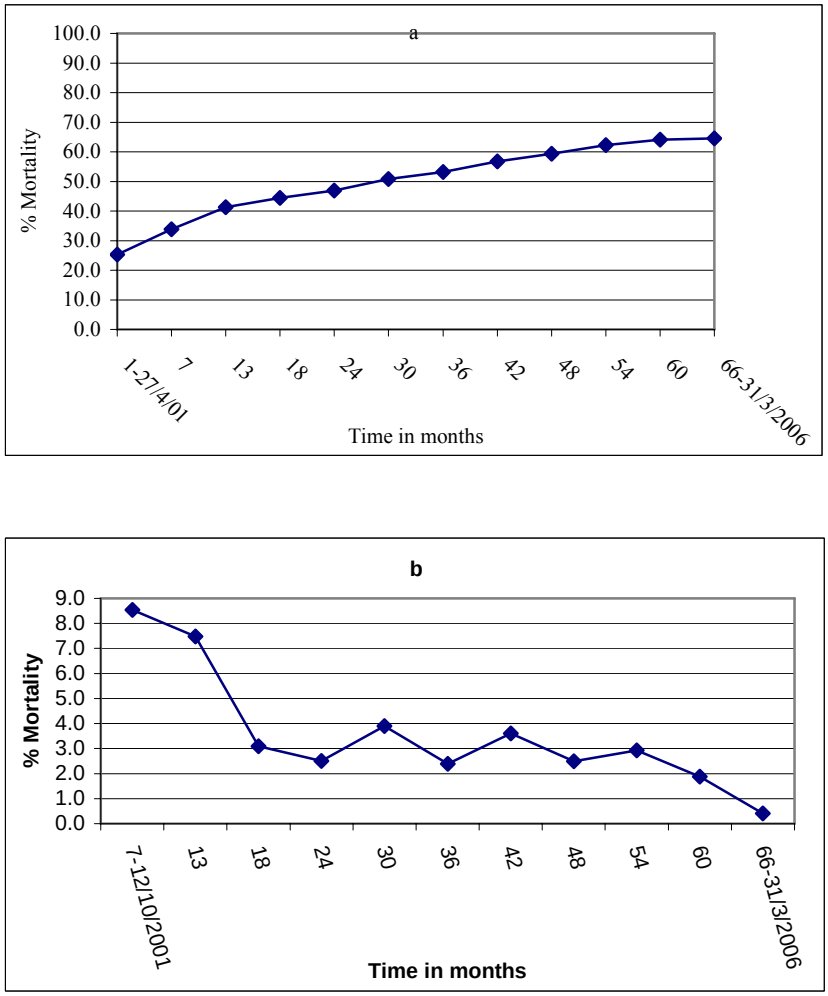


Figure 1.2: Coffee wilt disease progress curves and epidemic rates

2a) Increase of percent mortality, 2b) Percent new mortality

Spatial pattern

In Figure 1.3, maps with individual trees classified as either healthy, diseased or dead for the years 2001, 2002, 2003, 2004, 2005 and 2006 are shown. Trees affected by CWD aggregated

into clusters of varying sizes. The number of affected trees among the aggregates increased gradually over time. Areas of the experimental field planted with clones 1s/3, Q/3/4 and R/1/4, had very few affected trees. Parts of the experiment planted with clone J/1/1 did not have any trees with wilt symptoms. Analysis of variance performed on the disease severity data from the different assessment dates shows that variations due clones significant ($p<0.0001$) but variations due to blocks (replicate) were never significant

Semi-variance analysis performed on the disease severity data shows that overall, the exponential model fitted the actual data with host effects the best. The rss ranged from 0.058 to 0.510 for the 11 semi-variograms used to illustrate the spatial dependence of CWD (Table 1.1). The same model also provided the best fit, with the rss ranging from 0.032 to 0.136 for the 11 semi-variograms used to illustrate spatial dependence of CWD without host effects. Small nugget (error) effects were observed for all semi-variance analysis of disease spread measured with and without host effects (Table 1.1). The proportion (C/Co+C) of structural variance (C) to total variance (Co+C) for the disease measured with host effects ranged from 0.85, observed in June and November 2005 and March 2006, to 0.90 in April and October 2001 (Table 1.1). The proportion of spatial structural variance C/Co+C for disease measured without host effects ranged from 0.82 in June and December 2004 to 0.94 in December 2003, November 2005 and March 2006 (Table 1.1). A high proportion of spatial structural variance (C/Co+C) indicates that there is a strong correlation between trees for the spatial spread of coffee wilt disease.

The spatial dependence distance or in other words the effective range, increased from 1.9 trees in April 2001 to 3.3 trees in March 2006 (Table 1.1), showing that the disease spread from diseased trees to infect up to two neighbouring coffee trees away in April 2001 and up to four trees away by March 2006, in all direction.

Spatial dependence was also observed in the analysis without host effects (Table 1.1). Changes in spatial dependence distance over time for disease measured without host effects were smaller than observed for disease measured with host effect. The effective range for disease measured without host effects varied slightly between years, from 0.96 in March 2002 to 1.29 in April 2001. Indicating that in all occasions, the disease spread to infect up to one coffee tree away.

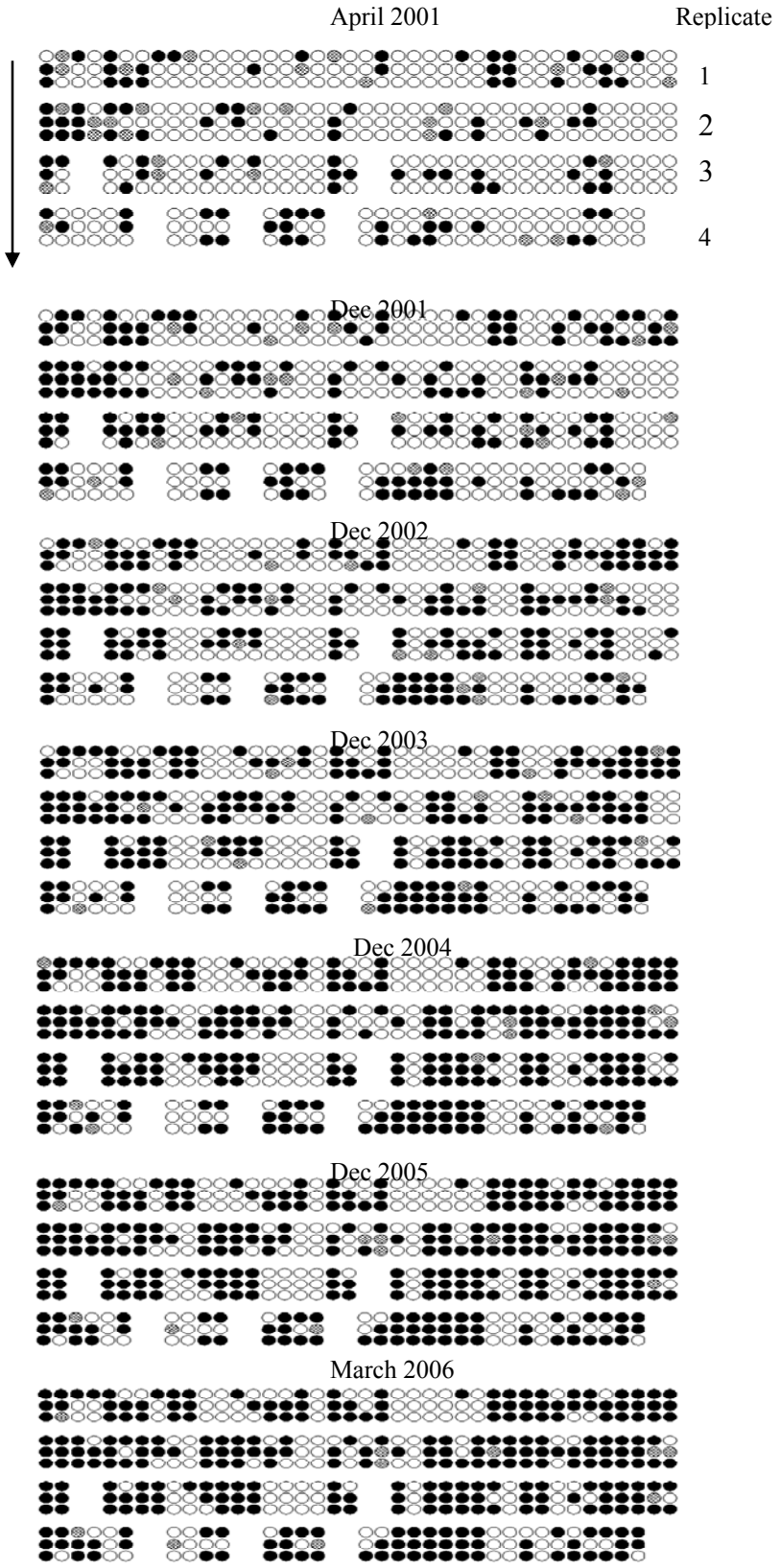


Figure 1.3: Pattern of diseased trees

Arrow is pointing down slope; Each tree is represented with a circle; Black circles are trees killed by CWD, Circle with cross squares show sick tree and white circles show uninfected trees; 4 replicates in the field separated by larger clear lines; 2 rowsx3 columns of circles in each replicate represent a clone; White gaps within replicates represent missing data; Trees uprooted prior to April 2001 are included among the dead

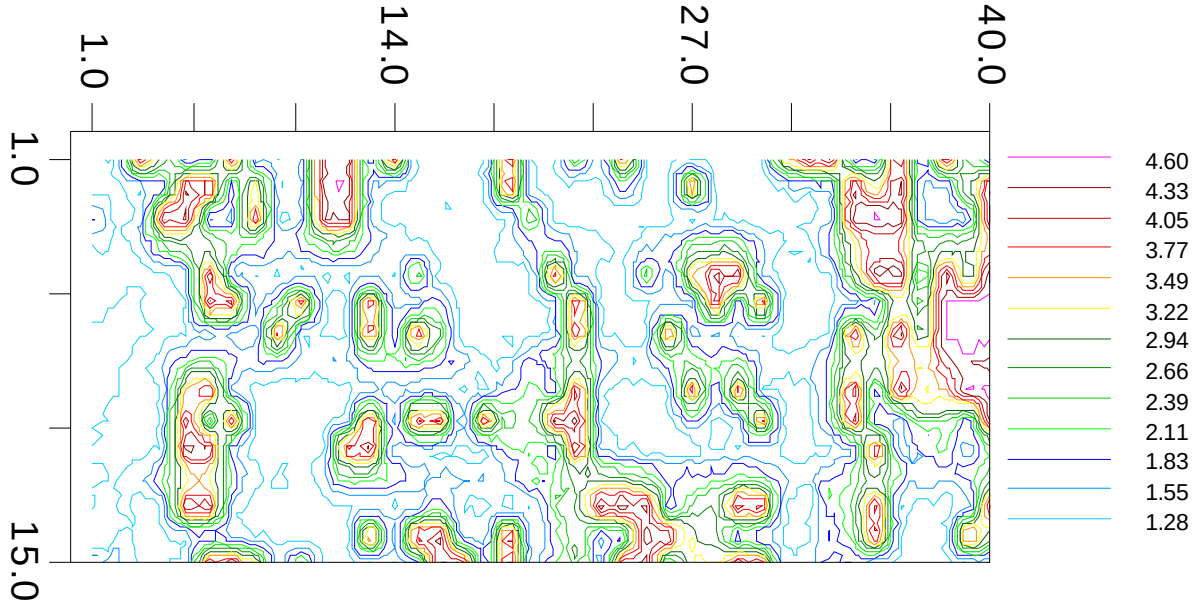
Table 1: **Variogram characteristics and model parameters of coffee wilt disease spread**

Date	Rss	C/C_o+C	Nugget (C_o)	Sample variance (C)	Sill (C_o+C)	Range (A_o)	Effective Range (A)
Measured with host effects							
April 2001	0.10	0.90	0.29	2.61	2.90	0.62	1.86
October 2001	0.17	0.90	0.48	4.13	4.61	0.69	2.07
March 2002	0.12	0.89	0.54	4.41	4.95	0.68	2.04
November 2002	0.17	0.88	0.63	4.76	5.39	0.76	2.28
May 2003	0.14	0.88	0.73	5.41	6.136	0.83	2.49
January 2004	0.24	0.87	0.75	5.19	5.94	0.88	2.64
July 2004	0.33	0.87	0.77	5.14	5.91	0.88	2.64
December 2004	0.43	0.86	0.83	4.90	5.73	0.99	2.97
June 2005	0.49	0.85	0.83	4.83	5.66	1.02	3.06
November 2005	0.51	0.85	0.81	4.74	5.55	1.07	3.21
March 2006	0.06	0.85	0.86	4.88	5.74	1.10	3.30.
Measured without host effects							
April 2001	0.04	0.92	0.20	2.18	2.38	0.34	1.02
October 2001	0.14	0.90	0.35	3.17	3.52	0.37	1.11
March 2002	0.08	0.89	0.39	3.17	3.56	0.26	0.78
November 2002	0.06	0.88	0.43	3.15	3.58	0.32	0.96
May 2003	0.04	0.87	0.46	3.04	3.50	0.39	1.19
January 2004	0.03	0.89	0.42	3.32	3.74	0.39	1.19
July 2004	0.04	0.89	0.42	3.30	3.72	0.34	1.02
December 2004	0.04	0.89	0.39	3.10	3.49	0.33	0.99
June 2005	0.04	0.88	0.40	3.07	3.47	0.32	0.96
November 2005	0.06	0.89	0.38	2.99	3.37	0.40	1.2
March 2006	0.06	0.88	0.41	3.10	3.51	0.43	1.29

Kriging maps revealed numerous disease foci of varying sizes widely scattered in all parts of the experiment at the beginning of assessment (Figure 1.4a). The disease foci expanded in all parts of the experiment and by March 2006, they had coalesced to form large continuous zones of high disease severity levels surrounding a few patches with lower disease severity levels or no disease (Figure 4b). The patches of low disease severity levels or no disease corresponded

to areas planted with clones J/1/1, Q/3/4, 1s/3 and R/14 as well as some areas with missing data. The change in size of disease foci measured without host effects, over the same period was smaller and some foci had small but noticeable displacement in location (data not shown).

a: clone & residuals in April 2001



b: clone & residuals in March 2006

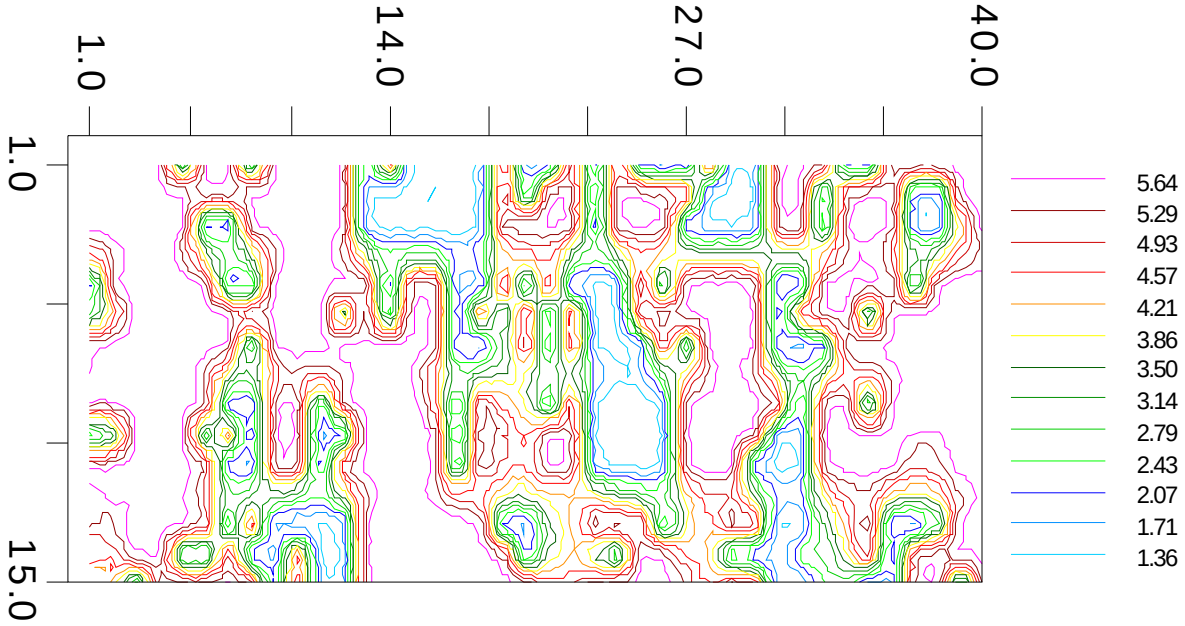


Figure 1.4: Kriging maps showing intensity of disease measured with host effects at different dates

Analysis of host influence

Mean disease severity indicated that most hosts (clones) were affected to some level by CWD in April 2001. The disease severity levels among the clones at this date varied from 0.0, for clones J/1/1 and Q/3/4, to 3.46 for clone P/3/6. These three hosts showed tree mortalities of 0.0%, 0.0% and 54.2%, respectively. By March 2006, disease severity levels among the coffee genotypes ranged from 0.0 for clone J/1/1, to 4.83 for clone C/1/7. Percentage tree mortality for these clones at this date was 0.0% for J/1/1 and 95.8% for clone C/1/7.

Discussion

Geo-statistics is useful in quantifying the degree and range of spatial dependence of variables (Ristaino and Gumpertz, 2000) and it has been used in plant pathology to quantitatively characterize changes in the spatial patterns of disease over time. Geo-statistics has been used to study the spatial distribution and temporal development for annual disease cycles such as Fusarium crown disease and root rot of tomato (Rekah et al., 1999) and late blight disease on tomato and potato (Jaime-Garcia et al., 2001). It has also been used to study spatial patterns of perennial diseases such as spear rot disease in oil palm (Van de Lande and Zadoks, 1999). In our study, geo-statistics was effectively used to analyze the spatial pattern of CWD, monitored over five years in a coffee plot in Uganda. Although the plot was not originally designed to study CWD, and studies did not commence directly with the arrival of this disease, we view that the data presented here provide valuable insight in CWD epidemics.

The study shows that at early stages of CWD epidemic, a more or less random distribution pattern was observed. In time, clusters of diseased and dead plants were formed, expanding in all directions (Figures 1.3 and 1.4). The high structural or sample variance derived from semi-variance analysis revealed that the spatial dependence for the disease spread is high, which implies that plant to plant infection plays a major role in CWD spread. However, the actual method(s) by which CWD spreads e.g. through root contact between diseased and healthy trees, by ascospores or conidia; is still unclear. Moreover, the source for initial infections which will start of an epidemic remains a key question to be answered by more in depth studies of the epidemiology of CWD.

The effective range, derived from semi-variance analysis of assessments with host effects indicated that diseased coffee trees can infect healthy coffee trees up to approximately 3 coffee trees (10 m) away. This dependence distance was initially shorter, between one and two coffee trees, but increased with increasing disease incidence. Rekah et al. (1999) found that the infection range of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the causal agent of crown and root rot of tomato, ranged between 1.1 and 4.4 meters in the exponential phase of the disease. Spatial dependence of *Phytophthora capsici* was found to reach 15 meters (Larkin et al., 1995).

The effective range calculated from assessment without host effects, of approximately one coffee tree (± 3 m), was shorter and more constant. Together with the result from the kriging analysis, which showed small, albeit measurable displacements of disease foci when comparing analyses with and without host effects, these results also show the importance of the coffee genotype in the spread of coffee wilt disease.

Information on the role of diseased trees in disease spread as well as knowledge on the effective range is important for designing effective coffee management strategies. Removal of infected trees as soon as the first symptoms become noticeable and consequently the possibility of disease spread is minimal will minimize build up of inoculum and slow down CWD epidemics. Our results also suggest that for uprooting to be effective, coffee trees up to 10 meters away from the initial disease focus have to be uprooted. As indicated in historical papers, rigorous systematic uprooting was used to eliminate the previous coffee wilt disease problem on *C. liberica* in Cameroon (Muller, 1997). From this point of view, uprooting can be applied in those regions where the disease is appearing for the first time but only when disease levels are still low, most likely below 10%, or when it is still localized. At higher disease levels, uprooting will be uneconomical since the infection foci will be many and scattered throughout the fields.

The disease progress curve (Figure 2a) illustrated that once CWD has invaded a *C. canephora* field, it will continue devastating the coffee trees until all susceptible trees are affected and will thus eventually die. The epidemic rate was highest, approximately 10% per annum, when mortality was between 25-45%. This seems logical, as at this stage there are sufficient inoculum sources and there are still many trees available for the inoculum to start new infections. The epidemic rate was reduced drastically as tree mortality exceeded 50%, since the number of susceptible trees had greatly reduced. This is typical for wilt diseases of many perennial tropical plants as observed for passion fruits and other perennial fruit crops (Ploetz,

1991, Ploetz, 2003, Ploetz 2006). Especially where soils are contaminated with the wilt pathogen, susceptible crops can often not be produced for decades. The overall 65% mortality observed in our field by the end of the study, within a period of seven years, illustrates the devastating effects of the disease. Moreover for some genotypes the mortality was as high as 96%. This confirms earlier reports that CWD is a very serious impediment to investment in coffee farming (Lukwago and Birikunzira, 1997; Rutherford, 2006) and underlines the urgent need for *C. canephora* resistant varieties since most of the Robusta coffee growing areas in Uganda and the DRC are infected with the coffee wilt disease.

The variability observed in disease progression and final disease levels on the *C. canephora* clones in this study illustrate that host genotypes influence spatial and temporal CWD development. This variability is attributed to variability in genetic resistance of host genotypes to the disease. Since kriging analysis showed disease foci to be present throughout the experimental field at the beginning of the assessment, thus there was sufficient inoculum to infect the plants in all parts of the study field; the genetic resistance should be the cause of the differential mortality observed among the clones in this field. This observation illustrates the presence of resistance among Uganda *C. canephora* genotypes. This phenomenon should be explored to identify more resistant clones required for re-planting country wide. As reported by Ploetz (2006), *Fusarium* wilt diseases are managed most effectively by using host resistance. Saccas (1956) and Meiffren (1961) mentioned that resistant varieties were replanted to control the previous coffee wilt disease epidemic in the DRC and Ivory Coast. This observation also indicates discrete patches of particular genotypes withstanding coffee wilt in a highly infected field can be explored for identifying resistant varieties in farmers' fields where the wilt has devastated most of the coffee trees.

It should be noted here that, although these studies generated valuable information which is pertinent for the disease control, future studies should target at generating information on other critically important aspects of the disease epidemiology such as the factors involved in disease transmission. This information is necessary for focusing and timing of phytosanitory interventions. It is also imperative that the resistant clones identified in this study are evaluated in different agro ecological areas to verify this resistance and determine their performance for other agronomic traits before they are availed to farms.

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CHAPTER 2

A new genetic diversity group from Uganda within *Coffea canephora* Pierre

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Abstract

For the purpose of identify sources of variability to control coffee wilt disease, which has devastated *Coffea canephora* in Uganda, wild and cultivated populations of this perennial crop were evaluated for their genetic diversity. One hundred and ninety six Ugandan *C. canephora* genotypes from 14 sites among two wild and one feral population plus two cultivated

populations were analyzed using 24 microsatellite markers. Eighteen of these markers were used to compare Ugandan populations with previously known genetic diversity groups of the species. Ugandan *C. canephora* was found to be highly diverse with a genetic sub-structure consisting of wild, feral and cultivated populations. Uganda *C. canephora* was found to be different from previously known diversity groups, implying it forms another diversity group within the species. In this study the relationships between cultivated, feral and wild Ugandan *C. canephora* populations were discussed in relation to available literature. Suggestions for conservation and rational utilization of this genetic diversity in relation to the development of wilt resistant varieties were made. The implications of our results to similar studies and breeding strategies for other tropical perennial tree crops were also discussed.

Introduction

Tree crops such as coffee, cacao, rubber tree and oil palm are of great economic importance in many tropical countries. For these perennial crops, resistance to pests and diseases and quality of the products are the main objectives of selection in national and international programs. Hence management of genetic resources of these crops is very important for breeding as they are being threatened by human activities and natural disasters in their natural habitats. But the management of these genetic resources is however expensive and demanding as they have to be maintained in field collections covering large areas, and evaluation of these field collections is a long term, tedious and costly process. Nevertheless, for coffee currently there is dire need for genetic resources to control coffee wilt disease.

Coffee belongs to the genus *Coffea*. This genus includes over 100 species, with a basic chromosome number of n=11 (Davis et al, 2006). *Coffea arabica* is a predominantly self pollinated tetraploid and self-fertile (Charrier and Berthaud, 1985) a cultivated species of this genus. This species is indigenous mainly in Ethiopian highlands. *Coffea canephora* is another cultivated species of the genus, which is predominantly cross pollinated and self-sterile diploid (2n=2x=22) (Charrier and Berthaud, 1985). Berthaud (1986) reported that pollen dispersal of *C. canephora* in forests, which is mainly by insects, can be up to two or three km distance and the dispersal of seeds, which is mainly due to animals especially mammals, can be up to five or 10 km away. *C. canephora* is indigenous to some areas of the tropical African Forest, stretching from West Africa through Cameroon, Central African Republic (CAR), Congo, the Democratic

Republic of Congo (DRC), Uganda, and up to Northern Angola. Since *Coffea* species are indigenous to Africa covering a wide geographical range, from Western Africa to Madagascar and exhibiting wide diversity range, they could be used as a model for studying evolution of diversity in other perennial tree crops in this zone.

Coffee is an extremely important commodity with nearly 7 million tons of green beans produced yearly in about 80 tropical countries (FAO 2006), however about 30 of these countries produce small quantities for local consumption. In terms of economic importance on international markets, it is the third agricultural product after oil palm and Soya bean, earning US $ 6-13 billion per year. Mainly two types of coffee are produced all over the tropical world: Arabica, produced from *Coffea arabica*, which grows well in higher altitude areas, and Robusta, produced from *Coffea canephora*, which is adapted to low and medium altitudes. Robusta coffee represents 35% of the global coffee production (ICO 2006).

In Uganda, coffee is the most important cash crop, both in terms of employment and value and, despite low and fluctuating world prices and diversification in exports, it still remains a major source of foreign currency, contributing 15-20% of foreign currency earnings yearly. Both Arabica and Robusta coffee are produced in Uganda, however, Robusta production is currently threatened by coffee wilt disease (CWD. This disease, which is caused by *Fusarium xylarioides,* became a serious problem of *C. canephora* and *C. liberica* spp. in Central and West African countries in 1930s to 1950s but it was controlled *on C. canephora* by replanting with resistant varieties (Meiffren, 1961; Flood and Brayford 1997; Muller 1997). Phytosanitory measures were used to control the disease on *C. liberica* spp. in Cameroon (Muller 1997). However in early 1980s this disease re-merged in the DRC from where it spread to Uganda and eventually to Tanzania. CWD was first reported in Uganda in 1993 and by 2002 it had spread to all Robusta producing zones affecting over 90% of farms and destroying about 45% of *C. canephora* trees nationwide (ODUOR *et al.* 2005). The disease is also affecting *Coffea canephora* gene pools in the Research stations and it was observed on wild coffee trees in Kibale and Itwara forests during surveys in 2004 and 2005. Currently there is an emergency in Uganda to develop CWD resistant varieties for controlling this disease. In this regard, *C. canephora* populations from two primary forests (Kibale and Itwara) and Kalangala islands in Lake Victoria in Uganda were explored for variability in search for potential sources of resistance to control CWD. The variability of these populations was studied using molecular markers.

Molecular markers are quick, dependable and relatively cheap tools for genetic analysis (Karp *et al*. 1996). Isozyme markers were the first to be used for this purpose. Discovery of these markers revolutionized the study of population genetics, as they allow gathering of the information within a relatively short time (Bernie 1998). The methodology was widely adopted and later it was used in association with other markers by many scientists (Beer et al. 1993; Gerdes and Tracy 1994; Hayati et al. 2004). Several other molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLPs) were developed. More recently microsatellite markers (Simple Sequence Repeats/SSRs) and Single Nucleotide Polymorphisms (SNPs) were adopted for studying genetic diversity and are now widely used. Since microsatellites are highly polymorphic, co-dominant and widely dispersed in genomes (Belaj et al. 2003) they have become popular valuable tools for genetic diversity and population genetics in plants.

Berthaud (1986) first described the genetic diversity of *C. canephora* using isozymes. He identified two diversity groups, a Congolese group, which comprised of genotypes from CAR, Congo basin and Cameroon and a Guinean group, which consisted of genotypes of wild origin from Ivory Coast. Montagnon et al. (1992), also using isozymes, proposed a substructure in the Congolese group with two sub-divisions SG1 & SG2. Dussert et al. (2003), using RFLP molecular markers placed *C. canephora* genotypes of cultivated and wild origins into five diversity groups, adding two groups B and C to the Congolese group. Recent studies using SSR markers on *C. canephora* among other coffee species (Poncet *et al.* 2004) and on *C. canephora* alone (Cubry et al. 2005) confirmed the structure described by Dussert et al. (2003).

This paper presents results of a study on genetic diversity of Ugandan *C. canephora* genotypes of wild and cultivated origins, in comparison to genotypes from the previously described diversity groups (Dussert et al. 2003). In this investigation, we addressed four questions: 1) what is the genetic diversity and structure of wild and cultivated Ugandan *C. canephora*? 2) Where are Ugandan genotypes located in the global *C. canephora* diversity? 3) What are the implications of the findings to the conservation and utilization of Ugandan *C. canephora* genetic resources in breeding for resistance against coffee wilt disease? And 4) what are the implications of the findings to the conservation strategies and utilization of genetic resources of other tropical perennial tree crops?

Materials and methods

For the purpose of studying Ugandan populations and testing relationships between cultivated and wild populations, a hierarchical sampling was performed in Uganda. It covered three regions, two of them consisted of wild populations and the third region is feral, which involved sampling previously cultivated and now forested areas on islands in Lake Victoria. For the first wild population from Itwara region, which covers an area of about 100 km², five sites separated by distances ranging from 0.6 to 10 km were sampled. In each site, seven to 18 individuals were sampled. For the second wild population from Kibale region, which covers an area of 500 km², four sites separated by distances of 7 to 19 km, four to 30 individuals per site were sampled. In both Kibale and Itwara regions, healthy looking trees estimated to be 40 to 70 years old were sampled. Sampling sites in these regions were located throughout the forests and at least one to five kilometres from the edges of the forest. Kibale and Itwara are primary forests considered to be a natural home of wild *C. canephora* (Maitland 1926; Thomas 1944). The two forests once formed a continuum but in the early 1900s areas in between were allocated to human settlement which led to their fragmentation and they are currently 30 km apart.

The third region, Kalangala, consisted of five sites separated by at least 10 km spread on two islands. The sites were located 0.5-2 kilometres from the edge of the islands. Three to 12 healthy looking trees, estimated to be over 40 years old were sampled per site. This region is a secondary forest, which regenerated from former cultivated areas and the population in here can thus be considered feral (Thomas 1935).

Among cultivated populations, individuals with two distinct tree architectures were sampled. The first type, called Erect, consists of genotypes which have inherently strong and erect stems. The second type, called Nganda, consists of genotypes with inherently small, flexible and spreading stems. Both types are old collections from farms which have undergone 2-3 generations of cultivation, as open pollinated seedlings, at Kawanda Agricultural Research Station (KARI). The two types of genotypes do not represent defined geographical origins but were considered as separate groups, equivalent to regions in this study. Individuals of each type were selected on the basis of historical records and field spot maps in addition to their phenotypic appearance.

To compare Ugandan *C. canephora* with known diversity groups of the species, previous analyses of Guinean genotypes and genotypes from Congolese regions SG1, SG2, B and C were

considered (Figures 2.1 and 2.2). Guinean and Congolese populations SG1, B and C genotypes are of wild origin while SG2 genotypes are cultivated.

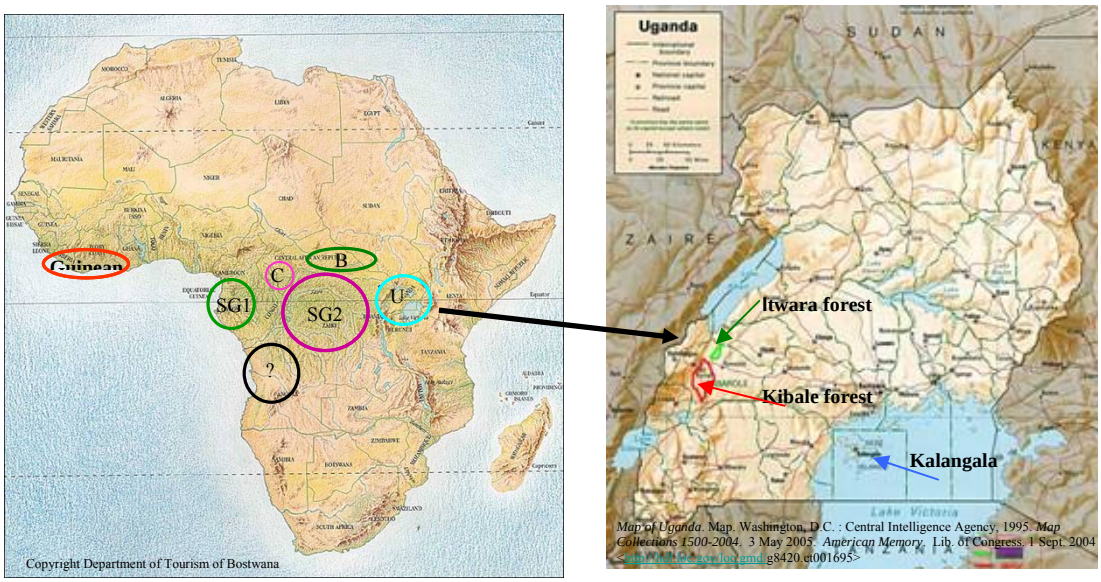


Figure 2.1- Geographical distribution of *C. canephora* Pierre diversity groups. Guinean region covers Ivory Coast and Guinea. Congolese regions (B, C, SG1, and SG2) are located in Central African Republic, Cameroon and the Congo basin. ? is an unexplored region located in Angola. Uganda's map shows the three surveyed regions: Kibale and Itwara forests, Kalangala islands.

DNA extraction: Genomic DNA was extracted from leaf powder of ground frozen leaves using MATAB buffer and the extraction method adapted from a procedure described for cocoa (Risterrucci et al. 2000). The DNA extracts were purified using the solution-based Promega's Wizard Genomic DNA Purification Kit (Cat# A1125). Concentration of the DNA extracts was estimated using a spectrophotometer before they were standardized at 0.5 ng/µl final work solution.

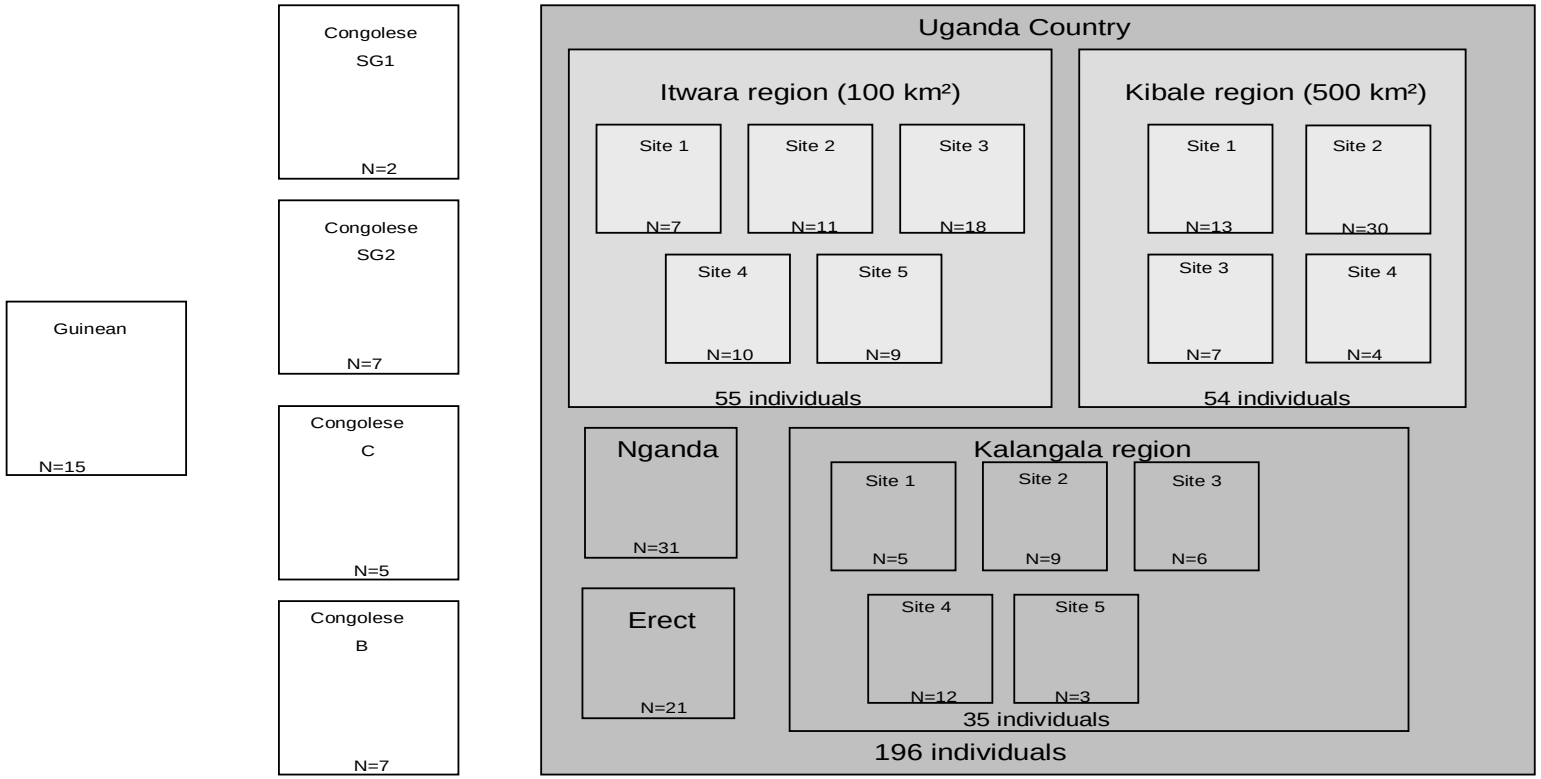


Figure 2.2 - Hierarchical representation of plant material sampling: country, regions and sites are identified. The number of individuals per region and site (N) is indicated in the boxes. Sampling surface for Ugandan forests is indicated in parenthesis

Table 2.1 - Microsatellite markers used for genotyping 232 *C. canephora* samples.

Marker code	LG	Repeated sequence (SSR)	Allele size (bp)	Forward primer	Reverse primer	EMBL database number	Reference
351	3	$(GT)^{13}$	304	AAGGATGGCAAGTGGATTTCT	GCAGCTCTTGATTGTAGTTTCGT	AM23551	DUFOUR *et al.* 2002
355	1	$(TG)^{15}$	177	CTATGATGTCTTCCAACCTTCTAAC	GGTCCAATTCTGTTTCAATTTC	AM231552	DUFOUR *et al.* 2002
358	13	$(CA)^{11}$	248	CATGCACTATTATGTTTGTGTTTT	TCTCGTCATATTTACAGGTAGGTT	AM231554	DUFOUR *et al.* 2002
364	6	$(A)^{21}$	90	AGAAGAATGAAGACGAAACACA	TAACGCCTGCCATCG	AM231556	DUFOUR *et al.* 2002
368	8	$(TG)^{13}$	160	CACATCTCCATCCATAACCATTT	TCCTACCTACTTGCCTGTGCT	AM231558	DUFOUR *et al.* 2002
384		$(AC)^{10}$	255	ACGCTATGACAAGGCAATGA	TGCAGTAGTTTCACCCTTTATCC	AM231560	DUFOUR *et al.* 2002
394	6	$(TG)^{9}$	124	GCCGTCTCGTATCCCTCA	GAAGCCAGAAAGTCAGTCACATAG	AM231563	DUFOUR *et al.* 2002
429	2	$(A)^{13}$	175	CATTCGATGCCAACAACCT	GGGTCAACGCTTCTCCTG	AM231565	DUFOUR *et al.* 2002
442	8	$(CA)^{19}$	227	CGCAAATCTGAGTATCCCAAC	TGGATCAACACTGCCCTTC	AM231566	DUFOUR *et al.* 2002
445		$(AC)^{10}$	274	CCACAGCTTGAATGACCAGA	AATTGACCAAGTAATCACCGACT	AM231567	DUFOUR *et al.* 2002
456	8	$(AC)^{14}$	297	TGGTTGTTTTCTTCCATCAATC	TCCAGTTTCCCACGCTCT	AM231568	DUFOUR *et al.* 2002
461	2	$(AC)^{9}$	461	CGGCTGTGACTGATGTG	AATTGCTAAGGGTCGAGAA	AM231570	DUFOUR *et al.* 2002
463	10	$(AC)^{8}$	227	CATTCTTCCCACGATTCTATCTC	GTGACTTTCGGTTGAAATACTGG	AM231571	DUFOUR *et al.* 2002
471	1	$(CT)^{12}$	301	TTACCTCCCGGCCAGAC	CAGGAGACCAAGACCTTAGCA	AM231572	DUFOUR *et al.* 2002
501	7	$(TG)^{8}$	343	CACCACCATCTAATGCACCT	CTGCACCAGCTAATTCAAGC	AM231576	DUFOUR *et al.* 2002
753	6	$(CA)^{15}$	294	GGAGACGCAGGTGGTAGAAG	TCGAGAAGTCTTGGGGTGTT	AJ308753	ROVELLI *et al.* 2000
755	1	$(CA)^{20}$	184	CCCTCCCTCTTTCTCCTCTC	TCTGGGTTTTCTGTGTTCTCG	AJ250258	COMBES *et al.* 2000
774	3	$(CT)^{5}(CA)^{7}$	228	GCCACAAGTTTCGTGCTTTT	GGGTGTCGGTGTAGGTGTATG	AJ308774	ROVELLI *et al.* 2000
779	7	$(TG)^{17}$	116	TCCCCCATCTTTTTCTTTCC	GGGAGTGTTTTTGTGTTGCTT	AJ308779	ROVELLI *et al.* 2000
782	5	$(GT)^{15}$	114	AAAGGAAAATTGTTGGCTCTGA	TCCACATACATTTCCCAGCA	AJ308782	ROVELLI *et al.*2000
790	1	$(GT)^{21}$	134	TTTTCTGGGTTTTCTGTGTTCTC	TAACTCTCCATTCCCGCATT	AJ308790	ROVELLI *et al.* 2000
837	2	$(TG)^{16}(GA)^{1}$	102	CTCGCTTTCACGCTCTCTCT	CGGTATGTTCCTCGTTCCTC	AJ308837	ROVELLI *et al.* 2000
DL013	2	$(CA)^{6}(CT)8$	267	AGAGGGATGTCAGCATAA	ATTTGTGTTTGGTAGATGTG	AJ871892	LEROY *et al.* 2005
DL025	1	$(C)^{17}$	197	TTGTTGAGAGTGGAGGA	CCAAAGACAGTGCAGTAA	AJ871902	LEROY *et al.* 2005

LG: Linkage group of the genetic map

SSR genotypes: Twenty four polymorphic microsatellite markers (SSR) widely distributed on *C. canephora* genome (Appendix 1), were used to genotype the 196 genotypes from Uganda (Table 2.1). Eighteen of these markers were used for analyzing individuals from the previously known diversity groups genotyped by Cubry et al. (2005). Microsatellite markers DL013 and DL025 were previously published from a BAC library developed for studying sugar metabolism in coffee (Leroy et al. 2005). Other marker sets came from SSR enriched library of *C. canephora* clone 126 (Dufour *et al.* 2002) and from enriched libraries of *C. arabica* var. c*aturra* (Combes et al. 2000; Rovelli et al. 2000). All PCR primers were defined using PRIMER3 software (Rozen and Shaletsky 2000). *C. arabica* primers were defined by Poncet et al. (2004) and primers from *C. canephora* libraries were defined by Berline (2002). The DL primers were defined by Leroy et al. (2005).

PCR amplification and visualization of the microsatellites: PCR reactions were performed in 10 µl, containing 2.5 ng of DNA, 1 mM Tris-HCl, 5 mM KCl, 2 mM MgCl2, 200 µM dNTP, 0.10 µM of reverse primer, 0.08 µM of forward primer tailed with M13 sequence, 0.10 µM of infrared fluorescently labelled M13 primer and 0.1 U of Taq DNA polymerase. PCR amplifications were run in an Eppendorf Ep384 thermocycler. Amplification program consisted in an initial denaturation cycle of 4 min at 94°, followed by 10 cycles of "Touch-Down" (45 sec at 94°, 1 min at 60° to 55° decreasing by 0.5° per cycle, and 1.5 min at 72°), 25 cycles (45 sec at 94°, 1 min at 55°, and 1.5 min at 72°) and ended with a last elongation step at 72° for 5 minutes.

Fluorescently labelled PCR products were analyzed by electrophoresis migration on a LiCor® 4300 automated sequencer with a 6.5% acrylamide gel. Gels' images were retrieved and annotated with manufacturer's program SAGA® GT Generation Two. Allele sizes were evaluated on the basis of allelic controls previously defined by Cubry et al. (2005). Data matrix was used for further analyses. All genotyping experiments were performed on the Genotyping Platform of Genopole Montpellier Languedoc-Roussillon, France.

Basic diversity analyses: Summary statistics, including number of alleles, observed and expected heterozygosity (gene diversity), were calculated for all groups of genotypes using PowerMarker (Liu and Muse 2005). These analyses were performed on Ugandan genotypes in comparison with previous values obtained for other genetic groups (Cubry et al. 2005).

CONVERT software (Glaubitz 2004) was used to point out private alleles in all regions and sites. This software was also used to format the data for other software analyses, including Fstat (Goudet 2001), Arlequin (Excoffier et al. 2005) and Structure (Pritchard et al. 2000).

Dissimilarity analysis: Dissimilarity matrix between individuals (in Ugandan samples using 24 SSR markers, comparison of Ugandan individuals with individuals of other regions using 18 out of the 24 markers), based on simple matching index, was computed with DARwin 5 (Perrier et al. 2003) taking into account missing data. A tree representation of the dissimilarities was obtained using the same software and weighted neighbor joining method (Saitou and Nei 1987). Robustness of the nodes was assessed by 1000 bootstraps.

Fixation and genetic differentiation analyses: At the different levels of sampling, Hardy-Weinberg equilibrium was tested using fixation indices within sites, regions or country (F_{IS}). Genetic differentiations between sites or region levels were estimated with F_{ST}. All *F*-statistics were estimated with Fstat software (Goudet 2001) using estimations proposed by Weir and Cockerham (1984), since Nei's *F*-statistics (Nei 1973) are less convenient when sample sizes vary a lot. Although SSR markers might not follow assumptions needed for *F*-statistics, especially in terms of mutation rate, these statistics were preferred to R_{ST} (Slatkin 1995), since in our case (low number of individuals in some cases, and low number of loci) they appear to be more robust (Gagiotti et al. 1999). Significance of all statistics was performed with 1,000 permutations (Fstat, Goudet 2001), and although genotypes and not alleles were permuted for testing the differentiation between levels, we called the statistics F_{ST} instead of the log-likelihood G (Goudet et al. 1996) used in the software.

In order to test whether genetic differentiation follows a pattern of isolation by distance, correlation between genetic distance matrixes (F_{ST} values between pair of sites) and geographic distances were tested with a Mantel test using GenAlEx software (Peakall and Smouse 2006). We applied data transformation proposed by Rousset (1997), to perform regression analysis between logarithm of the geographical distance and a genetic distance coefficient calculated as $F_{ST}/(1-F_{ST})$. This test was used to calculate correlation within Ugandan wild populations (Kibale and Itwara). Geographical distances between populations were computed using GPS coordinates for each site. The geographical distances between diversity regions for overall analysis were

estimated by comparing collection sites for groups B, C, with Ugandan wild populations. An average geographical centre of the group was used for regions SG1 and SG2.

Molecular variance analysis: The level at which major genetic differentiation occurs was estimated with hierarchical analyses of molecular variance using Arlequin software (Excoffier Et Al. 2005). Analyses were performed up to individual level, with an accepted level of missing data of 0.2 and tested with 1,000 permutations.

Population structure analysis: Finally, fine structure and relationships of Ugandan populations were analyzed with STRUCTURE software (Pritchard et al. 2000). STRUCTURE software was used to infer the population structure within Uganda, assign individuals to regions, and know more about admixtures between defined populations. A 50,000 burn-in period and 100,000 MCMC iterations were used for each run. We chose to take the default parameters with no prior information about the populations' origin in order to reallocate our individuals to groups. Simulations were made for a number of groups K varying from K = 1 to K = 20 with five repetitions for each values of K. Transformation of resulting data, following a method proposed by Evanno et al. (2005), taking into account rate of change in log probability of data between successive K values enhanced detection of optimal value of K.

Results

Basic diversity statistics: Table 2.2 presents a summary of the basic diversity statistics. The mean number of alleles is significantly higher (Wilcoxon two sample ranked test, $U=30$, $p<0.01$) in Ugandan regions compared to other diversity groups. Among Ugandan wild samples, Kibale and Itwara populations present lower values for observed heterozygosity and gene diversity than cultivated populations, including Kalangala region (Wilcoxon two-sample ranked test, $U=3$, $p<0.01$). Itwara region presents higher values for observed heterozygosity and gene diversity than Kibale region. The three cultivated and feral Ugandan populations present observed heterozygosity and gene diversity values close to values of SG2 cultivated group, and Kibale presents observed heterozygosity values close to values of known wild diversity populations (Guinean, Congolese B and C). These results are comparable to results obtained by Cubry et al. (2005).

Table 2.2 - Basic diversity statistics

	Region	No. of sites	No. of individuals	Observed Heterozygosity (SD)	Gene diversity (SD)	No of alleles (Mean No. of alleles per locus)	No. of private alleles (Ugandan regions)	No. of private alleles (all regions)
	Itwara	5	55	0.396 (0.044)	0.586 (0.041)	193 (8.04)	37	14
	Kibale	4	54	0.288 (0.043)	0.531 (0.051)	177 (7.38)	19	4
Uganda	Kalangala	5	35	0.405 (0.045)	0.628 (0.049)	206 (8.58)	34	17
	Nganda	1	31	0.407 (0.043)	0.623 (0.048)	194 (8.08)	14	7
	Erect	1	21	0.397 (0.045)	0.625 (0.048)	172 (7.17)	12	6
	Guinean	2	69	0.335(0.053)	0.524(0.060)	97 (5.40)		24
Others	SG2	4	25	0.405(0.054)	0.67(0.048)	125 (6.95)		12
	SG1	1	9	0.213(0.072)	0.337(0.059)	39 (2.17)		11
	Congolese B	1	39	0.283(0.0052)	0.416(0.055)	87 (4.84)		7
	Congolese C	1	10	0.320(0.053)	0.465(0.046)	52 (2.89)		14

Diversity statistics for Guinean and Congolese regions, adopted from CUBRY *et al*., 2005, were calculated using 18 markers. Number of private alleles across all regions was detected with 18 markers on 232 individuals. SD = standard deviation

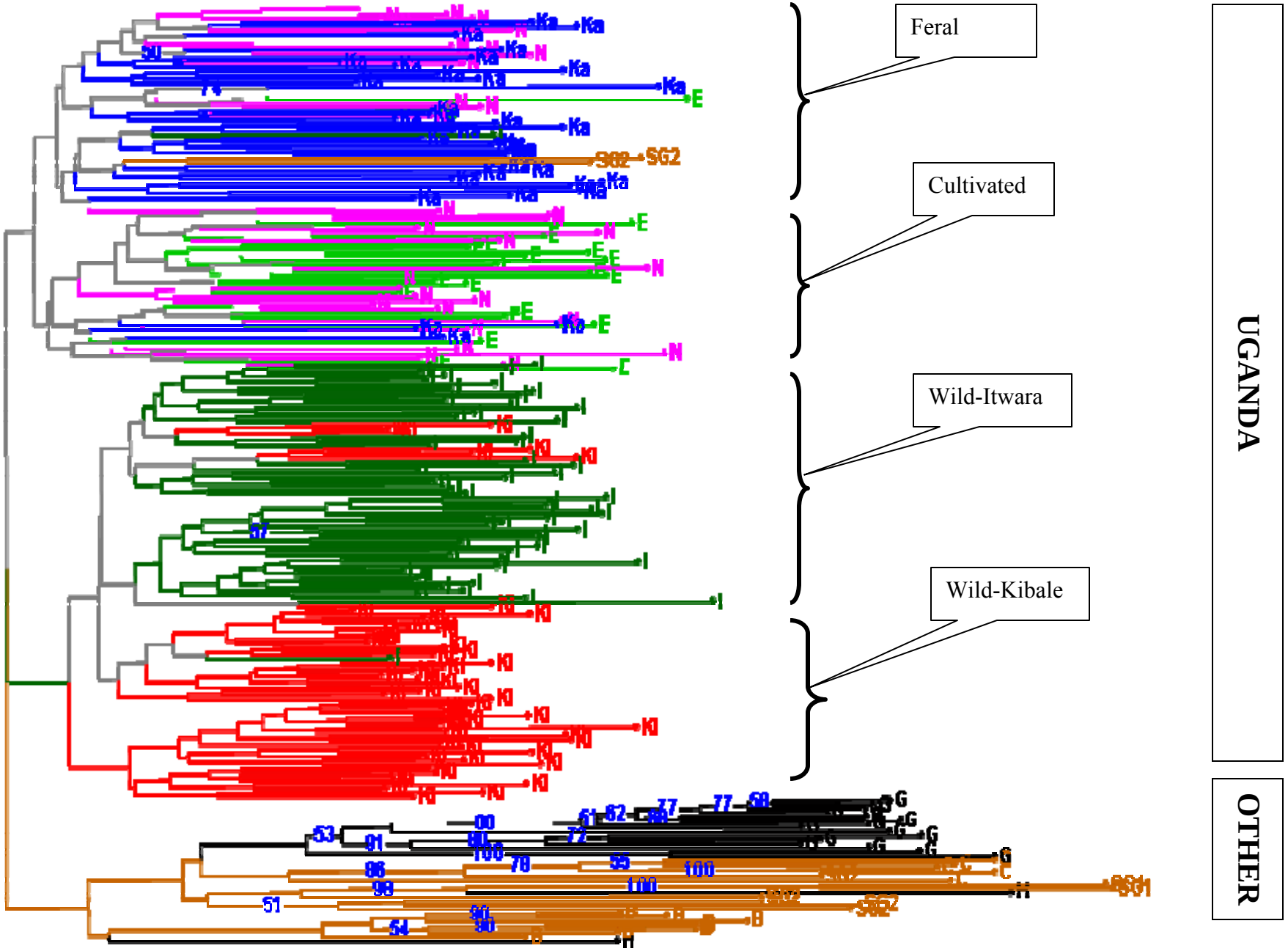
Among Ugandan regions, wild forests regions and Kalangala present the highest number of private alleles. It was noted that individuals harbouring these private alleles are less frequent than 10%, except for one locus in Itwara region. Individuals from wild Ugandan populations have different numbers of private alleles. If we consider all the regions, Guinean, Itwara and Kalangala regions present the highest number of private alleles. For five loci, over 50% of Guinean individuals harbour private alleles. For the locus 358, all the Guinean individuals harbour private alleles.

Genetic distances between individuals: When all samples were considered individually, Guinean and Congolese groups were clearly separated from genotypes of Ugandan origin (Figure 2.3), with the previously described structure being distinct. Uganda thus is a new genetic diversity compartment within *C. canephora*. Besides, among Ugandan samples, wild genotypes from Kibale and Itwara are separated from cultivated and feral genotypes. When only the 196 Ugandan genotypes are considered, (data not shown) four groups of genotypes are identified, discriminating Kibale, Itwara, and Kalangala from a group that includes Erect and Nganda individuals. Three Nganda genotypes and one Kalangala genotype are located close to Kibale genotypes.

Fixation indices and genetic differentiation: Among Uganda, all the F_{IS} values are significantly different from zero, except for two sites, one in Kibale and one in Itwara regions (Figure 2.4). This implies there is genetic structure in sites and regions.

In order to assess population differentiation, F_{ST} coefficients were estimated between sites for each region and between regions for Uganda. Considering all the ten regions or only the five Ugandan regions, the differentiation is significant, with F_{ST} always higher than 0.15 (Figure 2.4 and Table 2.3). If the three Ugandan geographical regions (Kibale, Itwara and Kalangala) are considered separately, high differences are observed. Kibale region exhibits a highly significant between sites F_{ST} coefficient (F_{ST} =0.17), meaning that this region is highly differentiated into sites. This is probably due to the large distances between sites and therefore less genetic exchanges. On the other hand, F_{ST} coefficients are not significant for Itwara forest and Kalangala region, meaning that sites sampled inside these regions are not genetically different. This is probably due to shorter distances between sites and therefore more genetic exchanges as compared to sites in Kibale region.

Figure 2.3 - Diversity tree of 232 individuals (simple matching distance based on 18 microsatellite loci, weighted neighbor joining). G = Guinean; SG1, SG2, B and C = Congolese regions; H = hybrids between Congolese and Guinean forms; I = Itwara forest; Ki = Kibale forest; N = Nganda, E = Erect; Ka = Kalangala islands. Bootstrap values (1000 permutations) over 50 are plotted



F_{ST} values for diversity regions excluding Uganda are superior to 0.20, indicating a high differentiation between regions (Table 2.4). Lowest F_{ST} values are observed between Congolese groups SG2 and C, these two regions being geographically the closest, with enhanced possible genetic exchange compared to other regions. Guinean region presents pair wise F_{ST} values with other regions ranging from 0.24 to 0.44, indicating high level of differentiation from other regions. SG1 region presents the highest F_{ST} values with other regions (0.33 to 0.63). Highest F_{ST} value is obtained between the Congolese SG1 and B groups. Considering Uganda as a whole in relation to other regions, it is important to note that pair wise F_{ST} values are always lower for Ugandan regions relating to SG2 than when relating to other regions. This implies that Ugandan *C. canephora* are genetically more related to SG2 region. F_{ST} values observed between the three cultivated Ugandan populations are low, ranging from 0.04 to 0.11, confirming their common genetic background. F_{ST} values for relationship between cultivated and wild populations are much higher, ranging from 0.16 to 0.24, but they remain lower than those obtained for wild populations with other diversity regions (0.28 to 0.59). Within Ugandan regions (Tables 2.3 and 2.4), pair wise F_{ST} values between sites are very low for Kalangala region (0.00 to 0.11) and Itwara (0.00 to 0.05), but quite high (0.11 to 0.35) between sites in Kibale region, confirming existence of a high differentiation between sites in Kibale. F_{ST} value of 0.10 is observed between wild Ugandan populations.

Ugandan wild populations showed a clear pattern of isolation by distance (P_{Mantel}=0.015, Table 2.5). Since these forests were separated at the beginning of the 20th Century, pollen and seed exchanges between the regions have been almost impossible. Itwara population showed a pattern of isolation by distance (P_{Mantel}=0.04, data not shown), whereas Kibale population did not show this pattern. Considering all the diversity regions, correlation between geographical distances and genetic distances were less evident than correlations within Uganda, since the probability of the correlation coefficient (0.132) is not significant.

Molecular variances: Results for analysis of molecular variance (AMOVA) showing genetic level at which main differentiations occur are presented in Table 2.6. Considering only wild Ugandan populations (Kibale and Itwara) with Congolese regions SG1, SG2, B, C and Guinean region (six defined groups), variation was mostly partitioned between regions (36.2%) and among individuals within regions (23.4%). When considering Uganda cultivated (Nganda and

Erect) and feral (Kalangala) versus wild samples partitioned into Uganda (Kibale and Itwara) and Guinean and Congolese regions (7 groups), results indicate a high percentage of the variation is explained by the groups defined (20.3%). Small percentage of the variation is explained by regions within groups (6.9%) and more than 70% of the variation is explained by individuals among regions and within individuals. This result indicates that the region structure is strong, but a large amount of the variation is at the individual level. Finally, taking into consideration only Ugandan samples (5 groups), the percentage of variation explained by regions is not high (13.5%), in comparison to variation among individuals within regions (19.9%) and within individuals (66.7%). When Erect and Nganda or all cultivated and feral genotypes are grouped, results indicate that less than 10% of the variation is explained by the groups, less than 7% is explained by regions within groups, and more than 80% is explained at the individual level (data not shown). These results indicate that cultivated Nganda and Erect genotypes from Uganda, which are closely related to SG2 group, don't confer a strong genetic structure within *C. canephora*, as compared to wild populations, including wild regions outside Uganda, that are clearly differentiated

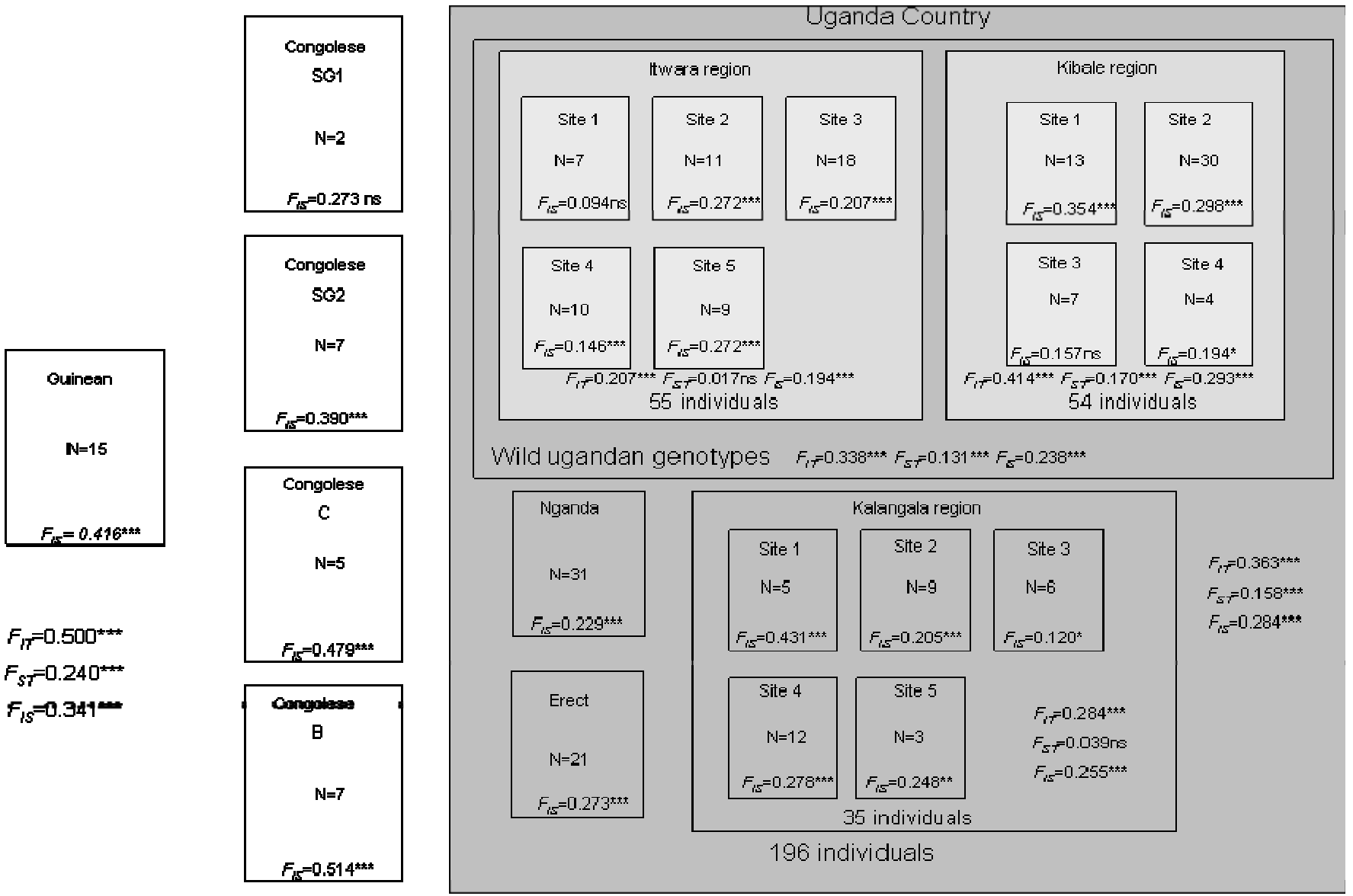


Figure 2.4: *F* statistics within and among countries, regions and sites. *FIS* values and significance for all countries, regions and sites are indicated in the corresponding boxes. *FIT*, *FST* and *FIS* are indicated for Ugandan regions including different sites, wild Ugandan genotypes and Uganda country. Overall values have been estimated considering the 10 regions. N indicates the sample size

Table 2.3 - Pair wise F_{ST} coefficients among *C. canephora* populations within Uganda

		Itwara					Kalangala					Kibale				Nganda
Region	**Site**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	
Erect		0.17ns	0.20	0.19ns	0.23ns	0.20	0.11	0.09	0.14	0.12ns	0.12ns	0.29	0.17	0.20ns	0.24ns	0.03ns
Itwara	1	0	0.01ns	0.01ns	0.05ns	0.01ns	0.16	0.15	0.20	0.17ns	0.15ns	0.24	0.16	0.10ns	0.18ns	0.13ns
	2		0	0.01ns	0.04ns	0.01ns	0.17	0.16	0.20	0.18	0.15	0.20	0.17	0.10	0.22	0.13
	3			0	0.03ns	0.00ns	0.16	0.16	0.19	0.18ns	0.15ns	0.26	0.18	0.10ns	0.19ns	0.14ns
	4				0	0.04ns	0.24	0.23	0.28	0.23ns	0.21ns	0.31	0.30	0.15ns	0.29ns	0.18ns
	5					0	0.18	0.17	0.21	0.19	0.16	0.22	0.18	0.12	0.21	0.16
Kalangala	1						0	0.00ns	0.06ns	0.01ns	0.00ns	0.36	0.26	0.20	0.30	0.08
	2							0	0.08ns	0.03ns	0.04ns	0.33	0.22	0.21	0.29	0.09
	3								0	0.11ns	0.03ns	0.36	0.28	0.23	0.30	0.12
	4									0	0.04ns	0.33	0.25	0.22ns	0.29ns	0.10ns
	5										0	0.26	0.19	0.18*	0.25ns	0.09ns
Kibale	1											0	0.35	0.18	0.32	0.19
	2												0	0.16	0.21	0.14
	3													0	0.11*	0.14*
	4														0	0.21ns

Significance of P value for 1,000 permutations is indicated: ns=not significant; *=significant ($p<0.05$)

Table 2.4 - F_{ST} pair wise cœfficients between *C. canephora* populations

	Nganda	Kibale	Itwara	Kalangala	B	SG2	SG1	C	G
Erect	0.04ns	0.24*	0.23*	0.11*	0.38*	0.19ns	0.48*	0.37*	0.33ns
Nganda	0	0.16*	0.16*	0.07*	0.35*	0.22ns	0.52*	0.39*	0.34ns
Kibale		0	0.10*	0.21*	0.42*	0.33*	0.59*	0.49*	0.43*
Itwara			0	0.18*	0.37*	0.28*	0.52*	0.43*	0.39*
Kalangala				0	0.33*	0.17ns	0.46*	0.35*	0.31*
B					0	0.29*	0.63*	0.46*	0.42*
SG2						0	0.33*	0.21ns	0.24ns
SG1							0	0.37*	0.44*
C								0	0.31*

Significance of P value for 1,000 permutations is indicated, ns=not significant; *=significant ($p<0.05$)

Table 2.5 – Isolation by distance pattern between all genetic groups and Ugandan regions

	R	P_{Mantel}	Statistic
Ugandan wild populations	0.411	0.015	F_{ST} matrix
All regions	0.497	0.132	F_{ST} matrix

Guinean genotypes, isolated from other groups, were not integrated in this study

Table 2.6 - Analysis of molecular variance (AMOVA)

Defined groups	Source of Variance	df	sm	Variance components	Percentage of explained variance
Wild from Uganda, Guinean, Congolese B, C & SG1	Among groups	5	298	2.23(<0.01)	36.2
	Among individuals	140	754	1.45(<0.01)	23.4
	Within individuals	146	365	2.50(<0.01)	40.4
Cultivated and feral from Uganda, wild from Uganda, Guinean, Congolese B, C, SG1 and SG2 populations defined within Ugandan groups (3 and 2 respectively)	Among groups	6	413	1.09(<0.01)	20.3
	Among populations within groups	3	97	0.37(<0.01)	6.98
	Among individuals within populations	223	1131	1.16(<0.01)	21.5
	Within individuals	233	643	2.76(<0.01)	51.3
All Ugandan regions	Among groups	4	287	0.85(<0.01)	13.5
	Among individuals within groups	192	1289	1.25(<0.01)	19.9
	Within individuals	197	829	4.21(<0.01)	66.7

df = degree of freedom; sm = sum of squares. P value for variance components is indicated in parenthesis

Population structure: Results of fine structure and relationships of Ugandan populations indicated four populations (Figure 2.5). Nganda and Erect populations constituted one group. Wild populations, Kibale and Itwara are clearly identified as different groups, with some level of admixture. We also observed some gene flows from Kibale region to Nganda-Erect group. Finally, Kalangala islands are identified as a specific group, with low admixture with other populations. Analysis performed with cultivated and feral populations (data not shown) discriminates Kalangala and Erect/Nganda populations. Analysis performed including SG2 region did not find admixtures with Ugandan populations (data not shown).

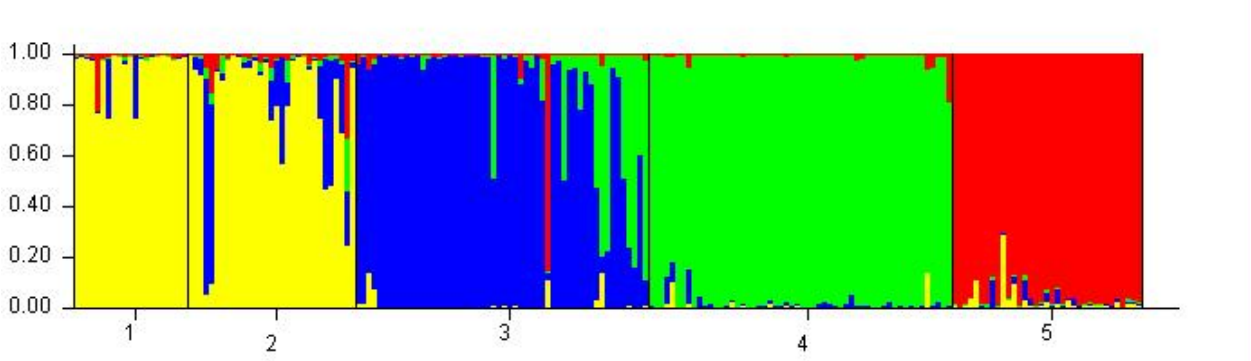


Figure 2.5 - Result of the population structure analysis within Uganda country. Four populations were identified: population A gathers Nganda (1) and Erect (2) regions. Population B is Kibale forest (3), population C is Itwara forest (4) and population D is Kalangala (5). Admixtures are presented by heterogeneous colours within populations.

Discussion

Diversity and structure of populations: The eighteen SSR markers used to analyze genetic diversity of *Coffea canephora* from different origins, clearly discriminated the different origins, confirming genetic diversity groups described in earlier studies using other molecular markers (Dussert *et al.* 2003) and pointed out the specificity and high diversity of *C. canephora* from Uganda (Figure 2.2).

Among Ugandan populations, wild and cultivated *C. canephora* are clearly identified (Figure 2.2 and Figure 2.5). The wild populations (Kibale and Itwara) exhibit contrasting results. Observed heterozygosity, gene diversity and allelic content are lower in Kibale than Itwara. This contrast could be partly due to sampling. Although nearly the same numbers of individuals were sampled for both of these regions, in Kibale a wider area was sampled and distances between sites were thus larger (7-19 km) as compared to 0.6-10 km for Itwara. It can also be noted that sampling between sites was not balanced. In Kibale a large part of individuals were sampled in one site (site 2) while the other sites provided a few individuals. Besides, genetic differentiation between sites is higher in Kibale than in Itwara, which can be related to the pattern of effective dispersion of pollen and seeds, sites having been chosen at distances larger than the effective neighbourhood size. However, the overall low number of private alleles and the level of introgression of Itwara region in Kibale and of Kibale genome into Nganda and Erect (Figure 2.5) suggest first that there is a directed gene flow from Itwara to Kibale, and second that Kibale samples were used as a source for cultivated populations. This has already been hypothesis for

the Nganda genotypes (Thomas 1935) and the Erect descendants of Maitland's selection tree 9 (Maitland 1926).

The feral population from Kalangala has a large number of alleles and private alleles. Structure is exhibited into the sites sampled on two islands, while no differentiation between sites is observed. While an insularity syndrome was expected (isolation of populations), at least three sources that provided planting materials for the old farms in Kalangala have been documented, first from the defunct Kasai forest (not sampled in this study) and from Nganda and Erect populations respectively, (Thomas 1935). Finally, Kalangala-Erect introgression is low (less than 5% in STRUCTURE analysis) while the effect of Kasai forest cannot be quantified.

Nganda and Erect present the same genetic background (Figures 2.2 and 2.5), probably because their phenotypic differences were not reflected in the genetic differentiation revealed by these by neutral markers. Similarities of Nganda and Erect populations can be explained by their cultivation history. This association can also be attributed to the fact that both populations have been cultivated closely in mixture for over three generations and open pollinated seedlings were used for planting new generations.

F_{IS} coefficients revealed a genetic structure within sites or regions. For the wild Ugandan populations, since *C. canephora* is strictly allogamous, this structure can be attributed to Wahlund effect. I;e. the structuring could be due to limited pollen and or seed inflow and thus the relatedness between individuals. The structure could also be due the founder effect, since most wild populations are normally in patches constituted from a small number of founder mother trees (Berthaud, 1986). For cultivated genotypes such as Nganda or Erect, it can be a bias related to the nature of the groups. For regions outside Uganda, all F_{IS} are highly significant and the same hypotheses apply. Note that F_{IS} value estimated for SG1 region, represented by only two samples is meaningless.

Analysis of molecular variance indicated a low percentage of variation explained by *C. canephora* populations from different Ugandan sources. These results show that all Ugandan populations have a common genetic background and suggest that genetic exchange could have occurred between these populations. At the species level, analyses of molecular variances confirmed high differentiation between regions. Percentage of variation explained by the diversity regions at global level is very high, confirming that a structure with six diversity

groups, the five already identified and wild Uganda should be considered for global *C. canephora* diversity.

The correlations between geographical and genetic distances revealed that wild Ugandan populations present a genetic differentiation related to geographical distance. Effective gene flow; which can occur through pollen dispersed mainly by insects (reported to be limited to a 3 km distance) and through seed dispersed mainly by mammals (limited to 10 km in forests (Berthaud, 1986) is thus limited. Mantel test correlations were not significant for the overall analysis, since geographical distances are very large and genetic exchanges between populations are impossible. Furthermore, the structure of sampling within regions was unbalanced. In Guinean region, where more than 20 forests covering Ivory Coast and part of Guinea have been prospected (Berthaud 1986; Le Pierres et al, 1990), *C. canephora* diversity observed in this region can be considered sufficiently studied and it is therefore correct. Within Congolese regions B and C, where only one site was prospected, their reported global diversity might not be very accurate. A wider and systematic sampling from these forests and others, including relict forests near sampled primary forest, for each Ugandan and the Congolese regions B and C would give more precise indications of diversity. A study of *C. canephora* populations in countries like the Congo, Gabon and Angola would also provide more information about the *C. canephora* genetic diversity and relationships between regions.

History and relationship of wild and cultivated population: Admixtures observed between Kibale and Itwara forests confirmed their previous connection, but it concerns less than 10% of the population, indicating that a separation distance of less than 30 kilometers is sufficient to prevent gene flow between wild *C. canephora* in forests.

Similarities in basic genetic diversity characteristics of Itwara and Kibale populations with those of known wild populations, Guinean and Congolese B and C, confirms that Kibale and Itwara *C. canephora* populations are wild. Relationships between Kibale forest and Nganda-Erect population point gene out from forests to farms in Uganda. In most African countries, cultivated coffee was first established using wild materials, which were later supplemented by introduced genotypes from other regions or countries and natural and artificial hybrids resulting from crosses between genotypes from different sources, leading to a complex mixture of genotypes (Montagnon et al, 1998). Historical papers from Uganda (Thomas 1944; Leakey 1970)

pointed out the difficulty to clearly trace the source of cultivated trees and to validate wild and cultivated *C. canephora* populations in Uganda. As reported in previous papers, *C. canephora* was being cultivated and traded in Uganda for use in traditional ceremonies earlier than 1800, long before commercial cultivations started (Maitland, 1926). The source of planting materials for these early planting has not been clear and Thomas (1935, 1944) hypothesized the wild origin of Nganda genotypes. Genetic relationships observed between Kibale and Nganda-Erect populations supports this hypothesis and points out Kibale as a likely source of early Nganda establishments in Uganda as suggested by Thomas (1935). The admixture observed presently is probably lower than what it was 150 years ago, at the beginning of *C. canephora* cultivation in Uganda, since Kibale forest is no longer used as a source of planting materials, except for a small number of natives leaving close to the forest. The propagation processes and mixed cultivation of Nganda and Erect genotypes in the research station fields over generations explains the separate evolution of Kibale and the Nganda-Erect populations and it also explains the complete genetic integration of Erect and Nganda populations.

The specificity of Kalangala region, which has a high number of alleles including private alleles, and its separation from other Ugandan cultivated populations, is particularly interesting in this study. Kalangala is one of the first centres of *C. canephora* cultivation in Uganda and planting materials for these early cultivations are reported to have originated from Kasai forest which was located on the mainland in Mukono district near Lake Victoria. But at the beginning of the 20th Century when commercial farming started, farmers were provided with seed from parents of Nganda phenotypes. After this first period of cultivation, coffee plantations were abandoned for 15 years and when cultivation resumed, farmers were supplied with seeds derived from Erect genotypes (Thomas 1947). However the latter introductions did not adapted well to conditions (low soil pH) on these Islands and was later abandoned by the farmers. Then, the old trees sampled for this study could have originated mainly from Kasai forest. This history of the coffee culture in this region and the geographical structure of the islands could explain the specificities observed in Kalangala *C. canephora* population. Results obtained in here also suggest there minor introgressions between main Kalangala *C. canephora* population, which is basically made up of genotypes originating from the defunct Kasai forest, and the Nganda and Erect genotypes types.

Two SG2 genotypes clustered with Ugandan cultivated genotypes, suggesting that these genotypes are genetically very close to Ugandan cultivated populations. It is possible that these two genotypes originated from Uganda or there could have been introgressions because of the geographical proximity between the two populations

From these studies it can be deduced that cultivated Ugandan coffee originated from wild material in forests covering parts of the country near Ruwenzori Mountain and areas near Lake Victoria. Genotypes from these sources have been cultivated in admixtures. Compared to other diversity groups, Ugandan *C. canephora* is highly diverse and rich in alleles, and it can be considered as another centre of diversity.

Implications for management and breeding: The variability among Ugandan *C. canephora* is potentially valuable for the national variety improvement programs. Preliminary tests on progenies of genotypes from Kibale, Itwara, Kalangala and Nganda populations for resistance to coffee wilt disease have revealed high but variable levels of resistance among Kalangala and Itwara progenies (data not shown). The results show that these populations are a very important potential source of resistance genes for developing wilt resistant varieties and can be a source of genes for improving other agronomic traits required in commercial varieties. High levels of resistance observed in Kalangala islands point out the relevance of surveying isolated cultivated areas for fixed alleles that are useful for plant breeding programs.

Since genetic differentiation among *C. canephora* populations is high, sampling a few genotypes from many populations would effectively capture enough natural genetic variability for conservation and utilization in future breeding programs. For the purpose of conserving Ugandan *C. canephora,* samples can be collected from Itwara, Kalangala and sites in Kibale in addition to other relict forests and isolated cultivated regions not included in this study. Because of threats from coffee wilt disease and other unforeseen natural disasters on *C. canephora* genetic resources, an international germplasm collection and conservation program with multiple conservation centres in different countries would preserve the genetic diversity for future coffee breeding activities.

The approach used in this study and the strategy proposed for conservation and utilization of genetic resources to control diseases and their use in breeding programs proposed in this study can be adopted for other tropical perennial tree crops in Africa. Our sampled Guinean and

Congolese regions are located in the Guinean-Congolese diversity zone which is made up of three centres of diversity related to the last glaciations (White 1979): the Guinean region, the Central continental region, and the coastal Atlantic region, the last one being the most valuable in terms of genetic diversity (Jolly et al, 1997). The results obtained in here on *C. canephora* confirm the importance of these refuge zones, which correspond to our Guinean, SG2 and SG1 regions respectively, (Maley 1996; Adams and Faure 1997), as important centres of diversity. In Uganda, some mountain forests remained during the last glaciations (Jolly et al, 1997) but it is not known if *C. canephora* genotypes were present in these areas. It is now established for coffee that the domestication was very rapid in countries like Ivory Coast and Uganda, where genetic diversity was completely mixed between wild and introduced genotypes. These results can be considered when studying other allogamous species in Africa such as *Cola* spp (Sie et al, 2005) and *Elaeis guineensis* (Ghesquiere, 1985). The approach can also be replicated for perennial tree crops in tropical America such as for *Theobroma cacao* (Motamayor et al, 2002) and *Hevea brasiliensis* (Besse et al, 1994). Surveying original wild and cultivated populations in repartition areas is necessary for a better understanding of the genetic diversity of species. From a genetic point of view, diversity groups have also been pointed out in plants like *Elaeis guineensis* and *Cola* spp, where a multi-species complex covers the same area covered by *C. canephora* in Africa as (Sie et al, 2005). Breeding of Robusta coffee (Leroy et al, 1993) and oil palm (Meunier and Gascon 1972) are already based on complementary characteristics of diversity groups related to geographical origins, which can also be applied to other perennial crops like *Cola* spp. Many trees of interest exist in the African tropical forest (Okafor 1984), and lessons from our study could be useful for studying these trees.

Acknowledgment

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CHAPTER 3

Assessment of resistance to coffee wilt disease caused by *Fusarium xylarioides* in *Coffea canephora*

1- Introduction

The re-emergence of coffee wilt (CWD) disease at the beginning of 1980s in DRC and its appearance in Uganda in 1993 and subsequent spread to Tanzania has led to loss of millions of *C. canephora* trees (Oduor, 2005). Because this disease was appearing in Uganda for the first time and C. *canephora* coffee trees in this country had never been subjected to it, no resistant varieties or control programmes had been developed. Experience from epidemics in 1930s to 1960s in CAR, DRC and Ivory Coast did not lead to development of any effective phytosanitory or chemical treatments for infected trees. In addition, it seems it is impossible to replant in infected soils until after several years.

In previous programmes set up in Uganda to improve the performance of *C. canephora* varieties for different agronomic traits, such as quality (cup & bean), yield and resistance to leaf rust (*Hemileia vastatrix* Berk & Br.) and red blister disease (*Cercospora coffeicola* Berk & Cooke), specific cross hybrids and clones were generated and planted for evaluation in field experimental plots at Kituza, the headquarters of Coffee Research Institute (CORI), (Uganda) in 1997. But in 1999, coffee wilt disease started affecting coffee trees in these trials. The trials were then monitored to follow the spread of the disease in the field and to study the trees for resistance against the disease. First observations very soon revealed that none of the commercial varieties was resistant. It was therefore apparent that searches for resistant varieties, as the most reliable option to control coffee wilt disease (Delassus, 1954), was necessary. Consequently, it became necessary to assess resistance of *C. anephora* genotypes in collection plots and different experimental trials at CORI, as part of the scheme for breeding for resistance to coffee wilt disease. During these assessments, it became apparent that reliable protocols were required for assessing and quantifying the resistance of these trees.

Given that different procedures and indicators have been used by different persons to determine resistance of coffee trees to coffee wilt disease (Meiffren (1961; Van der Graaff and Pieters, 1978; Bieysse, 2005; Musoli et al, 2001; Musoli, 2005), it is necessary to develop

reliable and harmonized procedures of assessing and measuring resistance to enhance a global understanding of the resistance.

In this study, different assessment protocols (resistance traits/indicators, pathogen isolates, plant materials (seedlings, cuttings, *Coffea* species), study environments (field, screen house, climatic chamber) and inoculation techniques were studied in relation to resistance of *C. canephora* trees to coffee wilt disease. The main objectives of the study were to: 1) Develop an effective and reliable protocol of quantifying resistance to coffee wilt disease. 2) Assess specificity of *C. canephora* and *C. Liberica* var. *dewevrei* (formally *C. liberica* type *excelsa*) to a range of *F. xylarioides* isolates. 3) Assess resistance of Ugandan wild *C. canephora* to coffee wilt disease. 4) Characterise resistance to coffee wilt disease in *C. canephora*.

2- Materials and methods

The studies were performed in three parts comprised of 7 experiments.

Part 1: **Assessment of resistance to coffee wilt disease:** This part comprised of experiments 1 to 4. In this part, *C. canephora* clones were assessed for resistance in the field and the results were compared with results of artificial inoculation obtained by different inoculation techniques on clones and open pollinated seedlings.

Part 2: Analysis of host specificity to *F. xylarioides* isolates: This part consisted of experiments 4 to 6. Coffee wilt disease was first reported Central Africa (1927) and the initial known host of *F. xylarioides* is *C. liberica* var. *dewevrei* (formally "excelsa"). Progressively, the disease appeared on *C. canephora* in Ivory Coast (1947), DRC (1949), Guinea (1958) (Guillemat, 1946; Fraselle, 1950; Saccas, 1951; Muller, 1997). In 1957 it was observed on *C. arabica* in Ethiopia (Lejeune, 1958). In order to characterize CWD resistance, it was necessary to define host specificity of the pathogen using isolates collected from the 3 *Coffea* species: CAB003 strain from *C. canephora*, "excelsa" DSMZ62457 strain from *C. liberica* var. *dewevrei* and CAB007 strain from *C. arabica*. Host specificity was assessed using open pollinated seedlings of *C. canephora* artificially inoculated with the 3 isolates. This study also included cross-inoculations of *C. canephora* and *C. liberica* var. *dewevrei* open pollinated seedlings using isolates from either species.

Part 3: Assessment of Ugandan *C. canephora* populations for resistance to coffee wilt disease: This part comprised of experiment 7. Wild *C. canephora* trees from primary forests and cultivated types ("Nganda" and "Erecta") plus plants isolated on islands in Lake Victoria were assessed for resistance to coffee wilt disease using *F. xylarioides C. canephora* strain. All plants studied in this experiment are open pollinated progenies, whose mother parents were used in the analysis for genetic diversity of Ugandan *C. canephora* given in chapter two of this Thesis.

2-1 Plant Materials

2-1.1: Part 1 (Experiments 1 to 4)

Experiments 1 to 3 were carried out at CORI. Experiment 4 was carried out at CIRAD. Studies in experiment 1, were conducted using 20 *C. canephora* genotypes raised from rooted stem cuttings and planted out in the field in 1997 (Table 3.1). Data from this trial was also used to analyze spatial and temporal spread of the disease described in chapter 1. Clones 1s/2, 1s/3, 223/32 and 257s/53 are among current commercial clones used in Uganda. The rest of the clones in this experiment were selected from progenies of specific crosses involving different parents whose resistance to coffee wilt disease was not known. The 20 clones were selected for yield, bean quality, suckering ability and resistance to leaf rust and red blister disease. This experiment was laid out in a randomized complete block design with four replicates. Each replicate was divided into 20 plots of six trees arranged in straight line patterns of 3 rows x 2 columns, spaced at 3 x 3 m. Each of the clones was randomly allocated and planted in a plot in each of the replicates. Clones Q/6/1 and Q/1/1 were only planted in replicates 1 & 2 due to insufficient planting materials. Their plots in replicates 3&4 remained without plants. Clones H/4/1 and R/14 were not planted in replicate 4 because of a similar reason. It was maintained following routine procedures used in Uganda for maintaining *C. canephora* gardens (MAAIF, 1995).

Table 3.1: *C. canephora* and *C. liberica* var. dewevrei genotypes and number of plants and *F. xylarioides* isolates studied in experiments 1 - 6

Location	**CORI**			**CIRAD**							
Experiment	**Expt1**	**Expt2**	**Expt3**	**Experiment 4**		**Experiment 5**				**Experiment 6**	
Plant material	**Clones**	**Rooted cuttings**	**Seedlings**	**Seedlings**		**Seedlings**		**Seedlings**		**Seedlings**	**Seedlings**
Isolate	**Field**	**Local 257/53**	**Local 257/53**	**CAB003**		**CAB007**		**DSMZ62457**		**CAB003**	**DSMZ62457**
Inoculation method	**Natural**	**Root dip**	**Root dip**	**Stem wounding**							
Parent clone				**Rep. 1**	**Rep. 2**	**Rep. 1**	**Rep. 2**	**Rep.1**	**Rep. 2**		
B/1/1	24		20	20	18	20	18		20		
B/2/1	24	7	20	20	17	20			19		
B/6/2	24		19	19	16	20	16		16		
C/1/7	24	11	20	20							
C/6/1	24		20	20	20	20		20			
E/3/2	24		20	17	19						
G/3/7	24	20	20	20	19	20			15		
H/4/1	18	20	20	19	19	20	12	20	18		
J/1/1	24	20			19	19			8	20	20
L/2/7	24	12	20	20	17						
P/3/6	12		16	20	20	20	20	20	20	20	
P/5/1	24				20						
Q/1/1	24		17	19	20	20	19	20	20		
Q/3/4	24	20		19	20						
Q/6/1	12		20	19	20			21	21		
R/1/4	18	9	20		12						
1s/2	24	12	20								
1s/3	24		20								
223/32	24	10	20								
257s/53	24	20	20								
Nemaya (Control)										20	20
C. liberica var *dewevri* 1734										18	17
" " " "1608										18	16
" " " "1609										15	12
" " " " 1450										15	14
" " " " 1724										13	15
" " " " 1727										11	9

In Experiment 2 (E2), 9 months old rooted stem cuttings from 11 out of the 20 clones in experiment 1 were artificially inoculated and assessed in the screen house (Table 3.1). Cuttings of the 9 missing clones failed to root at nursery stage.

For Experiment 3 (E3), 9 months old half sib progenies from 17 out of the 20 clones in experiment 1 were artificially inoculated and assessed in the screen house (Table 3.1). Seeds of the 3 missing clones failed to germinate.

Plant materials for these three experiments were raised in the nursery at CORI following coffee nursery routine procedures.

In Experiment 4 (E4), 9 months old half sib progenies of 15 *C. canephora* clones studied in experiment 1 were artificially inoculated and assessed under controlled climatic conditions in climatic chambers at CIRAD (Table 3.1). The progenies in this experiment were assessed in two tests (replicates). In the first test only 13 progenies were assessed. Seeds of 7 clones failed to germinate. In the second test 15 progenies were studied and seeds of 5 clones failed to germinate. The seedlings used in experiment 4 were raised in the glass house at CIRAD

2-1.2: Part II (Experiment 4 to 6)

All studies in part II were carried out at CIRAD. Data of experiment 4, which were used for analyzing resistance in part 1, were also used to study host specificity in this part.

In Experiment 5, 15 weeks old half sib progenies of 10 out of the 20 *C. canephora* clones studied in experiment 1 were assessed, after artificial inoculation with two *F. xylarioides* isolates and incubation under controlled climatic conditions (Table 3.1). Seeds of 10 clones failed to germinate. Nine progenies were inoculated with *F. xylarioides* isolate CAB007 (arabica strain). This inoculation was performed in two tests/replicates. The first test involved 9 progenies and the second test included only 5 progenies (Table 3.1). In the second inoculation, 10 progenies were inoculated with *F. xylarioides* isolate DSMZ62457 ("excelsa" strain). The seedlings in this inoculation were also assessed in two tests (replicates). The first test involved 5 progenies and the second test involved 9 progenies (Table 3.1).

In Experiment 6, 6 half sib progenies of *C. liberica* var. *dewevrei* and 3 half sib progenies of *C. canephora* in experiment 1 were assessed after inoculation with *F. xlarioides* isolates DSMZ62457 and CAB003 and incubation under controlled climatic conditions (Table 3.1). Both *C. canephora* and *C. liberica* var. *dewevrei* seedlings were 15 weeks old.

Seedlings studied in experiments 5 and 6 were raised in the glass house at CIRAD together with seedlings in experiment 4.

2-1.3: Part III (Experiment 7)

This part was carried out at CORI. In Experiment 7 (E7), 9 to 12 months old half sib progenies raised from open pollinated seeds of *C. canephora* mother trees in Kibale and Itwara wild forests, Kalangala islands in Lake Victoria and "Nganda" and "Erecta" phenotypes were studied in the screen house (Table 3.2). All mother trees of seedlings in this experiment were used in the analysis for genetic diversity of Ugandan *C. canephora* presented in chapter 2 of this thesis.

In Itwara forest, the mother trees were chosen from 4 sites separated by distances ranging from 0.6 to 10 km and the sites provided 1 to 4 progenies. In Kibale, mother trees were chosen from three sites separated by 7 to 19 km distances and the sites in this forest also provided 1 to 4 progenies per site. Sampled sites in both Itwara and Kibale forests were located throughout the forests.

In Kalangala, 4 sites separated by at least 10 km distances spread on 2 islands and located 0.5 to 2 kilometres from the edge of the islands provided 2 to 9 progenies per site.

The fourth source, Erecta provided 9 progenies and the fifth source, Nganda, provided 16 progenies. Both Erecta and Nganda are cultivated populations with distinct tree architectures. Erecta genotypes have strong erect stems and Nganda have flexible spreading stems. Both populations were obtained from germplasm collections at Kawanda Agricultural Research Station (KARI).

Each progeny (all populations) provided 6 to 20 seedlings for the studies. The seedlings were raised in the coffee nursery at CORI, following routine procedures of raising coffee seedlings.

Table 3.2: *C. canephora* populations and plants studied in Experiment 7

Source	Type of material	Sampled sites	No. of progenies	No. of plants
Itwara	Wild	4	10	110
Kibale	Wild	3	7	67
Kalangala	Feral	4	23	395
Nganda	Cultivated	1	16	311
Erect	Cultivated	1	9	177

2-2 Infection of plant materials

F. xylarioides isolates used for infecting plants in the different experiments are given in Table 3.3. Inoculation methods used in the various experiments are described below.

Table 3.3: *F. xylarioides* isolates used in experiments 2-7

Isolate	Origin	Origin host species	Year of deposit (mycotech)	collector	Taxonomic proposal (Lepoint)	Denomination adopted in here	Expt.
CAB003	Uganda	*C. canephora*	2000	Julie Flood	*F. congoensis*	*F. xylarioides canephora* strain	*E4 & E6*
CAB007	Ethiopia	*C. arabica*	2000	Girma Adugna	*F. abyssiniae*	*F. xylarioides arabica* strain	*E5 & E6*
DSMZ62457	CAR	*C. liberica var. dewevrei*	1971	J. Kranz	*F. xylarioides*	F. xylarioides "excelsa" strain	E5 & E6
257s/53	Uganda	*C. canephora*	N/A				E2, E3 & E7

2-2.1: Experiment 1: Field assessment for resistance to coffee wilt disease in C. canephora clones at CORI

Plants in this experiment were infected naturally by *F. xylarioides* inoculum available in the field.

2-2.2: Experiment 2 and 3: Assessment of resistance to coffee wilt disease using artificial inoculation on C. canephora clones (cuttings) and seedlings (CORI screen house)

Plants in these experiments were artificially inoculated by root dipping using a mono-conidia local inoculum of *F. xylarioides* isolated from infected stem parts of *C. canephora* clone 257s/53 (Table 3.3). The plants were stripped off all planting medium (soil) and their roots were washed with tap water before dipping the entire root section for 30 minutes into a water suspension inoculum at $1.0x10^6$ conidia ml^{-1}. After inoculation, the plants were re-planted in polythene pots filled with sterilized soil enriched with organic nutrients. Re-planted plants were kept within the screen house for incubation at room temperature. The plants were regularly watered with tap water as they were being monitored for CWD symptoms.

2-2.3: Experiment 4: Assessment of resistance to coffee wilt disease using artificial inoculation on C. canephora seedlings (CIRAD climatic chambers.

Plants in Experiment 4 were artificially inoculated, in two repeated tests, with a water suspension inoculum at $1.0x10^6$ conidia ml^{-1}, derived from a single conidia of *F. xylarioides* isolate CAB003 (Table 3). The isolate was isolated at CABI from the bark of infected *C. canephora* tree obtained from Uganda in 2000 (Bieysse, 2005). The plants were inoculated by wounding on the first upper internodes with a sterile scalpel and applying 1-2 drops of the inoculum into the wound using the scalpel. Inoculated plants were incubated in a room with temperature, relative humidity and light regulated at 25°C, 80% and 12h/12h lighting regime, respectively. The plants were watered twice a week with tap water using a hosepipe as they were being monitored regularly for wilt symptoms.

2-2.4: Experiment 5 (Assessment of specificity of C. canephora to F. xylarioides isolates using artificial inoculation on seedlings (CIRAD climatic chamber)

Seedlings in Experiment 5 were inoculated, in two repeated tests, by wounding as described for experiment 4. Water suspension inoculum of isolates, CAB007 *F. xylarioides* arabica strain and DSMZ62457 *F. xylarioides* "excelsa" strain, at a concentration of $1.0x10^6$ conidia ml^{-1} was used. CAB007 was isolated at CABI (U.K.) from the bark of infected *C. arabica* tree obtained from Ethiopia in 2000 (Bieysse, 2005). DSMZ62457 is a historical isolate from *C. liberica* var. *dewevrei,* which was collected from Central African Republic in the 1960s. After inoculations, the seedlings were incubated under controlled conditions as described in experiment 4.

2-2.5: Experiment 6 (Assessment of specificity of C. canephora and C. liberica var. dewevrei to F. xylarioides isolates originating from either species, using artificial inoculations on seedlings (CIRAD climatic chambers)

C. canephora and *C. liberica var.* dewevrei seedlings in experiment 6 were inoculated by piercing their stems with a seeker and applying about 2 drops of the inoculum prepared from either isolate CAB003 or DSMZ62457; as described in experiments 4 & 5, into the wound using an injection needle. After inoculations, the plants were incubated under controlled climatic conditions as descried for experiment 4.

2-2.6. Experiment 7 (Assessment of resistance in wild Ugandan C. canephora using artificial inoculation on seedlings at CORI)

Seedlings in this experiment were inoculated by root dipping using local isolate derived from *C. canephora* clone 257/53, prepared and applied as described in experiments 2 and 3. After inoculation, the plants were incubated in the screen house and monitored as described for experiments 2 and 3

2-3 Data collection

Plants in all experiments were assessed for resistance using a disease symptom severity scale. Because plants in the screen house and in the climatic chamber experiments were young and small with different stem architecture, symptoms on plants these experiments were different from those on plants in the field. Thus the scale used for field assessment was slightly different from that used in the screen house and climatic chambers. The two scales are however the same numerically and in logical progression of the symptoms.

2-3.1. Field data collection

Data collection and rating of the disease on the tree from the field trial (Experiment 1 of this chapter) is as described in chapter 2, since it is the same data that was used for analyzing spatial and temporal spread of the coffee wilt disease

2-3.2. Screen house and climatic chamber data collection

Data collection to assess plants in the screen house and climatic chambers for resistance to coffee wilt disease commenced at week 5 when wilt symptoms started showing. For experiments 2, 3 and 7 (CORI screen house), symptoms were assessed weekly and the assessment lasted up to 10 weeks after inoculation. For experiments 4 to 6 (CIRAD climatic chambers), data were scored after every two weeks and the assessment lasted up to 22 weeks after inoculation. Plants in experiments 2 to 7 were assessed on a symptom severity scale of 1 to 5, where: 1 = no disease, 2 = curling leaves and stunted growth, 3 = leaf drooping, weary and yellowing, 4 = leaf necrosis, leaf wilting, and abscission and 5 = plants are dead.

2-4. Data analysis

2-4.1. Part I: Assessment of resistance to CWD

2-4.1.1: Comparison of resistance traits

To identify the most suitable traits for assessing coffee trees for resistance to coffee wilt, data on four traits, i.e. percent plant mortality, mean plant defoliation (1-5 scale), disease period and area under disease progress curve (AUDPC), were derived from the disease data scored on the 1-5 defoliation scale for each of the clones in experiment 1. Percent plant mortality was calculated as a percentage proportion of plants that attained level 5 on the 1-5 plant defoliation scale, at each of the assessment dates. Mean plant defoliation was calculated as the average score of the 1-5 measurements for each of the clones at each assessment date. The disease period corresponds to average time lapse the disease took to progress from level 2 up to its last observation in level 4 on the 1-5 assessment scale. Disease period of each of the clones was calculated from scores of diseased and/or dead trees whose disease levels had progressed from scale 1 to 5 within the assessment period. The area under disease progress curve (AUDPC) was calculated for each of the clones from their respective disease progress curves plotted using percent mortality data. The percent mortality curves of all the clones were auto fitted onto non-linear model curves using Curve finder routine of CurveExpert software, version 1.34 (Daniel Hyams, 1997). MMF (Morgan-Mercer-Flodin) model auto fitted data of most clones and it was thus selected for modelling disease progress curves of all the clones. Area under each of the model disease progress curve (AUDPC) was calculated as a derivative from the MMF model function. AUDPC was calculated using MATLAB version 6.1 software.

Percent mortality and mean plant defoliation data of the last assessments, plus the area under the disease progress curves (AUDPC) and disease periods were used as measures of resistance of the 20 clones in experiment 1. Spearman correlation analysis was performed on these data to determine their correlations and relative suitability as determinants of resistance. Since values of AUDPCs were very large, they were transformed into square roots and the square root values were used in the correlation analysis.

2-4.1.2: Temporal disease progress

Disease progress curves plotted from percentage mortality data of each of the clones in experiment 1 was used to illustrate variations in temporal progression of coffee wilt disease among *C. canephora* genotypes. Data at six months intervals were used for plotting the curves because differences between data points at shorter intervals were too small.

2-4.1.3: Statistical analysis

To test the significance of variations observed between clones or progenies, analysis of variance (ANOVA) was performed on the plant defoliation data measured on the 1-5 scale for experiments 1-4 using the general linear model (GLM) of SAS program (SAS Institute Inc., Cary, NC, 1989). In experiment 1, the ANOVA was performed on data sets of all assessment dates. For experiment 2-3, the ANOVA was performed on data sets of assessments at week 10 from time of inoculation. For experiment 4, the ANOVA was performed on data sets of assessment at week 22 from time of inoculation. For these 3 experiments, Logistic regression analysis was performed on the progeny and clone percent mortality data using the generalized linear model (GENMOD) (Nelder and Wedderburn, 1972) procedure of the SAS software (SAS Institute Inc., Cary, NC, 1989), to rank resistance of the clones and progenies by their mortality. Logic link function was used in this analysis because of the binomial distribution of the percentages.

The clones were also grouped into non-overlapping resistance classes using their mean percent mortality rated on a 1-5, where 1 = resistant (0% mortality), 2 = moderately resistant (2-25% mortality), 3 = moderately susceptible (26-50% mortality), 4 = susceptible (51-75% mortality) and 5 = highly susceptible (76-100% mortality)

2-4.1.4. Comparison of resistance in the field and resistance in artificial inoculations

To determine the relationship between resistance in the field and resistance identified through artificial inoculations in the screen house and under controlled climatic conditions, Pearson correlation analysis was performed on percent mortality data of experiments 1 (field), 2, 3 (screen house) and 4 (climatic chamber). The correlations with experiment 4 (under controlled climatic conditions) were performed on percent mortality data of the two tests within this experiment independently, and also on their mean mortality data.

2-4.2: Part II. Host specificity to F. xylarioides isolates

Percent plant mortalities were calculated, for *C. canephora* progenies in all inoculations (*F. xylarioides* isolates) in experiments 5 and 6. Percent mortality was also calculated for *C. liberica* var. *dewevrei* progenies in experiment 6. Percent mortality in these experiments was also calculated from the symptom severity data as a percent proportion of plants that attained level 5 on the 1-5 disease assessment scale. The percent mortality data of assessment at week 22 after inoculation, for the two tests within experiment 5, together with percent mortality data for the two tests within experiment 4, also for assessment at week 22 after inoculation, were plotted into a histogram to illustrate responses of *C. canephora* to artificial infection by the three *F. xylarioides* isolates (CAB003, CAB007 and DSMZ62457). The percent mortality data at week 22 in experiment 6 were also plotted into a histogram to illustrate cross inoculation effects of *F. xylarioides* isolates CAB003 and DSMZ62457 on *C. canephora* and *C. liberica* var. *dewevrei*.

2-4.3: Part III: Assessment of resistance among Uganda wild and other C. populations

To test the significance of genetic variation between and within the half sib progenies from Kibale, Itwara, Kalangala, "Nganda" and "Erecta" *C. canephora* populations, analysis of variance was performed on the disease symptom severity data of experiment 7 using the general linear model (GLM) of the SAS program (SAS Institute Inc., Cary, NC, 1989). The analysis was performed on data at week 10 after inoculation. Student-Newman-Keuls mean separation test was performed on mean symptom severity data to rank resistance of the populations and progenies. Progenies within populations were also grouped into non-overlapping resistance classes using their mean percent mortality.

3. Results

3-1: Part I: Resistance to CWD

3-1.1: Field resistance

3-1.1.1: Suitability of resistance assessment traits/indicators

Mean plant defoliation measured on 1 – 5 scale for the last assessment, percent plant mortality of the last assessment, AUDPC and disease period for experiment 1 are shown in Table 3.4. Plant mortality ranged from 0.0% observed on clone J/1/1 to 95.8% observed on clone C/1/7. Mean plant defoliation 1-5 scale ranged from 1.0 (no disease) observed on clone J/1/1 to 4.83 observed

on clone C/1/7. Disease period ranged from 4 weeks observed for clone R/1/4 to 63 weeks observed for clone Q/1/1. Area under disease progress curve (AUDPC) ranged from 16 observed for clone R/1/4 to 78.7 observed for clone H/4/1. Spearman correlation analysis performed on these variables found a very strong relationship between percent plant mortality, mean plant defoliation and area under disease progress curves (AUDPC) (Table 3.5). Correlations between these parameters were highly significant ($p<0.0001$). Correlation between disease period and percent mortality and correlation between disease period and mean plant defoliation were weak, only significant at 6% probability. Coefficients of correlations for these two comparisons are negative (Table 3.5). Correlation between disease period and AUDPC is not significant.

Table 3.4: Resistance of *C. canephora* clones in Experiment 1 to CWD indicated by different traits

Clone	% Mortality (Last assessment)	1-5 mean defoliation (Last assessment)	*Disease period	**Square root of AUDPC
J/1/1	0.0a	1.00a	-	-
Q/3/4	4.2b	1.25a	58	-
1S/3	33.3c	2.67bc	22.2	16
R/1/4	33.3c	2.33b	4	26.8
B/2/1	50.0cd	3.25bcdef	23	36.2
Q/6/1	50.0cd	3.00bcd	--	-
C/6/1	54.2cd	3.13bcde	27.6	26.7
223/32	58.3cde	3.37bcdefg	34	43.9
L/2/7	62.5def	3.50bcdefg	16	49.3
Q/1/1	66.7defg	3.67bcdefg	63	39.6
B/1/1	75.0defgh	4.00cdefg	14	56.9
257/53	83.3efgh	4.33defg	12	66.5
G/3/7	83.3efgh	4.42defg	15	67
1S/2	87.5fgh	4.54efg	27.2	69.4
E/3/2	87.5fgh	4.50defg	17.2	76.2
P/5/1	87.5fgh	4.50defg	20	55.3
B/6/2	91.7gh	4.79fg	18.8	70
P/3/6	91.7gh	4.67fg	16.4	58.7
H/4/1	94.4gh	4.78g	14.2	78.7
C/1/7	95.8h	4.83g	10.8	71.4

**Disease period is in weeks. **AUDPC is a relative measure without units*

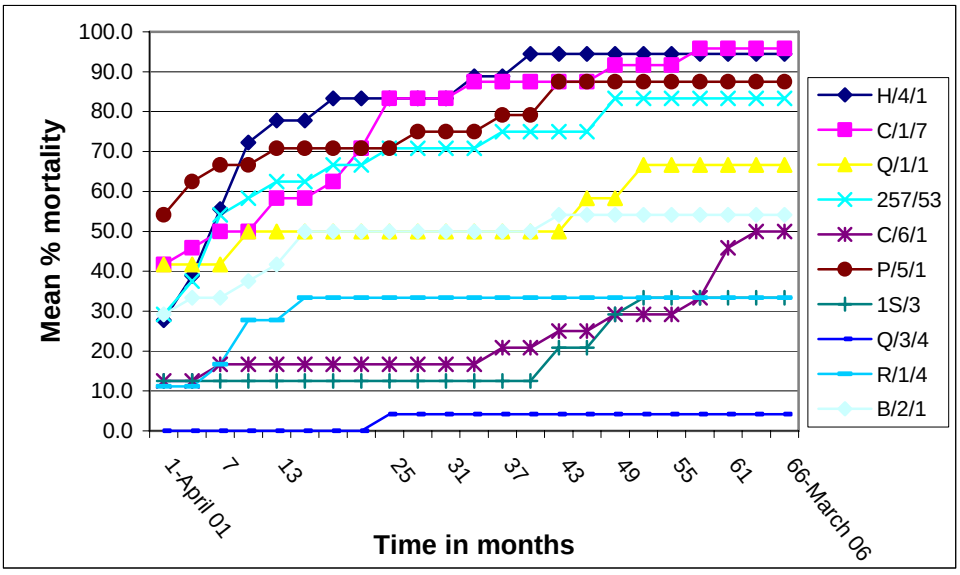
Table 3.5: Correlations of resistance on *C. canephora* clones in experiment 1 determined by different traits

	Mean defoliation (1-5 scale)	Disease period	Sqr. AUDPC
% plant mortality	0.997(<.0001)	-0.446 (0.064)	0.935(<.0001)
Disease severity (1-5 scale		-0.454 (0.059)	0.931(<.0001)
Disease period			-0.281 (0.274)

Sqr. AUDPC is coefficient of correlations performed on the square root of AUDPC, Figures in parentheses are probability values

3-1.1.2: Temporal disease progress

Curves showing progression of plant mortality among *C. canephora* clones caused by coffee wilt disease in Experiment 1 are given in figure 3.1. The curves show that the disease was at varying levels among clones when assessment begun in April 2001 and it progressed at varying rates to varying final levels by March 2006. It also shows that only clone J/1/1 did not succumb to coffee wilt disease throughout the assessment period. Mortality, varied from 0.0% observed on clone J/1/1 to 54.2% observed on clones P/3/6 and P/5/1, when assessment started in April 2001. The disease progressed rapidly among clones P/3/6, H/4/1, E/3/2, C/1/7 and B/6/2 and by March 2006 (fifth year of assessment), mortality of these clones ranged from 87.5% observed on clone E/3/2 to 95.8% observed on clone C/1/7. The disease progressed very slowly on clones Q/3/4 followed by R/1/4 and 1s/3 and by March 2006 (fifth year of assessment) their levels were varying from 1.3 to 2.3. The disease progressed moderately on most clones. Clone 1s/2 appeared to be resistant until April 2002, when its disease level rapidly increased and by March 2006, it was among the most affected.



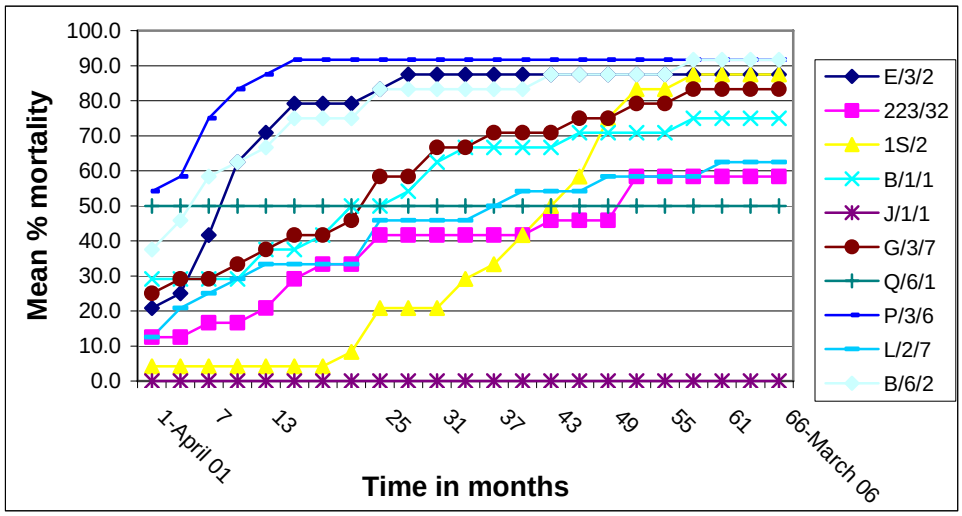


Figure 3.1: Progression of plant mortality among *C. canephora* clones in Experiment 1

3-1.1.3: Analysis of variances

Analysis of variance performed on the plant defoliation data of clones in Experiment 1 (field) found highly significant ($p<0.001$) genetic differences between clones for CWD resistance (Table 3.6, ANOVA of first and last assessments as example). Replicate effects were not significant in all analyses.

Table 3.6: Analysis of variance for coffee wilt disease severity on *C. canephora* clones in experiment 1

Date	Source	df	ss	ms	f-value	Pr>f	C.V
27/4/2001	Clone	19	235.56	12.4	4.89	***	76.1
	Replicate	3	1.85	0.62	0.24	ns	
	Error	421	1067.87	2.54			
31/3/2006	Clone	19	573.88	30.2	13.01	***	41.82
	Replicate	3	12.52	4.17	1.8	ns	
	Error	421	977.59	2.32			

C.V is Coefficient of variation
*** Highly significant at probability less than 0.01%
ns not significant at 5% probability

The logistic regression analysis performed on percent mortality data of the last assessments ranked clones in experiment 1 into 8 resistance classes. 6 of these classes overlapped (Table 3.7).

When the clones were grouped into distinct resistance classes using their percent mortality rated on a 1-5 scale, only clone J/1/1 was in the resistant class, clone Q/3/4 was in the moderately

resistant class, 4 clones (20%) were moderately susceptible, 5 clones (25%) were susceptible and 9 clones (45%) were highly susceptible. Clone J/1/1 was the most resistant and clones C/1/7, B/6/2, H/4/1 and P/3/6 were the most susceptible in this experiment.

3-1.2: Resistance under artificial inoculation

3-1.2.1: Resistance of rooted cuttings in the screen house at CORI

Variations were also observed for disease progression and final disease levels among rooted cuttings of *C. canephora* clones studied in the screen house (Experiment 2). Analysis of variance performed on disease symptom severity data at week 10 from time of inoculation found significant differences between the clones in this experiment (data not shown).

Logistic regression analysis performed on percent mortality data of assessment where ANOVA was performed (week 10) ranked clones in experiment 2 into 4 overlapping resistance classes (Table 3.7). Clones J/1/1, B/2/1 and Q/3/4 had least mortality in this experiment.

When clones in this experiment were grouped into distinct resistance classes using their percent mortality rated on the 1-5 scale, only clone B/2/1 (9%) was in resistant class, clones J/1/1, Q/3/4 and 257/53 (27.3%) were moderately resistant, 2 clones (18.2%) were moderately susceptible, 2 clones (18.2%) were susceptible and 3 clones (27.3%) were highly susceptible.

3-1.2.2: Resistance of seedling progenies in the screen house at CORI

Variations were observed for disease progression and final disease levels among the half sib progenies studied in the screen house (Experiment 3). Analysis of variance performed on the disease symptom severity data at week 10 from time of inoculation found significant difference between the progenies (data not shown).

Logistic regression analysis performed on the percent mortality ranked the progenies in experiment 3 into 6 overlapping resistance classes (Table 3.7). When progenies were grouped into distinct resistance classes using their percent plant mortality rated on the 1-5 scale, no progeny was classified as resistant, 4 progenies (23.5%) were classified as moderately resistant, 4 progenies (23.5%) were moderately susceptible, 6 progenies (35.3%) were susceptible and 3 progenies (17.7%) were in the highly susceptible class.

Table 3.7: Percent mortality of *C. canephora* clones in experiments 1& 2 and of progenies in experiments 3 & 4

Parent clone	E1 (Clones-field)	E2 (Cuttings-CORI)	E3 (Progenies-CORI)	$E4_1$ (Progenies-CIRAD)	$E4_2$ (Progenies-CIRAD)
J/1/1	0.0a	15.0b			30.0a
Q/3/4	4.2b	20.0b		10.0a	50.0ab
1S/3	33.3c		35.0abcd		
R/1/4	33.3c	44.4bc	35.0abcd		33.3ab
C/6/1	50.0cd		65.0def	10.0a	50.0ab
Q/6/1	50.0cd		80.0fg	32.0abc	45.0ab
B/2/1	54.2cd	0.0a	10.0a	35.0abcd	38.9ab
223/32	58.3cde	90.0d	65.0def		
L/2/7	62.5def	50.0bcd	25.0abc	30.0abc	41.2ab
Q/1/1	66.7defg		53.0cde	32.0abc	60.0ab
B/1/1	75.0defgh		85.0fg	15.0ab	50.0ab
257/53	83.3efgh	25.0b	40.0bcde		
G/3/7	83.33efgh	80.0cd	50.0cde	35.0abcd	63.2b
P/5/1	87.5fgh			40.0bcd	42.9ab
E/3/2	87.5fgh		25.0abc	41.0bcd	57.9ab
1S/2	87.5fgh	100e	20.0ab		
P/3/6	91.7gh		69.0efg	65.0d	65.0b
B/6/2	91.7gh		68.0efg	37.0abcd	56.3ab
H/4/1	94.4gh	70.0cd	60.0def	47.0cd	47.4ab
C/1/7	95.8h	63.6cd	95.0g		

4_1 and 4_2 are first and second tests within experiment 4, respectively

3-1.2.3: Resistance of seedling progenies in controlled climatic conditions

Variations were also observed for disease progression and final disease levels among progenies in the two tests of Experiment 4. Analysis of variance performed on disease symptom severity data at week 22 from time of inoculation found significant difference between progenies in both first and second tests of this experiment (data not shown). Logistic regression analysis performed percent plant mortality data of the two tests of experiment 4 ranked progenies in test one into 4 overlapping resistance classes and progenies in the second test were ranked into two overlapping resistance classes (Table 3.7).

3-1.3: Correlations of resistance in the field and in artificial inoculations

All rooted cuttings in Experiment 2 (screen house) and their open pollinated seedling progenies in Experiment 3 (screen house) and Experiment 4 (controlled climatic conditions) were differently affected by coffee wilt disease (Table 3.7). Clone J/1/1, which had no disease symptoms under field conditions (Experiment 1), developed the disease but to low levels of disease in the screen

house. Correlation coefficients showing a comparison of mortality among *C. canephora* clones in the field (E1) with mortality of their rooted cuttings in the screen house (Experiment 2) are given in table 8. Correlations coefficients showing a comparison of mortality among clones in experiments 1 (field) and 2 (cuttings in screen house) with mortality of their open pollinated seedling progenies in Experiment 3 (screen house) and Experiment 4 (controlled climatic conditions) are also given in table 3.8. Similarly, correlation coefficients showing a comparison of mortality among seedling progenies in the two tests of experiment 4 are also given in table 3.8.

There was significant (p = 0.002) correlation between plant mortality among clones in the field (E1) and mortality of their rooted cuttings in the screen house (E2). Correlations between mortality among open pollinated progenies in the screen house (E3) and mortality of their parents (clones) in the field (E1) and in the screen house (rooted cuttings-E2) were not significant. But correlations between mortality among progenies under controlled climatic conditions (E4) and mortality of their parents (clones) in the field (E1) were significant (p=0.010, 0.014 and 0.002 for tests 1, 2 and their mean, respectively). Correlation between mortality among progenies in the two tests (separately) within Experiment 4 (under controlled climatic conditions) and mortality of their parents in the screen house (rooted cuttings) was not significant but the correlation between mean mortality of the two tests of experiment 4 with mortality of their parents in the screen house was significant (p=0.004). Correlation between mortality among progenies in the first and second tests of experiment 4 (open pollinated seedlings under controlled climatic conditions) was not significant, but the correlation of the mortality of each of these tests with their mean morality was significant (p=0.001, p=0.002 for first and second tests respectively).

Table 3.8: Correlations of *C. canephora* resistance to CWD under field and artificial inoculations

	Screen house		**Climatic chamber**		
	Cuttings (E2)	**Progeny (E3)**	**Progeny ($E4_1$)**	**Progeny ($E4_2$)**	**Progeny (Mean of $E4_1$ & $E4_2$)**
Clone in field (E1)	0.965 (0.002) 6	0.132 (0.698) 11	0.708 (0.010) 12	0.617 (0.014) 15	0.744 (0.002) 15
Cuttings(E2)		0.791 (0.209) 4	0.879 (0.121) 4	0.645 (0.167) 6	0.951 (0.004) 6
Progeny (E3)			0.099 (0.798) 9	0.205 (0.546) 11	0.002 (0.995) 11
Progeny ($E4_1$)				0.344 (0.273) 12	0.900 (<0.0001) 12
Progeny ($E4_2$)					0.725 (0.002) 15

Figure in parentheses is the probability; $E4_1$ and $E4_2$ are tests 1 and 2 within experiment 4 respectively. The number below each correlation coefficient shows data pairs used to generate the coefficient immediately above it.

3.2: Part II: Host specificity to *F. xylarioides* isolates

3-2.1: Specificity of C. canephora

A histogram showing percent mortalities among *C. canephora* seedling progenies at week 22 after inoculation with *F. xylarioides canephora* strain CAB003 in experiment 4 and *C. arabica* strain CAB007 and "excelsa" strain DSMZ62457 in experiment 5 is given in figure 3.2. Percent mortality shown for CAB003 is a mean for the two tests within experiment 4. The histogram shows clear differences in levels of mortality caused by the three isolates among these progenies. The level of mortality caused by isolate CAB003 varied from 30% among *C. canephora* progenies J/1/1 and C/6/1 to 65% on progeny P/3/6.

"Excelsa" strain DSMZ62457 caused mortality to progenies H/4/1 and G/3/7 in Experiment 5. Progeny H/4/1 had 7.8% seedling mortality and mortality among progeny G/3/7 was 6.7%. Progeny H/4/1 was affected in both tests of experiment 5. Progeny G/3/7 was involved only in the second test. There was no mortality realised among progenies inoculated with *C. arabica* strain CAB007.

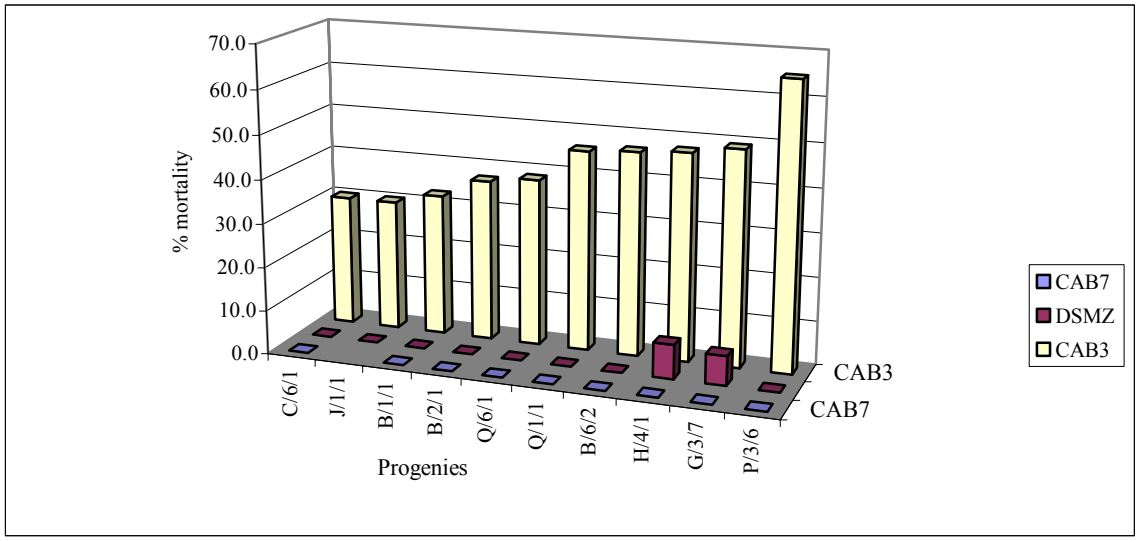


Figure 3.2: Percent mortality of *C. canephora* seedlings inoculated with *F. xylarioides* isolates CAB003 (E4), CAB007 (E5) and DSMZ62457 (E5).

3-2.2: Cross specificity of C. canephora and C. liberica var. dewevrei to F. xylarioides strains from either Coffea species.

Percent mortality among *C. canephora* and *C. liberica* var. *dewevrei* half sib progenies inoculated with *F. xylarioides* strains CAB003 and DSMZ62457 in Experiment 6 is shown in figure 3.3.

Strain DSMZ62457 caused severe but variable mortality among *C. liberica* var. dewevrei progenies. Mortality caused by DSMZ62457 in these progenies ranged from 69.2% observed on progeny 1724 to 100% observed on progeny 1609. This strain did not cause mortality in *C. canephora* progenies.

Strain CAB003 caused mortality among *C. canephora* progenies, which ranged from 2.5 observed on progeny J/1/1 to 30% observed on progeny P/3/6. These plant mortality levels are relatively lower than those observed for the same isolate on the same progenies in experiment 4. This strain did not did not cause mortality to *C. liberica* var. dewevrei.

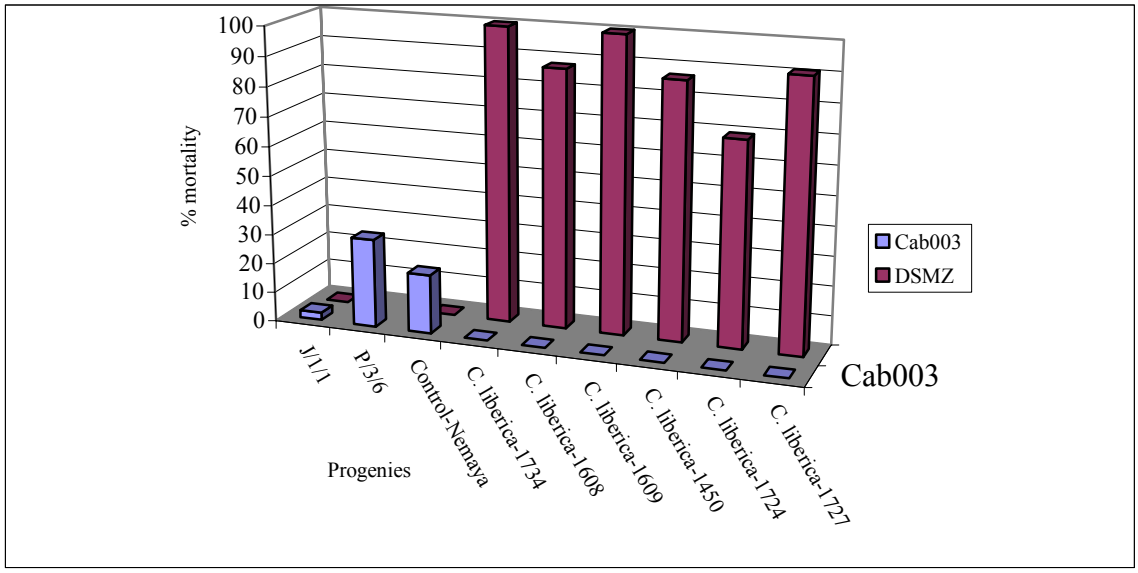


Figure 3.3: Percent mortality among *C. canephora* and *C.* liberica var. *dewevrei* seedlings infected with *F. xylarioides* strains CAB003 and DSM62457 in experiment 6

Nemaya is *C. canephora* genotype obtained from CIRAD greenhouse.

3-3: Part III: Coffee wilt disease resistance among *C. canephora* populations

Studies conducted on open pollinated seedling progenies in experiment 7 show that disease severity varied between and among *C. canephora* populations (Table 3.9 and Figure 3.4). Mean disease symptom severity was 1.0 (no disease), 1.0, 1.1, 1.2 and 1.1 for progenies from Itwara, Kalangala, "Nganda", "Erecta" and Kibale respectively, when assessment started at the fifth week from time of inoculation. Symptoms were not observed among progenies from Itwara and Kalangala until the sixth week from time of inoculation. Once started, the disease progressed at varying rates to varying levels for progenies of different population. It progressed rapidly among "Nganda" and "Erecta"

progenies. By week 10 after inoculation, disease severity among Nganda and Erecta populations was 4.3 and 3.9 respectively. The disease progressed slowly among progenies from Itwara and Kalangala and by week 10 after inoculation, disease symptom severity among progenies from these populations was 1.7 and 2.0 respectively. Disease progressed moderately among progenies from Kibale forest. Variations were also observed between progenies within each of these populations.

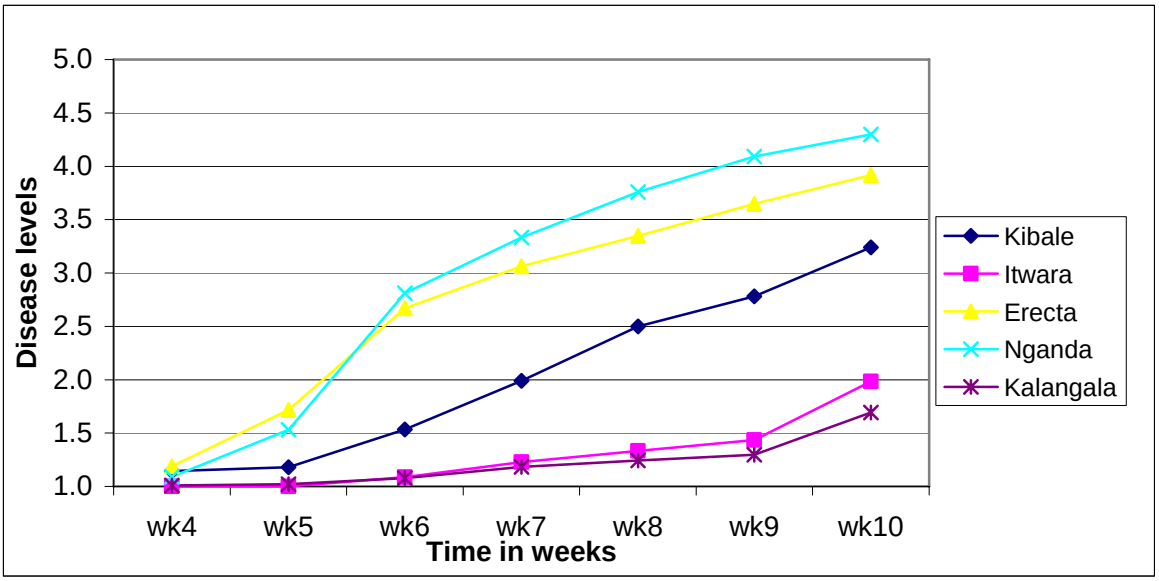


Figure 3.4: Progression of coffee wilt disease among *C. canephora* open pollinated populations studied in Experiment 7.

Analysis of variance performed on disease symptom severity data at week 10 from time of inoculation found significant ($p<0.0001$) genetic differences between populations ($p<0.0001$, Table 3.9). There were significant genetic differences between progenies within Kibale, Kalangala, Nganda and Erecta populations. Differences between Itwara progenies were not significant.

Student-Newman-Keuls mean separation tests performed on mean disease symptom severity data ranked these populations into 3 resistance classes (Tables 3.9 & 3.10). Similar tests performed on mean disease symptom severity data of progenies within populations ranked progenies from Kalangala into three overlapping resistance classes (Table 3.10). Progenies from Kibale, Erecta and Nganda population were also ranked into three overlapping resistance classes

(Table 3.10). Progenies from Itwara were not ranked since the differences were not statistically significant.

Grouping of progenies within populations of this experiment into distinct resistance classes using their mean percent mortality data rated on a 1-5 scale, grouped Itwara progenies into 2 (20%) resistant, 4 (40%) moderately resistant and 4 (40%) moderately susceptible. No progenies in Itwara population were in susceptible or highly susceptible classes. Kalangala progenies were grouped into 4 (17.4%) resistant class, 17 (73.9%) moderately resistant and 2 (8.7%) moderately susceptible. Also there were no progenies in susceptible and highly susceptible classes for Kalangala population. Kibale progenies were grouped into 1 (14.3%) resistant, 2 (28.6%) moderately resistant, 2 (28.6%) moderately susceptible, 1 (14.3%) susceptible and 1 (14.3%) highly susceptible. Three (3) (18.8%) "Nganda" progenies were in moderately susceptible class, 8 (50%) were in susceptible class and 5 (31.2%) were highly susceptible. There were no Nganda progenies in resistant and moderately resistant classes. Two (2) (22.2%) "Erecta" progenies were in moderately resistant class, 2 (22.2%) were in moderately susceptible class, 2(22.2%) were susceptible and 3 (33.3%) were highly susceptible. There were no Erecta progenies in resistant class.

Table 3.9: ANOVA for disease symptom severity on *C. canephora* progenies in experiment 7

Source	df	ms	ss	f value	pr	C.V	Rank
Population	4	1484.7	371.2	242.1	<0.0001	41.4	
Nganda Progeny	15	69.8	4.7	4.4	<0.0001	23.9	4.3a
Erecta Progeny	8	126.7	15.8	13.8	<0.0001	27.3	3.9b
Kibale Progeny	6	78.4	13.1	7.7	<0.0001	35.03	3.7b
Itwara Progeny	9	38.2	4.2	1.7	0.08	79.9	2.0c
Kalangala Progeny	22	113.3	5.1	2.8	<0.0001	78.6	1.7c

Table 3.10: Mean disease symptom severity among C. canephora populations in experiment 7

Kalangala		Itwara		Kibale		Erecta		Nganda	
Progeny	**Mean**	**Progeny**	**Mean**	**Progeny**	**Mean**	**Progeny**	**Mean**	**Progeny**	**Mean**
UW218	3.1a	UW154	3.0a	UW010	4.85a	UE006	4.75a	UN001	4.9a
UW204	3.0ab	CPT11 TR2	2.5a	UW004	4.50a	UE005	4.7a	UN003	4.9a
UW199	2.3abc	UW155	2.3a	UW009	4.16ab	UE031	4.63a	UN008	4.9a
UW191	2.1abc	UW146	2.1a	UW012	3.57abc	UE016	4.45ab	UN017	4.8a
UW189	2.0abc	UW106	2.1a	UW005	2.86abc	UE024	4.32ab	UN011	4.74a
UW205	2.0abc	UW123	2.1a	UW022	2.33bc	UE011	3.7bc	UN013	4.6ab
UW212	1.9abc	UW090	1.8a	UW008	1.67c	UE020	3.60bc	UN024	4.5ab
UW210	1.8abc	UW098	1.7a			UE010	3.00c	UN006	4.5ab
UW219	1.8abc	UW136	1.1a			UE012	2.11d	UN010	4.3abc
UW183	1.8abc	UW091	1.1a					UN007	4.2abc
UW180	1.7abc							UN020	4.0abc
UW181	1.5abc							UN016	3.9abc
UW185	1.5abc							UN018	3.9abc
UW215	1.5abc							UN005	3.9abc
UW211	1.4abc							UN015	3.6bc
UW201	1.3bc							UN004	3.4c
UW206	1.3bc								
UW182	1.3bc								
UW217	1.3bc								
UW203	1.3bc								
UW209	1.2bc								
UW194	1.2c								
UW198	1.0c								
Population mean	**1.7c**		**2.0c**		**3.4b**		**3.9b**		**4.3a**

4.0 Discussion

4.1: Appropriateness of traits/indicators for measuring resistance

In this study very strong significant ($p<0.0001$) correlations were observed between Percent Plant Mortality, Plant defoliation (symptom severity) measured on 1-5 scale and Area under Disease Progress Curve (AUDPC). This shows that these traits can similarly be used to measure coffee wilt disease resistance in *C. canephora* plants. Percent mortality is considered the best because it represents actual contrasting situations (dead or alive) of infected plants. It was noted from these studies that, AUDPCs derived from percent mortality curves are as good as percent mortality for measuring resistance, however modelling field data to calculate AUDPC requires at least 3 or more data plotting points. Therefore AUDPCs can not be used to measure resistance in genotypes with fewer disease data plotting points such as it was observed for clones Q/3/4, Q/6/1 and the resistant clone J/1/1 in this study. Secondly, lengthy calculations are involved before deriving AUDPC and therefore this method is not convenient.

There was weak (significant at 6% probability) correlation between Disease Period and Percent Plant Mortality and Plant defoliation (1-5 scale). This suggests that Disease period can not be similarly used as the latter two traits to measure CWD resistance in *C. canephora*. Besides it did not have a significant correlation with AUDPC, thus it is not a good trait for this purpose. However, their negative correlation coefficients suggest that susceptible genotypes with high disease levels have short disease periods. I.e. susceptible plants tend to die faster than relatively resistant plants. This seem to correspond well for most clones in experiment 1, but clones 1s/2 and Q/1/1 which had high (87.5% and 66.7% plant mortality, respectively) final disease levels also had relatively long Disease Periods. Clone R/1/4 with relatively low (33.3% plant mortality) final disease level had a very short Disease period (one month). This suggests that resistance to coffee wilt disease among *C. canephora* genotypes is controlled by different resistance mechanism. However this point needs to be investigated further.

4.2: Field resistance

Analysis of variance revealed significant genetic differences among *C. canephora* clones for resistance to coffee wilt disease in the field. Logistic regression analysis revealed that variations among the *C. canephora* clones for resistance to CWD in the field were quantitative in nature and clone J/1/1 was the most resistant with no disease, followed by clone Q/3/4 with about 4% percent plant mortality. Clones C/1/7, H/4/1, B/6/2 and P/3/6 were among the most susceptible clones in the field.

4.3: Comparison of resistance in the field and artificial inoculations

Field resistance was confirmed in artificial inoculation performed on rooted cuttings in the screen house at CORI, although disease levels on the clones were not exactly the same. Moreover, the correlation between mortality among clones in the field and in the screen house (rooted cuttings) was significant ($p=0.006$). This indicates that field and screen house assessments give comparable resistance results and both protocols can reliably be used for assessing resistance to CWD in *C. canephora*.

Strong differences were, however, observed on particular genotypes for disease levels in the field and screen house. It was noted that clone B/2/1 which is classified as susceptible in the field and whose progenies were classified as moderately susceptible in the climatic chambers was

resistant in the screen house. Its progenies were also classified as moderately resistant in the screen house. While some genotypes such as C/1/7 and 1s/2 had more disease in the field than in the screen house. This could be due to different mechanism used by genotype for resisting penetration of the fungus in aerial and subterranean plant parts.

It was observed that traits/indicators of resistance to coffee wilt disease considered in this study were explicitly expressed in the field, green house and under controlled climatic conditions. This shows that field, screen house and climatic chambers conditions allow appropriate expression of this disease therefore permit distinguishing of resistance of different genotypes. The three environments are co-supplementary for assessing CWD resistance.

It was also observed that these traits were explicitly expressed irrespective of the infection technique. These results illustrate that infections through root dipping, scalpel wounds and stem wounding by drilling are effective and give results that are comparable to field infection. Inoculation by root dipping is adopted for assessing resistance to coffee wilt disease among germplasm of different coffee species (*C. canephora*; *C. arabica*, Arabusta) and plant material types (rooted cuttings or seedlings) at CORI (Musoli et al., 2001). Wounding by stem drilling is an inoculation technique used at CIRAD and scalpel stem wounding is a technique adapted for studies on arabica (Girma and Hindorf, 2001). For all types of infections, results were obtained quickly and at far less cost as compared to field assessment. This implies artificial inoculations can be used to reduce time and cost of initial assessment of resistance.

Results on correlations involving half sib progenies, rooted cuttings and clones in the field suggest that rooted cuttings are better than half sib progenies for assessing (field or in artificial inoculations) resistance of specific *C. canephora* genotypes to CWD. This may be due to the fact that *C. canephora* is predominantly an out breeder and highly heterozygous, therefore progenies are segregating populations whose individuals are different from their parents. Therefore resistance among progenies may not substantially represent resistance of their parents. As seen from studies on heritability of CWD resistance given in chapter 4 of this thesis, transmission of resistance to progenies ranges from low to medium, thus weak correlations between parents and progenies and among progenies are expected.

4.4: Host specificity

Results of host specificity studies revealed that *F. xylarioides* isolates collected from *C. canephora* (CAB003), *C. arabica* (CAB007) and *C. liberica* var. *dewevrei* (DSMZ62457) induce CWD symptoms in *C. canephora*, but only C. *canephora* strain CAB003 and "excelsa" strain DSMZ62457 are lethal to this species. These results confirm earlier reports that *arabica* strain CAB007 is not lethal to *C. canephora* (Bieysse, 2005). DNA analysis of *F. xylarioides* isolate diversity using microsetallite markers revealed genetic differences between the three strains (Bieysse, 2005). Lepoint (2005) using reproduction tests, reported the three isolates to be non-mating and proposed them to considered as different biological species within the *Fusrium* genus (*Lepoint, 2006*). These variations could be the reason for observed differential reactions on *C. canephora*. The results of this study have also revealed that arabica strain CAB007 is host specific. This observation concurs with field observations from areas where there are mixed cultivations of *C. arabica* and *C. canephora* in Ethiopia, where only the former species is affected by the coffee wilt disease (Girma and Hindorf, 2001). Extension reports from areas of similar cultivations in Uganda and DRC indicate that only *C. canephora* is affected, suggesting the *F. xylarioides* canephora strain does not affect *C. arabica*.

Strain DSMZ62457 causes mortality to both *C. liberica* var. *dewevrei* and *C. canephora*. Bieysse (2006) reports this strain causing mortality on *C. arabica* seedlings in climatic chambers. This shows that DSMZ62457 is a broad spectrum strain. This should be a point of concern because it implies *C. canephora* and *C. arabica* varieties being developed for commercial cultivation in wider geographical localities in Africa should be evaluated for resistance to both their specific strains plus DSMZ62457. It also highlights the threat paused by DSMZ62457, in case of genetic modifications that could transform it into a more aggressive form.

These results imply that either coffee wilt disease scourge that destroyed trees of *C. canephora* and *C. liberica* spp. in West and Central African countries during the 1920s to 1960s is different from the current scourge killing C. canephora trees in DRC, Uganda and Tanzania or the two disease types were present by then, since Meiffren (1961) reported differential responses within genotypes in different localities within the same region. However, it is apparent that the disease in Ethiopia is different from the disease that affected trees of *C. canephora* and *C. liberica* spp. during the early scourge in West and Central Africa.

It was noted that disease levels on *C. canephora* progenies common to experiments 6 and 4 are not the same. Low disease levels in experiment 6 could be due inefficiency of the climatic chamber to optimally control temperature and relative humidity within set limits during the experimentation period of this experiment.

4.5: CWD resistance among Ugandan C. canephora populations

There are significant genetic differences for resistance between different Ugandan *C. canephora* populations (Kalangala islands, Itwara and Kibale primary forests, Nganda and Erect phenotypes). This suggests that genes for CWD resistance are differently distributed among *C. canephora* populations in Uganda. Kalangala (Island) and Itwara (wild forest) populations which were found to be most resistant are anticipated to have highest number of resistant genotypes and or resistance genes. High levels of resistance observed among Kalangala (Island) and Itwara (wild forest) highlights the need for exploring different *C. canephora* germplasm sources for resistance to develop commercial CWD resistant varieties. It also highlighted the likely hood that these populations are valuable sources of genes for improving other agronomic traits of *C. canephora* varieties.

Nevertheless high intra population variability was observed. Kalangala and Itwara, which were overall most resistant populations, had highest intra population variations. This highlights the need for conducting CWD tests and targeting resistant genotypes in populations for resistance for developing CWD resistant varieties. This further confirms variable distribution of resistance genes among *C. canephora genotypes*.

4.6: Classification of CWD resistance

The quantitative nature of mortality observed among *C. canephora* clones and progenies in this study together with their differential disease periods and responses to aerial and subterranean infections suggests that resistance is controlled by many genes, which are variably distributed among genotypes. Most resistant genotypes such as J/1/1 and Q/3/4 are assumed to have most of the resistance genes or the most effective resistance gene. Quantitative resistance or horizontal resistance exist for many crop plant diseases (Van der Plank, 1963; Agrios, 1997). Horizontal resistance has been used to control some major diseases of many crop plants, e.g. late blight in Potato caused by *Phytophthora infestans* (Lanbeo et al., 1996), Brown rust of wheat caused by

Puccinia rocondita (Milliano et al., 1986) with variable success. However, as stated by Van der plank (1963 horizontal resistance can be influenced by the environment. This underlines the need to validate resistance of potential commercial varieties in multi agro-ecological *C. canephora* growing environments, before they are released to farmers.

4.7: Conclusion

Through these studies, plant mortality was identified as the best trait for measuring CWD resistance in *C. canephora*. *C. canephora* clones J/1/1 and Q/3/4 were identified to be resistant to CWD. Since yield and resistance to leaf rust and red blister disease of these clones seem to be satisfactory (Musoli, 2005), they can now be made available for validation, including assessment of other agronomic traits, in on-farm multi-location field trials. They can also be made available for farmer re-plantings restricted to Mukono districts. As observations in this study seems to suggest that resistance to CWD in *C. canephora* is polygenic and by the fact that such resistance can be unstable over a range of environments, it is possible that these clones might not exhibit the same level of resistance exhibited at Kituza if they are cultivated in an environment which favours the pathogen. Therefore distribution of these clones for farmer replanting should, at the moment be limited to districts with weather patterns nearly or similar to that of Mukono district, pending results of on-farm multi-location trials in different agro-ecological areas.

It was also validated that current Ugandan *C. canephora* commercial clones 1s/2 and 257s/53 are very susceptible to CWD and therefore they are not suitable for establishing commercial farm plantings.

Through these studies it was hypothesised that CWD resistance could be controlled by many genes. And that different genotypes could be having different resistance mechanisms and defence against penetrations of the wilt fungus. But further studies should be conducted using appropriate experiments to validate these observations. Further studies should also be conducted to validate the CWD resistance defence mechanism used by different coffee species and genotypes within this species.

It was noted from these studies that *F. xylarioides* strain DSMZ62457 is a broad spectrum isolate that infects mainly *C. liberica* var. *dewevrei* and also the two commercial *Coffea* species. Therefore legitimate *C. canephora* or *C. arabica* commercial varieties will have to be validated for resistance against different *F. xylarioides* strains if they are to be used in country infected with

different strains. A laboratory in a neutral/non-coffee growing country is required for validations and to causing existence of multiple strains within coffee producing countries.

Through these studies, it was noted that CWD resistance is variably available in *C. canephora* population within the country. Individuals whose progenies were tested in here and found resistant should be collected for use in further evaluation towards developing resistant commercial varieties.

CHAPTER 4

Inheritance of resistance to coffee wilt disease (*Fusarium xylarioides* Steyaert) in *Coffea canephora* Pierre

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Summary

- Use of genetic resistance is the only way of effectively controlling coffee wilt disease (CWD) caused by *F. xylarioides*, which has devastated coffee in East and Central Africa.
- Resistance to coffee wilt disease in *Coffea canephora* and its inheritance were investigated using a 10 parent half diallel progeny and 20 clones plus their half sib progenies. Heritability and genetic gain for resistance to CWD were calculated within a disease range of 50-65% tree mortality
- There were significant quantitative genetic difference among clones and progenies for CWD resistance. General combining ability calculated within the optimal disease range from the half diallel was significant. The specific combining ability was not significant at plant mortality higher than 57%. Broad sense heritability for the same analysis within the same disease range was moderate (0.267-0.332) and corresponding narrow sense heritability was low (0.112-0.117).
- Resistance to CWD in *C. canephora* is genetically controlled and the control is polygenic. Inheritance of the resistance to CWD in *C. canephora* is moderate and additive

Key words

Inheritance, resistance, *Coffea canephora*, wilt disease, *Fusarium xylarioides*

Introduction

Coffee is an important beverage and commercial crop produced in about 80 tropical countries. 50 of these countries export nearly 7 million tons of green beans every year (FAO, 2006). Globally coffee is the third most important commercial crop, after oil palm and Soya bean, earning with annual export values varying from US $6-13 billion (FAO, 2006). It is produced mainly from two *Coffea* species, *Coffea arabica* L and *Coffea canephora,* among more than 100 species of the genus (Davis *et al.*, 2006; Sonke *et al*., 2006). *Coffea arabica* L is an inbreeding self fertile allotetraploid, which produces Arabica coffee. *Coffea canephora* Pierre is a self-sterile diploid, which produces Robusta coffee. *C. canephora*, which grows well from sea level to 1600 meters above sea level within the tropics and contributes about 35% of the world coffee production (ICO, 2006), is being devastated by coffee wilt disease (CWD) in East and Central Africa.

Coffee wilt disease is caused by *Fusarium xylarioides* Steyaert. It is a vascular disease with symptoms, which progress from inward curling and wilting of leaves to die-back and death of affected trees (Fraselle, 1950; Saccas, 1951; Musoli *et al*, 2001). Symptoms may suddenly occur all over the stem but usually they start on a single or a few primary branches on one side of the stem and progress laterally until all branches are affected. Symptoms on trees with multi-stems first appear on a single stem and progresses laterally until all stems are affected. CWD disease kills its host at all ages and within a short period (Musoli, 2007). Time lapse from appearance of first symptoms to the death of infected mature *C. canephora* trees in the field vary from one to 15 months.

Symptoms on young plants (6-12 months old) in nurseries or screen house are slightly different. They progress from inward curling of leaves starting with youngest leaf pair followed by stunted stem and leaf growth and then leaf drooping, wearying and yellowing. Yellowing of leaf veins and yellowing and swellings in upper stem internodes may occur. At advanced stage, leaves wilt starting from leaf margins advancing inwards before abscission and dropping or they may abscise and drop without wilting, leaving the plant bear before it wilts to death.

CWD was first reported in 1927 on *Coffea liberica* var. dewevrei (formerly var. excelsa, Davis et al, 2006) in Central African Republic, where it destroyed plantations of this species during the 1930s to 1950s (Fraselle, 1950; Saccas, 1951; Muller, 1997). In the 1940s and 1950s it destroyed *C. canephora* plantations in the Democratic Republic of Congo (DRC). During the same period, this disease destroyed *C. liberica* var. dewevrei in Cameroon. In Ivory Coast the disease destroyed *C. liberica* var. dewevrei and *C. canephora* (Meffrein, 1957). CWD was first reported on *C. arabica* in Ethiopia in 1957 (Lejeune, 1958).

The disease was controlled in Central and West African countries in the 1950s and 1960s by planting resistant varieties and cultural practices (Muller, 1997). But in the 1980's new CWD outbreaks were reported on *C. canephora* in DRC (Flood & Brayford, 1997), from where it spread to Uganda and Tanzania. By 2002 CWD was in all *C. canephora* growing areas of Uganda and over 90% of farms were affected, with an overall average disease incidence of 44.5% nationwide (Oduor et al., 2005). In the same year CWD was found on farms in the DRC, Ethiopia and Tanzania.

The cause of the new CWD outbreaks is not known but now there is a dire need to control this disease in all areas where it exists and prevent it from spreading to new places. However, currently there are no effective control measures. Attempts in the past to use chemical

controls failed (Muller, 1997). Phytosanitory measures, which are currently being emphasized, are ineffective to prevent the disease spread and are difficult to implement (Wetala *et al*., 2000). Effective control of CWD will again depend on re-planting affected areas with resistant varieties (Delassus, 1954) but currently there are no resistant commercial varieties for re-planting. It is absolutely necessary to develop new CWD resistant varieties.

Breeding for CWD resistance is now the main objective of *C. canephora* improvement program in Uganda but genetic systems controlling CWD resistance in this species are not known. Knowledge on inheritance of resistance against CWD is vital for designing and implementing an effective breeding programme since genetic mechanism that control resistance to *Fusarium* wilt diseases have been reported to differ between crop species (Kumar, 1998; Boyhan et al, 2003; Perchepied and Pitrat, 2004; McGrath, et al., 2007), between cultivars within crops (Cross et al., 2000) and within a variety (de Franqueville and de Greef, 1987), due to interactions between the host, the pathogen and the environment.

This study was carried out to generate knowledge on inheritance of resistance to CWD as a prerequisite for breeding strategies to develop varieties with effective and durable resistance. Specific objectives of the study were to: 1) Understand inheritance of resistance to CWD in *Coffea canephora* and, 2) Propose a strategy for breeding *C. canephora* commercial varieties resistant to CWD for Uganda.

Materials and methods

Plant materials

Experiments were carried out at Kituza, the head quarters of Coffee Research Institute (CORI), in Mukono district in Central Uganda, located 25 kilometres east of the Capital, Kampala. A three part study was performed using:

i) 5 year old plants of full sib diallel progenies and their clonal parents evaluated in the field (Experiment 1).
ii) 4 year old trees of clones evaluated in the field (Experiment 2).
iii) 9 month old half sib progenies of the same clones used in experiment 2 and rooted cuttings of these mother clones evaluated in the screen house (Experiment 3).

Coffee wilt disease was not a serious problem in Uganda at the time of planting experiments 1 & 2 and it did not exist at CORI by then. However it became necessary to know resistance of genotypes

in these experiments when the disease started affecting them in 1999. Studies in experiment 3 (screen house) were carried out to validate and supplement information generated from field experiments 1 and 2.

Diallel progenies studied in experiment 1 were generated from a cross involving 10 parents (Table 4.1). Parents 1s/2, 1s/3, 1s/6, 223/32, 257s/53 and 258s/24 are current commercial clones selected for high yields, good cup and seed qualities and resistance to leaf rust (*Hemileia vastatrix*) and red blister disease (*Cercospora coffeicola*). Parents 259s/56 and 236/26 are high yielding and have good bean and cup qualities but they are susceptible to leaf rust. Parent 245/62 was selected for its resistance to leaf rust; however, it is a low yielder and has inferior cup quality. With the exception of parent J1/14, all the parent clones were selected among open pollinated progenies collected from farms in different parts of Uganda in the 1930s-1950s (Thomas, 1947; Leakey, 1970). Parent J1/14, which is resistant to leaf rust, is a single tree selection among open pollinated progenies introduced from Indonesia in the 1930s.

Table 4.1: *C. canephora* diallel progenies and number of plants studied in experiment 1

Parents	1s/2	1s/3	1s/6	223/32	257s/53	258s/24	236/26	259s/56	245/65	J1/14
1s/2		12		12	12					
1s/3				12				12		12
1s/6		12		12	12			12		12
223/32			12			12				
257s/53										
258s/24			12	12				12	12	
236/26	12							12	12	12
259/56	12				12	12			12	
245/62	12	12	12	12		12				
J1/14	12	12				12		12	12	

These diallel progenies were generated before CWD became a serious problem in Uganda and the resistance of their parents to CWD was not known prior to selection. Seedlings of the 35 diallel progenies in Experiment 1 were raised in the coffee nursery before planting out in the field (Table 4.1). Rooted cuttings of the 10 parents were also raised in the coffee nursery for inclusion in the experiment. This experiment was planted in May 1997. It was laid out in a randomized complete block design with two replicates running across the slope and each replicate was divided into 45 plots of six trees arranged in single straight line pattern at a spacing of 3 x 3 m. Each of the hybrid progenies and parent clones were randomly allocated and planted in plots in each

of the replicates. Two guard lines of mixed open pollinated seedlings of none experimental materials were planted all around the experiment and a row of similar materials was planted in between the two replicates.

The 20 clones studied in experiment 2 are listed in table 4.2. Clones 1s/2, 1s/3, 223/32 and 257s/53 are the commercial clones described in experiment 1. The rest of the clones in this experiment are single tree selections from progenies of specific crosses involving different parents (Table 4.2). They were selected for being high yielding, having good cup and seed qualities and resistance to leaf rust and red blister disease. They were selected before wilt became a problem in Uganda and the resistance of their parents against CWD is also not known. Experiment 2 was laid out in a randomized complete block design with four replicates running across the slope and each replicate divided into 20 plots of six trees arranged in straight line patterns of 3rows x 2columns, spaced at 3 x 3 m. Each of the clones was randomly allocated and planted in a plot in each of the replicates. The experiment was surrounded by two guard lines of none experimental materials and a guard line in between replicates of similar materials. This experiment was planted in October 1997.

Table 4.2: Genealogy and number of plants of *C. canephora* clones studied in experiment 2 and their rooted cuttings and half sib progenies in experiment 3

Clone	Type of source material	Source collection/progeny	No. of plants in experiment 2	No. plants in experiments 3	No. of seedlings in experiment 3
1s/2	Open pollinated	Farm collections	24	12	20
1s/3	Open pollinated	Farm collections	24		20
223/32	Open pollinated	Farm collections	24	10	20
257/53	Open pollinated	Farm collections	24	20	20
B/1/1	Specific hybrid	1s/6x4/6	24		20
B/2/1	Specific hybrid	1s/6x4/6	24	7	20
B/6/2	Specific hybrid	1s/6x4/6	24		19
C/1/7	Specific hybrid	1s/6x245/62	24	11	20
C/6/1	Specific hybrid	1s/6x245/62	24		20
E/3/2	Specific hybrid	1s/2x245/62	24		20
G/3/7	Specific hybrid	227/61x1s/2	24	20	20
H/4/1	Specific hybrid	257/18x1s/2	18	20	20
J/1/1	Specific hybrid	227/61x1s/6	24	20	
L/2/7	Specific hybrid	4/6x227/61	24	12	20
P/3/6	Specific hybrid	218/32x1s/6	24		16
P/5/1	Specific hybrid	218/32x1s/6	24		
Q/1/1	Specific hybrid	224/64x1s/6	12		17
Q/3/4	Specific hybrid	224/64x1s/6	24	20	
Q/6/1	Specific hybrid	224/64x1s/6	12		20
R/1/4	Specific hybrid		18	9	20

Both experiments 1 and 2 were weeded, trained, pruned and supplied with organic and inorganic fertilizers following routine procedures of maintaining *C. canephora* gardens in Uganda

Half sib progenies studied in Experiment 3 were raised from open pollinated seeds of 17 out of the 20 *C. canephora* clones in experiment 2 (Table 2). Seeds of the remaining 3 clones failed to germinate. Rooted nodal cuttings studied in experiment 3 were raised from 11 out of the 20 clones in experiment 2 (Table 2). Cuttings of the remaining 9 clones failed to root.

Data collection

Coffee wilt disease did not exist at CORI in 1997 when field experiments 1 and 2 were planted. Coffee plants in these experiments started showing CWD symptoms in 1999. The plants were naturally infected by *F. xylarioides* inoculum available in the field. Assessing plants in Experiment 1 for resistance to coffee wilt disease commenced in December 2002, when they were 5 years old. Assessments on plants in Experiment 2 started in April 2001. Each plant was assessed on a disease symptom severity scale. The symptom severity scale used for rating plants in the field was based on plant defoliation due coffee wilt disease. The defoliation was measured using a 1 to 5 scale, where 1 = no disease, 2 = 1-25% defoliation, 3 = 26-50% defoliation, 4 = 51-75% defoliation, 5 = 76-100% defoliation. Data was recorded twice every month from time of commencement and lasted up to March 2006. A total of 81 data sets were collected for experiment 1 and 130 records were collected for experiment 2. Trees at level five of the assessment scale were classified as dead.

Plants in experiment 3 (screen house) were artificially inoculated with *F. xylarioides* by root dip method. The plants were stripped off all planting medium (soil) and their roots were washed using tap water before dipping their entire root section for 30 minutes into a *F. xylarioides* suspension containing $1.0x10^6$ conidia ml^{-1}. The suspension was prepared using *F. xylarioides* field isolate derived from infected stem parts of *C. canephora* clone 257/53. After inoculation, the plants were re-planted in polythene pots filled with fresh soil, enriched with organic nutrients and kept in the screen house for incubation at ambient temperature. These plants were regularly watered with tap water using a watering can and monitored for disease symptoms. Plants in the screen house were assessed for coffee wilt disease symptoms starting at week 5 from the time of inoculation, when CWD symptoms were first observed on them.

Because plants studied in the screen house are young and small with an architecture which is different from that of big plants in fields, the appearance and progression of CWD symptoms on these plants (screen house) is slightly different. Thus plants in the screen house

experiment were rated using a different disease symptom severity. The two scales (field and screen house) were however numerically similar. Plants in experiment 3 were rated according to the chronological appearance of characteristic wilt symptoms. Symptom progression on plants in this experiment were also rated on a scale of 1 to 5, where: 1 = no disease, 2 = curling leaves and stunted growth, 3 = leaf drooping, weary and yellowing, 4 = leaf necrosis, leaf wilting, and abscission and 5 = plants are dead. Assessment of experiment 3 lasted up to 10 weeks from the time of inoculation.

Data analysis

Analysis for phenotypic variations

Analysis of variance (ANOVA) were performed on the plant defoliation severity data using the general linear model (GLM) of the SAS program (SAS Institute Inc., Cary, NC, 1989) to determine statistical significance of observed variations in experiments 1 and 2. ANOVA for experiment 1 was performed on parents and their diallel progeny data separately. ANOVA for the diallel progeny data was performed to decompose replicate, family and replicate x family interaction effects. ANOVA for parents' data in Experiment 1 and clones in experiment 2 was performed to decompose only replicates and clone effects. All ANOVA were performed on data at monthly intervals including the first and last assessments.

Student-Newman-Keuls mean separation tests were performed on all experimental data sets where ANOVA was performed to rank the parents and progenies in experiment 1 and clones in experiment 2 into resistance classes using their defoliation severity means.

Percent plant mortality was calculated from all plant defoliation data sets were ANOVA was performed for experiments 1 & 2. The mortality was calculated as a percentage proportion of all plants in the experiment that had reached level 5 of the plant defoliation scale. The percent mortality data was used in determining appropriate disease levels at which to calculate genetic variances in the two experiments.

Parents in experiment 1 and their progenies and clones in experiment 2 were also classified into non-overlapping resistance classes using percent mortality data on a 1-5 scale, where 1 = resistant (0% mortality), 2 = moderately resistant (2-25% mortality), 3 = moderately susceptible (26-50% mortality), 4 = susceptible (51-75% mortality) and 5 = highly susceptible (76-100% mortality).

For experiment 3, ANOVA was performed on all disease symptom severity data. Student-Newman-Keuls mean separation tests were performed on data sets where ANOVA were significant to rank the half sib progeny and clone (cuttings) resistance in this experiment using their mean disease symptom severity data. Percent plant mortality was calculated for all the symptom severity data sets where ANOVA were performed. The mortality was calculated as a percentage proportion of all plants in the assessment that had reached level 5 of the symptom severity scale. The percent plant mortality data was used in determining the appropriate disease levels at which to calculate genetic variances and estimate narrow sense heritability.

Analysis for genetic variations

Diallel analyses were performed to calculate variance components and narrow (h^2_n) and broad (h^2_b) sense heritabilities from plant defoliation data on diallel progenies in experiment 1 for the first assessment and assessments after every month up to March 2006. The calculations were performed with Diogene quantitative genetics software (Baradat & Labbé, 1995) using Garretsen and Keuls random model adapted to incomplete diallel (Keuls & Garretsen, 1977):

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the value of the trait for the individual corresponding to the cross between parents i and j. g_i and gj are the general combining ability (GCA) of the parents. S_{ij} is the specific combining ability (SCA) of the cross between i-th and j-th parents. ε_{ijk} is the residual term. Since maternal effects are known to be low for many *C canephora* traits (Montagnon, 2000), in this analysis we pooled data of the reciprocal crosses assuming that maternal effects were insignificant. Broad sense heritability ($h^2_{b)}$ was calculated from the variances given by the half diallel analysis as: $h^2_b = V_g/V_p$, where V_g is the genotypic variance and V_p is the phenotypic variance components. Narrow sense heritability (h^2_n) was calculated as: $h^2_n = V_a/V_{p,}$ where V_a is the additive variance component. The confidence intervals (95%) for these estimations were obtained by the Jackknife method (Shao & Tu, 1995).

Because of the need to determine the disease levels at which there would be effective response to selection (improved resistance), correlation between genetic effects at different assessment dates were calculated. The correlations were calculated for total genetic effects and additive and dominance effects separately, of assessments at 6 months intervals.

Broad sense heritability values were also estimated from plant defoliation data of Experiment 2. These values along with associated confidence intervals (95%) were calculated from monthly data starting with the first assessment (April 2001) up to March 2006, using SAS software and the Wald model (Agresti & Coull, 1998).

Two narrow sense heritability values were estimated from percent plant mortality data of experiment 3. These values were calculated from data of experiment 3 at week 10 from time of inoculation and from data on the last assessment of experiment 2. The values were calculated by regressing percent mortality data of half sib progenies in experiment 3 onto percent mortality data of their parent clones (i) in experiment 2 and (ii) within experiment 3. The heritability values were then derived from the slope of the regression as: $b = {}^1/_2 h^2_n$ (Falconer, 1996), where b is the slope of the .i.e. h^2_n is twice the slope of the regression. The regression was performed using SAS software.

Results

Phenotypic variations

Diallel parents

Parents of diallel progenies in experiment 1 (field) were differently affected by coffee wilt disease (Table 4.3). Mortality among parents at the beginning of assessment ranged from 33.3% observed on parents 236/26 and 258s/24 to 91.7% observed on parents 257/53 and J1/14. Overall mean mortality of parents at this date was 52.5%. Disease progressed differently among parents and by March 2006, their mortality ranged from 33.3% observed on parent 236/26 to 100% observed on parent 257/53. Overall mean mortality of parents at this date was 70.8% (Table 4.3). When the parents were classified by their percent mortality at the last assessment, parents 236/26, 258s/24 and 223/32 were in the moderately susceptible class, 1s/2 and 1s/3 were in the susceptible class and the rest of the parents were in the highly susceptible class. Parent 236 was the overall least affected and parent 257/53 was the overall most affected.

Analysis of variance performed on plant defoliation data (1-5 scale) found highly significant ($p<0.0001$) differences between these parents for CWD susceptibility/resistance (Table 4.4, ANOVAs of first and last assessments as example). Replicates/blocks effects were never significant.

Student-Newman-Keuls mean separation test performed on the mean plant defoliation data ranked parents into 3 overlapping classes a, b and c for all data sets.

Diallel progenies

Diallel progenies in experiment 1 were also differently affected by coffee wilt disease (Table 4.3). Mortality among these progenies at the beginning of assessment ranged from 16.7% observed on progenies 236/26 x 1s/2 and 245/62 x 258/24 to 91.7% observed on progenies J1/14 x 259s/56, J1/14 x 1s/3, 1s/6 x J1/14 and 245/62 x 223/32 with an overall mean mortality of 56.7%. The disease progressed differently among progenies and by March 2006, their mortality ranged from 41.7% observed on progeny 236/26 x 1s/2 to 100% observed on progenies J1/14 x 259s/56, J1/14 x 1s/3, J1/14 x 258s/24, 1s/6 x J1/14 and 259s/56 x 1s/2, with an overall mean mortality of 79.8% (Table 4.3). Only 2 (5.7%) progenies were classified as moderately susceptible, 14 (40%) progenies were classified as susceptible and 19 (54.3%) progenies were classified as highly susceptible. Most of the progenies of highly susceptible parents such as J1/14, 257s/53 are in the highly susceptible and susceptible classes. Progenies of the moderately susceptible parent 236/26 are in the moderately susceptible and susceptible classes. All progenies had higher mortality than their least susceptible parent. Majority of progenies had higher mortality than their mid parents and in some cases the progenies were more susceptible than their most susceptible parent. The overall mean mortality of the progenies was slightly higher than overall mean mortality of the parents.

Coefficients of phenotypic variation (C.V) for plant defoliation among diallel progenies in Experiment 1 gradually decreased from 53.3% (high) observed in December 2002 to 36.3% (medium) observed in March 2006 (Table 4.4). Analysis of variance performed on the plant defoliation data found highly significant ($p<0.0001$) differences between these progenies (Table 4.4, ANOVAs of first assessment and assessments at every six months as example). Replicates/blocks effects were also never significant for this analysis and family x replicate interactions were also never significant.

Student-Newman-Keuls mean separation test performed on the plant mean defoliation data ranked the progenies in experiment 1 into 3 overlapping classes for assessments carried out in Dec 2002 to April 2003 (Data not shown). For assessments after this date, the progenies were ranked into two overlapping classes.

Table 4.3: Percent mortality among *C. canephora* clones used as parents of diallel progenies in field experiment 1

	Assessment dates							
Parent	Dec 2002	June 2003	Dec 2003	June 2004	Dec 2004	June 2005	Dec 2005	March 2006
257s/53	91.7	91.7	91.7	100.0	100.0	100.0	100.0	100.00
245/62	83.3	83.3	83.3	83.3	83.3	83.3	91.7	91.67
J1/14	91.7	91.7	91.7	91.7	91.7	91.7	91.7	91.67
1s/6	41.7	50.0	66.7	66.7	83.3	91.7	91.7	91.67
259s/56	75.0	83.3	83.3	83.3	83.3	83.3	83.3	83.33
1s/2	16.7	25.0	25.0	25.0	50.0	58.3	66.7	66.67
1s/3	16.7	16.7	25.0	33.3	50.0	58.3	58.3	58.33
223/32	41.7	50.0	50.0	50.0	50.0	50.0	50.0	50.00
258s/24	33.3	33.3	33.3	33.3	33.3	33.3	41.7	41.67
236/26	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.33
Mean	**52.5**	**55.8**	**58.3**	**60.0**	**65.8**	**68.3**	**70.8**	**70.8**
Progenies								
J1/14x259s/56	91.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0
J1/14x1s/3	91.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0
J1/14x258s/24	75.0	91.7	100.0	100.0	100.0	100.0	100.0	100.0
1s/6xJ1/14	91.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0
259s/56x1s/2	66.7	91.7	91.7	100.0	100.0	100.0	100.0	100.0
245/62x223/32	91.7	91.7	91.7	91.7	91.7	91.7	91.7	91.7
258s/24x245/62	58.3	75.0	75.0	75.0	91.7	91.7	91.7	91.7
J1/14x245/62	66.7	75.0	91.7	91.7	91.7	91.7	91.7	91.7
J1/14x1s/2	75.0	83.3	91.7	91.7	91.7	91.7	91.7	91.7
1s/6x223/32	58.3	58.3	75.0	75.0	91.7	91.7	91.7	91.7
259s/56x257s/53	33.3	66.7	83.3	83.3	91.7	91.7	91.7	91.7
259s/56x258s/24	75.0	83.3	83.3	83.3	83.3	91.7	91.7	91.7
1s/2x223/32	75.0	83.3	83.3	83.3	83.3	91.7	91.7	91.7
258s/24x1s/6	41.7	50.0	50.0	75.0	83.3	83.3	91.7	91.7
245/62x1s/3	50.0	58.3	75.0	83.3	83.3	83.3	83.3	83.3
1s/6x259s/56	66.7	83.3	83.3	83.3	83.3	83.3	83.3	83.3
1s/6x257s/53	75.0	83.3	83.3	83.3	83.3	83.3	83.3	83.3
236/26X245/62	66.7	66.7	75.0	75.0	75.0	75.0	83.3	83.3
236/26xJ1/14	66.7	75.0	75.0	75.0	75.0	75.0	83.3	83.3
245/62x1s/6	25.0	25.0	50.0	66.7	75.0	75.0	75.0	75.0
259s/56x245/62	58.3	58.3	66.7	66.7	75.0	75.0	75.0	75.0
258s/24x259s/56	50.0	58.3	58.3	58.3	75.0	75.0	75.0	75.0
1s/3x259s/56	50.0	50.0	50.0	50.0	50.0	66.7	75.0	75.0
1s/2x1s/3	33.3	58.3	75.0	75.0	75.0	75.0	75.0	75.0
245/62x1s/2	25.0	41.7	50.0	50.0	58.3	66.7	66.7	66.7
1s/3xJ1/14	58.3	58.3	58.3	58.3	58.3	66.7	66.7	66.7
1s/6x1s/3	50.0	58.3	66.7	66.7	66.7	66.7	66.7	66.7
223/32x1s/6	41.7	50.0	58.3	66.7	66.7	66.7	66.7	66.7
1s/2x257s/53	66.7	66.7	66.7	66.7	66.7	66.7	66.7	66.7
258s/24x223/32	41.7	41.7	41.7	50.0	50.0	50.0	66.7	66.7
223/32x258s/24	58.3	66.7	66.7	66.7	66.7	66.7	66.7	66.7
1s/3x223/32	25.0	41.7	50.0	50.0	58.3	58.3	58.3	58.3
236/26x259s/56	50.0	50.0	58.3	58.3	58.3	58.3	58.3	58.3
245/62x258s/24	16.7	33.3	33.3	41.7	50.0	50.0	50.0	50.0
236/26x1s/2	16.7	25.0	33.3	41.7	41.7	41.7	41.7	41.7
Mean	**56.7**	**65.7**	**71.2**	**73.8**	**76.9**	**78.3**	**79.8**	**79.8**

Table 4.4: Analysis of variances for defoliation caused by CWD among *C. canephora* full sib progenies in experiment 1and their parents

Date	Defoliation severity (1-5 scale)	% plant mortality	Replicate			Family/Parents			Replicate x family			C.V
			df	F	% P value	df	F	% P value	df	F	% P value	
Parents												
2-Dec	3.1	52.5	1	1.4	23.8	9	5.98	<0.0001				54.1
6-Mar	3.8	70.8	1	0.01	91.3	9	3.52	<0.0001				43.8
Progenies												
2-Dec	3.4	56.7	1	2.504	13.43ns	34	2.804	0.000****	34	1.289	12.29ns	53.3
3-Jun	3.7	65.7	1	1.14	28.64ns	34	2.53	0.001****	34	1.352	7.90ns	48.0
3-Dec	3.9	71.2	1	0.056	80.79ns	34	2.245	0.015***	34	1.102	28.32ns	41.9
4-Jun	4.0	73.8	1	0.001	97.46ns	34	2.036	0.084**	34	1.073	32.06ns	41.0
4-Dec	4.1	76.9	1	0.033	84.97ns	34	1.998	0.113**	34	0.909	57.24ns	38.9
5-Jun	4.1	78.3	1	0.046	82.57ns	34	1.863	0.319**	34	0.859	65.32ns	38.4
5-Nov	4.2	79.8	1	0.314	58.25ns	34	1.649	1.485*	34	0.878	62.33ns	37.3
6-Mar	4 2	79.8	1	0.331	57.26ns	34	1.624	1.768*	34	1.026	38.71ns	36.3

Date refers to assessment date; df is degree of freedom; ns is not significant; * is significant at 5% probability; ** is significant at 1% probability; *** is significant at 0.1 percent; **** is significant at less than 0.01 percent

Clones (Experiment 2)

Clones in experiment 2 (field) were also differently affected by CWD (Table 4.5). Mortality among these clones at the beginning of assessment ranged from 0% observed on clones J/1/1 and Q/3/4 to 54.2% observed on clones P/3/6 and P/5/1, with an overall mean at this date of 25.3 (Table 4.5). By March 2006 mortality in these clones ranged from 0% observed on clone J/1/1 to 95.8% observed on clone C/1/7 and their overall mean at this date was 64.5%. In March 2006, clones 1s/3, 223/32, 1s/2 and 257/53, which are part of the parents in experiment 1, had slightly lower disease levels in this experiment than in experiment 1 but 257/53 and 1s/2 remained more susceptible than the other two, as observed in experiment 1.

Coefficients of phenotypic variation (C.V) for plant defoliation among clones in Experiment 2 gradually decreased from 76.1% (very high) observed in April 2001 to 41.8% (high) observed in March 2006 (Table 4.6). Analysis of variance performed on plant defoliation data found highly significant ($p<0.001$) differences between these clones for all data sets analyzed (Table 4.6, ANOVA of first and last assessments as example). Differences between replicates in experiment 2 were never significant.

Student-Newman-Keuls mean separation tests performed on mean plant defoliation data grouped the clones into overlapping classes that varied from 4 to 7 depending on the assessment date (Data not shown). Clone J/1/1 did not succumb to the disease throughout the assessment period.

Rooted cuttings (Experiment 3)

Clones (rooted cuttings) in experiment 3 were also differently affected by coffee wilt disease. Plant mortality among clones in this experiment started at week 7 after inoculation. The disease progressed differently among clones and by week 10, mortality ranged from 0% observed on clone B/2/1 to 100% observed on clone 1s/2, with an overall mean mortality at this date of 50.7%. In comparison with results in the other experiments, clone B/2/1 which was moderately susceptible in the field (Experiment 2) was resistant in the screen house. Clone 257s/53 which was highly susceptible in the fields (Experiment 1 and Experiment 2) was moderately resistant in the screen house. Clone 223/32 which was moderately susceptible in the field was highly susceptible in the screen house.

Table 4.5: Percent mortality among *C. canephora* clones studied in field experiment 2

Clone	April 01	June 01	Dec 01	June 02	Dec 02	June 03	Dec 03	June 04	Dec 04	June 05	Dec 05	March 06
C/1/7	41.7	45.8	50.0	58.3	70.8	83.3	87.5	87.5	91.7	91.7	95.8	95.8
H/4/1	27.8	38.9	72.2	77.8	83.3	83.3	88.9	94.4	94.4	94.4	94.4	94.4
P/3/6	54.2	58.3	83.3	91.7	91.7	91.7	91.7	91.7	91.7	91.7	91.7	91.7
B/6/2	37.5	41.7	62.5	75.0	75.0	83.3	83.3	87.5	87.5	87.5	91.7	91.7
P/5/1	54.2	62.5	66.7	70.8	70.8	75.0	79.2	87.5	87.5	87.5	87.5	87.5
E/3/2	20.8	25.0	54.2	79.2	79.2	87.5	87.5	87.5	87.5	87.5	87.5	87.5
1S/2	4.2	4.2	4.2	4.2	8.3	20.8	33.3	50.0	75.0	83.3	87.5	87.5
257/53	29.2	37.5	58.3	62.5	70.8	70.8	70.8	75.0	83.3	83.3	83.3	83.3
G/3/7	25.0	25.0	33.3	41.7	50.0	58.3	70.8	70.8	75.0	79.2	83.3	83.3
B/1/1	29.2	29.2	29.2	37.5	50.0	54.2	66.7	66.7	70.8	70.8	75.0	75.0
Q/1/1	41.7	41.7	50.0	50.0	50.0	50.0	50.0	50.0	58.3	66.7	66.7	66.7
L/2/7	12.5	20.8	29.2	33.3	33.3	45.8	45.8	54.2	58.3	58.3	62.5	62.5
223/32	12.5	12.5	16.7	29.2	33.3	41.7	41.7	45.8	45.8	58.3	58.3	58.3
B/2/1	29.2	33.3	37.5	50.0	50.0	50.0	50.0	54.2	54.2	54.2	54.2	54.2
C/6/1	12.5	12.5	16.7	16.7	16.7	16.7	16.7	25.0	29.2	29.2	50.0	50.0
Q/6/1	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
1S/3	12.5	12.5	12.5	12.5	12.5	12.5	12.5	20.8	29.2	33.3	33.3	33.3
R/1/4	11.1	11.1	27.8	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
Q/3/4	0.0	0.0	0.0	0.0	0.0	4.2	4.2	4.2	4.2	4.2	4.2	4.2
J/1/1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	25.3	28.1	**37.7**	**43.7**	**46.5**	**50.6**	**53.2**	**56.8**	**60.3**	**62.2**	**64.5**	**64.5**

Table 4.6: Heritability and analysis of variances for plant defoliation due to CWD among *C. canephora* clones in experiment 2

Disease levels			ANOVA							Heritability
Date	Plant defoliation (1-5 scale)	% plant mortality	Replicate			Clone				h^2_b & confidence intervals
			df	F	% P value	df	F	% P value	C.V	
1-Apr	2.1	25.3	3	0.24	87.7ns	19	4.89	<0.0001	76.1	0.148 (0.05-0.25)
1-Jun	2.2	28.1	3	0.19	90.3ns	19	7.59	<0.0001	67.7	0.18 (0.06-0.29)
1-Dec	2.8	37.7	3	0.3	82.7ns	19	9.63	<0.0001	62.2	0.241(0.11-0.38)
2-Jun	3	43.7	3	0.71	54.5ns	19	10.48	<0.0001	58.9	0.274 (0.13-0.42)
2-Dec	3.2	46.5	3	0.94	42.2ns	19	10.44	<0.0001	55.8	0.295 (0.15-0.44)
3-Jun	3.5	50.6	3	0.8	49.2ns	19	11.58	<0.0001	52.5	0.312 (0.16-0.46)
3-Dec	3.7	53.2	3	0.88	45.3ns	19	12.19	<0.0001	49.9	0.331 (0.17-0.49)
4-Jun	3.8	56.8	3	1.34	26.2ns	19	12.16	<0.0001	48.2	0.333 (0.18-0.49)
4-Dec	4	60.3	3	2.16	9.2ns	19	12.87	<0.0001	44.8	0.349 (0.19-0.51)
5-Jun	4.1	62.2	3	1.53	20.7ns	19	12.79	<0.0001	43.6	0.354 (0.19-0.51)
5-Dec	4.2	64.5	3	1.68	17ns	19	13.08	<0.0001	42.1	0.355 (0.19-0.51)
6-Mar	4.3	65.5	3	1.8	15ns	19	13.01	<0.0001	41.8	0.347 (0.19-0.51)

Date refers to assessment date; df is degree of freedom; ns is not significant; <0.0001 is significant at less than 0.01 percent; h^2_b is broad sense heritability

Table 4.7: Analysis of variance for disease symptom severity on cuttings of clones and their seedling progenies in experiment 3

Experiment	Assessment date	Assessment record	Source	df	f-value	Pr>f	C.V.	Mean mortality
3	12/6/2006	6 (Week 10)	Clone	10	7.76	p<0.0001	48.4	50.7%
3	12/6/2006	6 (Week 10)	Progeny	15	5.37	p<0.0001	44.9	51.8%

C.V. is coefficient of variation; df is degree of freedom; p<0.0001 is significant at less than 0.001 percent

Analysis of variance performed on the disease symptom severity data found significant ($p<.0001$) differences between clones for assessments at weeks 7 to 10 from time of inoculation (Table 4.7, ANOVA for assessment at week 10 as example). Differences for assessments at weeks 5-6 were not significant (Data not shown).

Student-Newman-Keuls mean separation test performed on the mean symptom severity data where the ANOVA was significant (weeks 7-10) ranked the clones into three overlapping classes a, b and c for all data sets (Data not shown).

Half sibs (Experiment 3)

Half sib progenies studied in experiment 3 were also differently affected by coffee wilt disease. Plant mortality among progenies in this experiment started at week 6 from time of inoculation. By week 10 mortality among these progenies ranged from 10% observed on progeny B/2/1 to 95% observed on progeny C/1/7, with an overall mean of 51.8. In comparison to their parents in field experiment 2 and within the screen house experiment 3, progenies of clone B/2/1 which is moderately susceptible in field experiment 2, were moderately resistant. Progenies of clone 1s/2, which is highly susceptible in field experiment 2, were moderately resistant.

Analysis of variance performed on the disease symptom severity data found highly significant ($p<0.0001$) differences between progenies (Table 4.4) for assessments also at weeks 7 to 10 from the time of inoculation (Table 4.7, ANOVA for week as example). Differences in weeks 5-6 were not significant.

Student-Newman-Keuls mean separation test performed on mean disease symptom severity data where the ANOVA was significant grouped the progenies into three overlapping classes a, b and (Data not shown).

Genetic variations and genetic control

General and specific combining abilities plus dominance, additive and environmental variances together with associated narrow and broad sense heritabilities and their confidence intervals estimated from experiment 1 are given in Table 4.8 (for first assessment and subsequently 6, plus March 2006). Significant general combining ability (GCA) was observed for all assessments on these progenies. GCA progressively decreased from 4.2 (significant at less than 0.01%) observed in December 2002 to 2.6 (significant at 1%) observed in March 2006. Significant differences

were observed for parents' general combining ability effects (Table 4.9). Susceptible parents J1/14 and 259s/56 consistently had highest positive GCA throughout the assessment period followed. Moderately susceptible parents 236/26 and 1s/3 consistently had highest negative GCA throughout the assessment period.

Specific combining ability was significant (5% probability) only in the first assessment implying variability between crosses due to interaction of additive effects among parents was significant for this assessment only.

Generally, additive and dominance variances calculated for these data sets were low compared to the environmental variance (Table 4.8). Plant mortality increased with subsequent assessments leading to reduced genetic variance components and increase of environmental variance. Correlation coefficients for phenotypic effects varied from 0.617 observed for correlation between the assessment in December 2002 and March 2006 to 0.992 for the correlation between assessment in December 2005 and March 2006. The correlations were stronger for closer assessment dates. Correlation coefficients for total genetic effects varied from 0.596 for correlation between assessment in December 2002 and March 2006 to 1.0 for correlation between assessments in December 2005 and March 2006. Genetic correlation coefficients were higher for closer assessments and this relationship became stronger between later assessments (high disease levels). Correlation coefficients for additive effects were always 1.0 or varied slightly. Lowest coefficient was 0.955 observed for correlation between June 2004 and December 2005. There were medium to high correlations for dominance effects in assessments up to June 2004. There after there were no relationships between assessment dates for this trait.

Broad sense heritability (h^2_b) estimate values progressively reduced with each successive assessment, from 0.332 (medium) observed in the first assessment to 0.09 (low) observed in the last assessment. Their corresponding confidence intervals were large and they included zero, except for the first assessment. Narrow sense heritability (h^2_n) estimates were low. Changes in h^2_n estimates over time were minor, from 0.112 observed in the first assessment to 0.091 observed in the last assessments. These results underline a possibility of low genetic gains from selection of hybrids in this particular experiment.

Table 4.8: Variance components and heritability estimates from diallel analysis of plant defoliation data in experiment 1

Date	% mortality	SCA	GCA	V_d	V_a	V_g	V_e	V_p	h^2_b	h^2_n	% morality
Dec-02	56.7	1.841**	4. 199****	12.6	6.44	19.04	19.27	57.35	0.332 (0.04-0.62)	0.112 (0.00-0.25)	56.7
Jun-03	65.7	1.564ns	3.964****	7.66	6.00	13.65	23.82	51.13	0.267 (0.00-0.53)	0.117 (0.00-0.25)	65.7
Dec-03	71.2	1.399ns	3.695***	4.74	5.05	9.79	24.73	44.31	0.220 (0.00-0.48)	0.114 (0.00-0.24)	71.2
Jun-04	73.8	1.192ns	3.388***	2.26	4.80	7.06	28.92	43.04	0.164 (0.00-0.41)	0.111 (0.00-0.23)	73.8
Dec-04	76.9	1.051ns	3.192***	0.57	4.52	5.10	30.57	40.76	0.125 (0.00-0.36)	0.110 (0.00-0.23)	76.9
Jun-05	78.3	0.978ns	3.093**	0.00	4.40	4.40	31.18	39.98	0.110 (0.00-0.24)	0.110 (0.00-0.22)	78.3
Nov-05	79.8	1.549ns	2.822**	0.00	3.98	3.98	30.69	38.65	0.103 (0.00-0.21)	0.102 (0.00-0.21)	79.8
Mar-06	79.8	0.90ns	2.618**	0.00	3.41	3.41	30.68	37.5	0.090 (0.00-0.19)	0.090 (0.00-0.19)	77.9

is significant at 5% probability; ** is significant at 1%; *** is significant at 0.1 percent; **** is significant at 0.01; V_d is variance due to dominance; V_a is additive variance (breeding value); V_g is total genotypic variance; V_p is phenotypic variance; V_e is environmental variance; SCA is specific combining ability; GCA is general combining ability; h^2_n is narrow sense heritability; h^2_b is broad sense heritability. Figure in parenthesis are confidence interval

Table 4.9: General combining ability for parents of *C. canephora* diallel progenies studied in experiment 1

Parent	Dec-02	Jun-03	Dec-03	Jun-04	Dec-04	Jun-05	Dec-05	Mar-06
J1/14	1.10a	1.01a	0.88a	0.81a	0.69a	0.68a	0.64a	0.60a
259s/56	0.31b	0.31b	0.26b	0.20b	0.24ab	0.28ab	0.26ab	0.24ab
257/53	0.26b	0.21b	0.17b	0.06b	0.01ab	-0.02ab	-0.04ab	0.02ab
223/32	0.22b	0.09bc	0.01bc	0.00b	-0.08ab	-0.05ab	0.00ab	-0.03ab
245/62	-0.15b	-0.16bc	-0.01bc	0.03b	0.10ab	0.07ab	0.04ab	0.04ab
1s/6	-0.20b	-0.13bc	-0.01bc	0.14b	0.13ab	0.08ab	0.04ab	0.03ab
1s/2	-0.32b	-0.09bc	-0.70bc	-0.04b	-0.03ab	-0.05ab	-0.06ab	-0.03ab
258/24	-0.38b	-0.33bc	-0.37bc	-0.38bc	-0.25bc	-0.27b	-0.17b	-0.15ab
1s/3	-0.54b	-0.73bc	-0.47bc	-0.51bc	-0.50bc	-0.39bc	-0.40bc	-0.40bc
236/26	-0.48b	-0.50c	-0.77c	-0.75c	-0.85c	-0.88c	-0.84c	-0.78c

Broad sense heritability (h^2_b) values estimated from clones in experiment 2 are given in Table 4.6. The broad sense heritability estimates from this experiment progressively increased from 0.148 observed in April 2001to 0.355 observed in November and December 2005. h^2_b estimates after December 2005 slightly declined to 0.347 observed in March 2006. Corresponding confidence intervals were large but different from zero.

Narrow sense heritability values estimated by regression of percent mortality data of half sib progenies onto mortality of their parents in the field (experiment 2) was 0.584. While narrow sense heritability estimated from regression of mortality of the half sib progenies in this experiment (screen house) onto mortality of their parents within the screen house (Experiment 3) was 1.03 (1). However regression coefficients for both of these heritability estimates were not significant (p=0.276 and p=0.259), implying the confidence interval for these regression are not different from zero.

Discussion

No disease has been reported to devastate *C. canephora* as coffee wilt disease. Yet very few studies have been carried out on this deadly disease, devastating a very important crop in global commerce and beverages. Majority of the previous studies on CWD focused on the disease pathogen (Rutherford, 2006; Geiser MD *et al*., 2005, Lepoint, 2005) and the disease effects on

farms (Flood & Brayford, 1997; Muller, 1997; Oduor *et al*., 2005). Few studies have reported existence of host resistance (Meffrein, 1957; Delassus, 1954) among *C. canephora* genotypes in west and central Africa. But no study has ever given information on inheritability of resistance to CWD. This study revealed that resistance/susceptibility to CWD in *C. canephora* is genetically controlled. In our study, replicate effects in field experiments were never significant; implying most of the observed phenotypic variation is due to genetic differences between genotypes and progenies. It also shows that the field heterogeneity was properly controlled by the replications and randomisation and that the inoculum availability in different parts of the field did not vary substantial to affect the results.

The significant phenotypic differences observed among parents of the diallel progenies in experiment 1 shows that there are significant genetic variations among these parents for CWD resistance/susceptibility. By March 2006, CWD resistance/susceptibility of these parents varied quantitatively from 33.3% mortality observed on parent 236/26 (most resistant) to 91.7% mortality observed on parents J1/14 and 1s/6 (most susceptible) and Student-Newman-Keuls mean separation test ranked in overlapping resistance classes. None of these parents was completely resistant or completely susceptible. This suggests that resistance among these genotypes is controlled by many genes and moderately susceptible parent 236/26 has more or the most effective resistance genes than the highly susceptible parents 257s/53, 245/62, J1/14 and 1s/6 (Van der Plank, 1963).

There were also significant genetic differences for CWD resistance/susceptibility among the diallel progenies in experiment 1. Because of the low resistance of the parents, overall level of CWD resistance among these progenies was low. By March 2006, CWD resistance among progenies also varied quantitatively from 41.3% mortality observed on progeny 236/26 x 1s/2 (most resistant) to 100% mortality observed on progenies J1/14 x 259s/56, J1/14 x 1s/3, J1/14 x 258s/24, 1s/6 x J1/14 and 245/62 x 223/32 (most susceptible). The progenies were also ranked in overlapping resistance classes, suggesting that resistance among progenies is also controlled by many genes, which act additively.

Significant genetic differences were also observed among clones in field experiment 2. Clones J/1/1 and Q/3/4, in this experiment, were clearly resistant and clones C/1/7 and H/4/2 were clearly susceptible. Other clones were quantitatively distributed in between these extremes with

varying levels of resistance. Screen house studies (experiment 3) confirmed the quantitative nature of resistance among these genotypes and that clones J/1/1 and Q/3/4 were the most resistant.

Quantitative variations between progenies and or clones in these studies suggest that resistance to coffee wilt disease in *C. canephora* is controlled by many genes, which are differently distributed among genotypes. Most resistant genotypes J/1/1 and Q/3/4 are presumed to have the highest number of the resistance genes (Agrios, 1997). This concurs with results of resistance tests performed on open pollinated progenies of individuals from different sources (Kalangala islands, Itwara and Kibale primary forests, Nganda and erect phenotypes), conducted in an experiment outside the current study, which also showed that the resistance genes are differently distributed among *C. canephora* populations (P. Musoli, unpublished). Quantitative resistance has been reported for many crop plant diseases such as brown rust of wheat caused by *Puccinia recondita* (Milliano et al., 1986), Late blight caused by *Phytophthro infestans* in potato (Colon et al., 1995) and South American leaf blight caused by *Microcyclus ulei* in rubber tree (Lespinasse et al. 2000; Le Guen *et al*. 2006), rice blast in rice (Zahirul et al. 2005). Studying heredity of resistance controlled by many genes can be complex due to compounding effects of interactions between the host and pathogens and geographical locations. Such difficulties have been encountered for *F*. *Fusarium oxysporum* f. sp. *vasinfectum* on Cotton (*Gossypium hirsutum*) (Ulloa et al, 2006). Given that *C. canephora* is highly heterozygous and heterogeneous and very diverse geographically and therefore complex to understand genetically, the genetic controlled of CWD resistance should be confirmed in more elaborate studies. Studies using progenies from distinctively resistant and susceptible parents will provide more information and probably lead to confirmation of the genetic control of CWD resistance in *C. canephora* in observed in this study. Progenies of *C. canephora* double haploid parents, which are less heterozygous, although they are normally less vigorous (Lashermes et al, 1993), would be better for these studies.

Resistant and susceptible genotypes are distinguished when there is sufficient disease; determined from the level of symptom severity on susceptible genotypes or disease incidence. In this study, disease level of 55-65% plant mortality was considered logical for contrasting resistant and susceptible genotypes or progenies. This threshold was also considered convenient for similar assessment on black pod disease caused by *Phytophthora megakarya* in cocoa (Berry & Cilas, 1994). Disease levels below this range would be inappropriate because the inoculum under natural field infections, as indicated by Zadoks & Schein (1979), takes long to reach quantities required for

a simultaneous and possibly homogeneous challenge on many host trees. Thus assessment for resistance at low disease levels would lead to falsely considering unchallenged/escapes trees as resistant. High intra genotype or progeny variations at lower disease level caused by insufficient infections could lead to high coefficients of phenotypic variations that can compound the difficulty to detect genotype effects. Analyses of variance in this study revealed high coefficients of phenotypic variation (C.V) for assessments at lower disease levels, which (C.V) decreased with increase in disease levels. In field experiment 2, where assessment started at 25% plant mortality, the C.V ranged from about 50 to 76 for assessments at less than 55% plant mortality (Table 4.6). Secondly, partial resistance, as it appears to be for CWD among genotypes in this study is relative with small differences between genotypes which are difficult to measure and classification of resistant/susceptible can be arbitrary. A threshold disease level is required to make such classifications. Disease levels higher than the range used in this study were considered inappropriate as moderately resistant genotypes can also develop the disease due to high inoculum pressure (Agrios, 1997). Incomplete or polygenic resistance is known not to completely stop the disease but it is valuable for delaying disease development (Van der Plank, 1963, Agrios, 1997). Therefore a disease range of 56-65% plant mortality is the best compromise. Indeed *C. canephora* genotypes which have withstood CWD up to such levels and for this long in experimental fields of this study should be considered resistant enough to enable farmers gain from their investment, if they planted some of them.

Broad sense heritability calculated from diallel progenies within the disease range of 55-65% plant mortality was moderate and it decreased from 0.332 observed at 56.7% mortality to 0.267 observed at 65% mortality. This is because the genetic variance drastically continued to decrease as plants continued to die. For this particular experiment where the progenies are susceptible, because their parents were also susceptible, it would not be possible to estimate heritability at higher disease levels since even trees of the moderately resistant progenies will have died. This shows that CWD resistance in parents of these progenies is heritable but the genetic gains from this resistance would only delays the disease epidemic. However CWD resistance among individual trees within the progenies could be explored using artificial tests on their clonal propagules and individuals found resistant can be propagation as resistant clones.

High correlations for phenotypic and genetic effects between different assessment dates, calculated from diallel progenies in experiment 1 shows that resistance observed at 56.7%

mortality, at the beginning of assessment for this trial, has a high relationship with the resistance observed five years later at 79.8% mortality. This implies selection for CWD resistance at this date and disease level would be effective. However at this early selection the dominance and additive genetic effects are compounded. Dominance effects are minimal at disease levels high than 70% mortality. Parents variably contributed to the phenotypic effects of their diallel progenies. Effects of the most susceptible parents were always positively higher. Effects of the most resistant parents were always negatively higher. Effects of moderately resistant/susceptible parents were always moderate. This implies that progenies of the most susceptible parents were often the most susceptible at all assessment dates and progenies of the most resistant parents were often the most resistant at all assessment dates. Progenies of the moderately susceptible parents were either among the most susceptible or among the most resistant or moderately resistant/susceptible.

Broad sense heritability calculated from clones in experiment 2 within the disease range of 55-65% was medium and it increased from 0.333 observed at 56.8% to 0.355 observed at 64.5% mortality and slightly decreased to 0.347 observed at 65.5% mortality. This shows that CWD resistance in these clones is heritable and the resistant clones (J/1/1 and Q/3/4) in this experiment can be selected for globally improving CWD resistance in *C. canephora*. Besides clones J/1/1 and Q/3/4 and to a lesser extent R/1/4 and 1s/3, with realised resistance, in this experiment can be selected and propagated vegetatively.

Broad sense heritability estimated from regression of mortality of half-sib progenies onto mortality of their parents in the screen house (1.0) was higher than heritability estimated from regression of the progeny means onto parental means in the field (0.584). This is expected since the screen house experiments are closely related in a way that plant materials used in both experiments were of the same age and infection protocol and care for the material during experiment period was the same. Secondly it can be noted that both values are high, however, their regressions coefficients are not significant, implying that the confidence intervals therefore the heritability is not significantly different from zero. The differential responses to CWD under field and screen house by some clones and their progenies should be the cause of the large confidence intervals and hence weak relationship observed between the progenies and their parents in the field. Progenies of clone B/2/1, which is susceptible in the field, were found resistant and inversely, progenies of clone 1s/2, which is highly susceptible in the field, were

only moderately susceptible. Differential responses were also observed for clone B/2/1 in the field and its rooted cuttings in the screen house. This could imply that clones have different defence mechanisms against penetration by the fungus. Deferential defence mechanisms within genotypes against penetration by fungal pathogens have been reported for *Fusarium oxysporum* f. sp. vasinfectum in Cotton (*Gossypium hirsutum*) (Bugbee and Sappenfiled (1968). The high confidence interval observed between progenies and their parents within screen house studies are due to the small number of parents involved in this regression. Only 9 parents and progenies were involved.

As far as we know, this is the first study on heritability of coffee wilt disease resistance in *C. canephora.* However, low to moderate narrow and broad sense heritabilities have been reported for other quantitative traits in this crop (Leroy et al., 1994, Cilas et al., 2006; Montagnon et al., 2003). Since CWD resistance and the traits studied in earlier studies are all important consideration for commercial varieties, it will be interesting to study the relationships between resistance and these other traits.

Low to moderate heritability has been reported for partial resistance in some other crops (Flavio et al., 2003) and for most of them, developing resistant varieties through transgression was difficult. Studies on inheritance to *Fusarium* wilts in many crops have been complicated by interaction between the host and different races or pathogens and geographical localities, especially in field studies (Kumar, 1998; Boyhan et al, 2003; Perchepied and Pitrat, 2004; McGrath, et al., 2007), Cross et al., 2000, de Franqueville and de Greef, 1987.

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CHAPTER 5

General discussion and conclusions

5.1 General discussion

Present work was carried out to contribute effectively to the control of coffee wilt disease (CWD) in Africa, particularly in Uganda, where the disease has destroyed about 50% of *C. canephora* trees and it is continuing to ravage the crop. The work was carried out to particularly generate information on (i) spatial and temporal evolution of CWD in fields in Uganda (ii) diversity of genetic resources available for resistance to combat CWD (iii) genetic control of CWD resistance and (iv) prospects for resistance to coffee wilt disease in *C. canephora.* A number of findings emerged from this work that have important implications for (i) managing CWD in *C. canephora* using phytosanitory practices (Chapter 1) and genetic resistance (Chapter 4) in view of interactions of the host with its pathogen and the agro-ecological environment (Chapter 3) and for (ii) managing genetic resources available for controlling coffee wilt disease and other genetic improvements in *C. canephora* (Chapter 5)

5.1.1 Spatial- temporal spread of CWD

Analysis of disease data collected from *C. canephora* trees in fields at the Coffee Research Institute-Kituza in Uganda revealed that coffee wilt disease spreads gradually and continuously among *C. canephora* gardens as it kills all susceptible trees. Since CWD emerged in experimental fields were these studies were conducted in 1999, overall tree mortality progressed from 0 to 65%. However, on some varieties the disease progressed up to 96% mortality, while on others the mortality remained at 0%. As Kituza has an agro-ecological environment considered ideal for *C. canephora* cultivation in Uganda (received an average of 1155mm/year of rainfall during 2000-2005, has high relative humidity and daily temperature ranges from 25-28°C), it can be deduced from the observation at Kituza that CWD can devastate this crop in all parts of the country with similar conditions. This underlines the high mortality observed among *C. canephora* gardens in many parts of Uganda (Oduor, 2005) and it demonstrates the need for resistant varieties. Kituza weather pattern is probably not very different from that in other *C. canephora* growing parts in Africa, given that this crop is mainly adapted to areas with tropical

climate. High mortality due to CWD was also reported on *C. canephora* in some regions of the Democratic Republic of Congo ((Flood, 1997; 2001; Oduor, 2005)

Genotypes that withstood infection in the fields where this study was conducted, during the seven years of exposure to this pathogen, illustrated their resistance to this disease and highlights that CWD can be controlled in Uganda through host resistance.

This study also revealed that CWD spreads from infected *C. canephora* trees to cause new infections of up to three trees away, forming aggregated disease patterns. Nucleated disease spread has been reported for a number of other diseases. Rekah et al. (1999) found that the infection range of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, the causal agent of crown and root rot of tomato, ranged between 1.1 and 4.4 meters in the exponential phase of the disease. Spatial dependence of *Phytophthora capsici* reached 15 meters (Larkin et al., 1995). These observations imply that, for effective control of coffee wilt disease by uprooting infected trees, up to 3 healthy looking trees neighbouring a diseased tree should also be uprooted on first observation of CWD symptoms. This can also be considered precautionary given that the disease incubation period is normally long (Saccas, 1951, 1956) and therefore the healthy looking trees could be infected. Secondly, the actual mechanism of tree to tree infection is not known (either by water run off, splash, canopy contact or root contact) and therefore a three coffee tree distance ensures that uninfected trees are out of range from this obvious inoculum source.

Practical implication of these results is that this intervention is only limited to low disease levels, at probably 5% incidence. At higher disease incidence, diseased trees will be too many and uprooting becomes too costly (In Uganda it costs about US $0.5 to uproot a diseased coffee tree). Secondly, at a disease incidence of more than 5% infection points are many and uprooting 3 trees in all direction of each of these initial points will results into uprooting a large proportion of the crop, leaving the field virtually void of coffee trees. Lack of such information may have contributed to the failure of intervention by uprooting in Uganda (Wetala et al., 2000), where uprooting trees with disease symptoms was emphasized and irrespective of the disease incidence in the field.

5.1.2 Genetic diversity of C. canephora genetic resources

Molecular analysis of *C. canephora* populations using microsetallite markers revealed high genetic diversity between Ugandan *C. canephora* populations from different geographical

locations (Itwara, Kibale and Kalangala) and cultivated types (Nganda and Erect). Variability between cultivated types Nganda and Erect and feral population (Kalangala) however was not significant. Differences between genotypes from different locations within Kibale region were significant. The variability between and among wild and feral populations illustrated the potential of these populations as sources of new genes of interest to the breeding program. CWD resistance tests conducted on these populations (Chapter 3 of this thesis) showed that cultivated populations were susceptible and wild, particularly Itwara, and feral populations contain resistant trees whose resistance genes can be incorporated in commercial varieties.

Genetic differences were observed between Ugandan *C. canephora* and previously known genetic diversity groups within the species (Dussert, 2003). Ugandan *C. canephora* genotypes are significantly different from genotypes from Guinean, Congolese B, C and SG1 groups, originating from regions where coffee wilt disease was prevalent in the 1920s to 1960s. Since CWD was controlled in West African countries and Cameroon using resistant varieties developed from SG2 group, this population is a potential source of resistance for improving CWD resistance in *C. canephora* commercial varieties in Uganda, if the populations bear genes for resistance to *F. xylarioides* strain on *C. canephora* in Uganda. These populations are also a potential source of other new genes which may be of interest.

5.1.3 Resistance to coffee wilt disease in C. canephora

Resistance of *C. canephora* genotypes to CWD in the field showed a range of percentage mortality from 0 to 96%. Disease period (duration from appearance of first symptoms to death of the tree) was also variable between clones, ranging from one to 16 months. Generally resistant genotypes had long disease periods, however certain susceptible genotypes (Q/1/1) had exceptionally long disease periods and also certain resistant genotypes (R/1/4) had very short disease periods. These observations suggest there is variation in resistance mechanisms among the genotypes, which implies different genes conditions the resistance and in different ways and thus further supporting the hypothesis of resistance to CWD in *C. canephora* being polygenic. But with clones J/1/1 and Q/3/4 completely resistant to this disease in the field, the nature of resistance can be confirmed after analyse of progenies derived from specific crosses of resistant/susceptible genotypes.

A comparison of CWD resistance among *C. canephora* genotypes in the field and under artificial inoculations in the screen house and climatic chambers revealed that field, screen house and climatic chamber results are comparable and convenient for pre-selection test. However, differential reaction was observed with clone B2/1 between aerial inoculation by stem wounding and inoculation by root wounding (root dipping). This suggests there are different mechanisms of resistance expressed by the ability of plant to resist penetration of the fungus either through roots or aerial parts. Bugbee and Sappenfiled (1968) reported a similar resistance mechanism for *Fusarium oxysporum* f. sp. vasinfectum in Cotton (*Gossypium hirsutum*).

5.1.4 Heritability of CWD resistance

Analysis of disease data on *C. canephora* half diallel progenies found significant general combining ability for CWD susceptibility/resistance, implying there is additive genetic relationship between parents and offspring for CWD resistance. Broad sense heritability estimated from the same progenies at a disease level of 55-65% plant mortality was moderate (0.27-0.33) and corresponding narrow sense heritability was low (less than 0.15). Broad sense heritability estimated from an array of clones, within the same disease range, was medium (31-35). This shows that CWD resistance is heritable and therefore progenies of crosses between susceptible and resistant progenitors are expected to have better resistance than their susceptible parents. Thus resistance of Uganda current commercial clones, which are susceptible to CWD, can be improved through hybridisation with resistant genotypes. Bearing in mind that *C. canephora* is predominantly out breeding and these clones are heterozygous, progenies of theses crosses are expected to be heterogeneous. Therefore it is not likely that all individuals in a progeny of a cross between resistant and susceptible parent shall have CWD resistance to equal and acceptable levels and at the same time retain all qualities of the commercial parent. The progenies can therefore be evaluated as individual trees for all traits equally and resistant individuals, which have the other traits within acceptable limits, will be selected. These individuals will be multiplied as clones for assessment in multi-location field trials before they are released to farmers.

5.1.5 Host specificity

A study of interaction of *C. canephora* with *F. xylarioides* strains collected from *C. arabica, C. liberica* var. *dewevrei* and from within C. *canephora* revealed differential reaction by the host.

Strain from *C. arabica* was not lethal to *C. canephora* but the strain from *C. liberica* var. *dewevrei* is lethal to this species. Level of mortality induced by the strain from *C. liberica* var. *dewevrei* is however far less than that caused by the strain from within *C. canephora*. This revealed that *F. xylarioides* strains on *C. arabica* in Ethiopia and the historical strain on *C. liberica* originating from Central African Republic are different from the strain affecting *C. canephora* in Uganda, and although the strain from *C. liberica* var. dewevrei induces negligible mortality, there is a likelihood that this strain can at one time become a serious pest of *C. canephora*. In this specific study the strain from *C. canephora* was not lethal to *C. liberica* var. *dewevrei*, however earlier studies (Bieysse, 2005) showed that this strain causes minor mortality in *C. liberica* var. *dewevrei*.

In Uganda *C. liberica* var.*dewevrei* trees have remained healthy in fields were *C. canephora* has been destroyed by CWD in many parts of the country (Musoli, CORI, personal observations). However trees of one local variety, *C. liberica* var.*dewevrei* type "bwamba", have died due to coffee wilt disease in CORI coffee germplasm collections at Kawanda Agricultural Research Institute. Probably there are differential reactions to CWD infections among *C. liberica* ssp. in Uganda.

Differential host-pathogen interactions have been reported for many *Fusarium* species such as *F. oxysporum* Schlechtend. Fr. Forma *specialis phaseoli* Kendrick and Synder in common beans (*Phaseolus vulgaris*) (Cross et al, 2000), *F. Fusarium oxysporum* f. sp. vasinfectum on Cotton (*Gossypium hirsutum*) (Ulloa et al, 2006), *Fusarium oxysporum* f. sp. *cicer* on chickpea (*Cicer arietinum*) (Kumar, 1998). These interactions are normally a big hindrance to breeding disease resistant varieties.

5.1.6 Perspectives for breeding for resistance and other variety improvements in Uganda

Vast knowledge and skills were acquired from this study, which can be applied in programmes aiming to effectively control coffee wilt disease and restore coffee productivity in Uganda. Besides, tangible resistant clones were identified, which should be immediately evaluated for adaptation and adoption. Highlights of putting knowledge and skills acquired in this study into perspective are given below.

Adaptation and adoption trials for resistant clones identified in this study

Among genotypes studied in here, clone J/1/1 was completely resistant to CWD in the field and clone Q/3/4 was highly resistant. The two clones are known to yield on average 3.3 and 2.7 metric tones (five year average) of clean coffee per hectare per annum, respectively. Since they were initially selected for having good quality and being resistant to leaf rust and red blister disease, it implies they almost have all traits required of a commercial variety and they should be readily available for mass propagation. Buts as their initial assessments were carried out only in one site (Kituza), it is apparent that the quality, yield and other important traits need to be validated in multi-location trials in different *C. canephora* growing agro-ecological areas within the country.

It is also apparent that two clones are many enough for massive replanting in all *C. canephora* growing areas within Ugandan because of two reasons: First, host resistance is a function of the host, pathogen and the environmental interactions. Therefore it is not obvious that the two clones will be resistant to the same levels if they are cultivated in agro-ecological conditions that are different from those at Kituza, especially if the resistance is partial/horizontal as observed on clone Q/3/4 in the field and on both clones in the screen house. Thus these clones will have to be re-evaluated for CWD resistance in on-farm multi-location trials under different agro-ecological conditions before they are released to the farming community. Secondly, large scale coffee culture based on only two clones will be vulnerable in case of another disease or pest outbreak. Also, due to the out breeding nature of *C. canephora,* it is recommendable to plant at least five different clones for successful pollinations and fruiting and hence good yields. To avoid such risks, in the short run, the best of the moderately susceptible clones such as R/1/4 and commercial clones 258s/24 and 1s/3 should be included to broaden the genetic base, as more resistant clones are awaited from the germplasm screening programme.

Therefore the first activity will be to multiply CWD resistant clones identified in these studies for validating their performance in different *C. canephora* agro-ecological areas within Uganda (Figure 5.1). The clones shall be multiplied either as rooted nodal cuttings or tissue culture plantlets generated through somatic embryogenesis or by both methods. The facilities and expertise for both multiplication methods are available in Uganda.

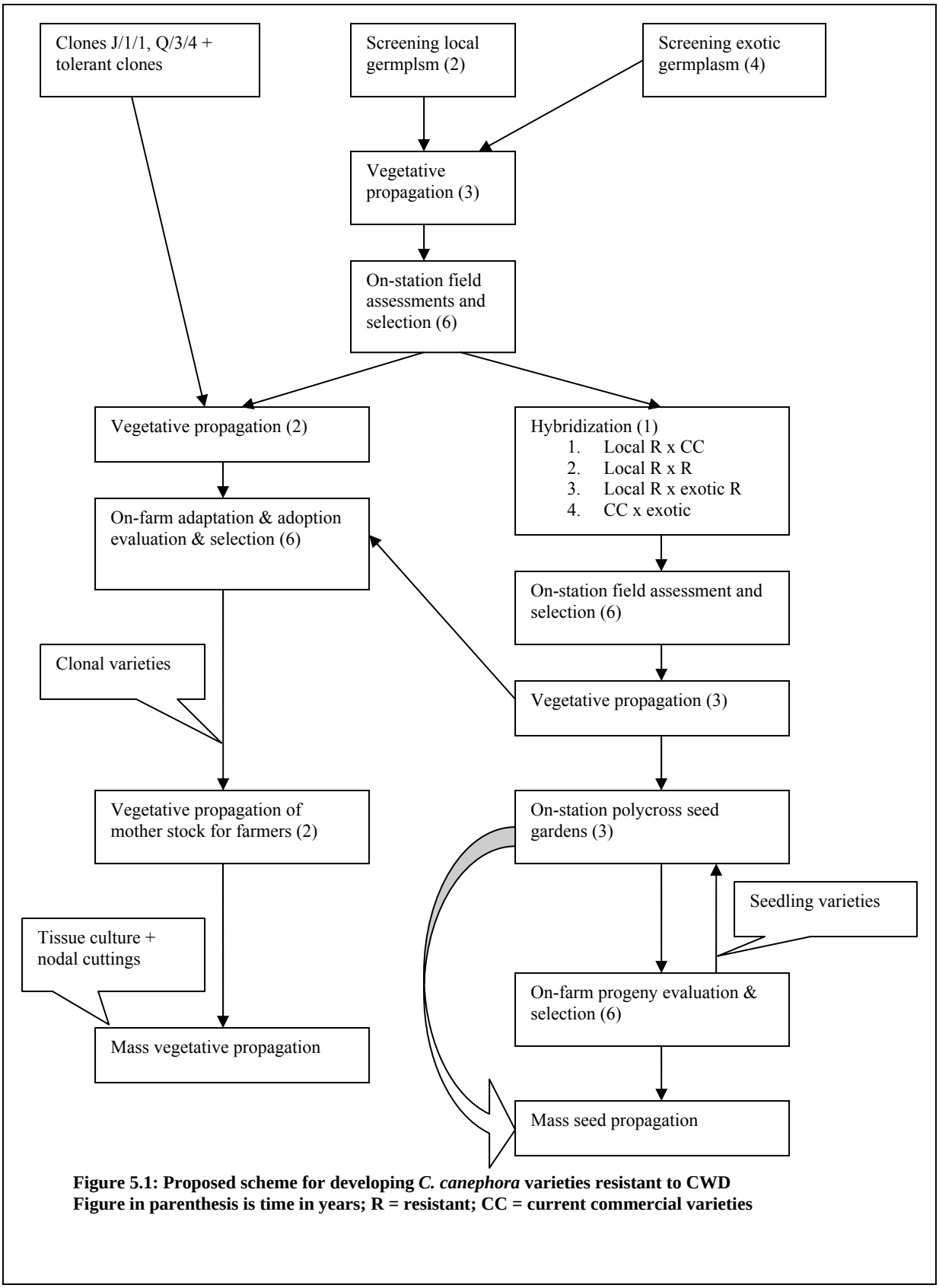

Figure 5.1: Proposed scheme for developing *C. canephora* varieties resistant to CWD
Figure in parenthesis is time in years; R = resistant; CC = current commercial varieties

Identifying more CWD resistant clones

A more sustainable approach shall be to identify more CWD resistant clones and give out only the CWD resistant clones to farmers in sufficient numbers (Figure 5.1). Through large scale germplasm screening using artificial inoculations in the screen house and field confirmation more, CWD resistant genotypes shall be identified to supplement the two resistant clones described above. Many local germplasm sources should be prospected for this purpose however the immediate source for this analysis shall be mother plants of resistant half sib progenies sampled from Kalangala Island on Lake Victoria, Itwara and Kibale forests. Primary screening will be conducted in the green house, since studies in here have shown that resistance identified through artificial inoculation in the screen house is correlated to resistance under natural infections in the field. Another advantage is that screen house results will be achieved within a year as compared to five or so years for the field studies (excluding time for raising plant materials) and also many genotypes can be handled in a small space and at a lesser cost. Resistant genotypes identified through this screening shall be planted in mother gardens for vegetative propagation. Clones raised from these mother gardens shall then be planted and evaluated in on-station field trials for CWD resistance and other agronomic traits. Promising clones from the on-station trials shall also be propagated vegetatively for planting in multi-location trials, where they shall be assessed for adaptation to different agro-ecological areas. Clones with satisfactory performance (high yield, good cup and seed qualities, resistant to CWD and other major coffee disease) in multi-location trials shall be released as clonal varieties for farmers' use.

Hybridisation programmes

Although CWD has taken precedent, yield and quality are very important aspects of the coffee variety improvement programme in Uganda. Hence specific crosses of CWD resistant genotypes with the current commercial clones shall be carried out to combine CWD resistance with the high yields (2.5 tons of clean coffee per hectare per annum) (Kibirige et al, 1993), good bean qualities (18-22g hundred beans weight, over 90% retained by screen 18/64) and good cup qualities (Leakey, 1970) of the commercial clones (Figure 5.1). Bearing in mind that *C. canephora* genotypes are predominantly out breeders and highly heterozygous, progenies of theses crosses are therefore expected to be very heterogeneous. Therefore it is not likely that

entire progeny of a cross will have improved CWD resistance to acceptable levels and at the same time retain all qualities of the commercial parent. Progenies will therefore be planted and evaluated in on-station field trials as individual trees for all traits equally and genotypes within acceptable limits will be selected. Selected individuals will be multiplied as clones and planted in multi-location trials for re- assessment for adaptation and adoption. Good performing clones will be selected for release to farmers as clonal varieties.

Hybridization shall also be conducted involving CWD resistant parents with complimentary traits. Such hybrid progenies shall be evaluated as individual trees for resistance against CWD and for field performance in other traits. Good performing progenies shall be selected, multiplied vegetatively and planted in multi-location trials for adaptation and adoption tests. Good performing clones shall be selected and release to farmers as clonal varieties.

Where entire progenies of resistant/resistant parents perform well, parents of such progenies shall be planted in polycross seed gardens for production of seeds to be given out to farmers. It is anticipated that progenies involving parents from different populations shall benefit from hybrid vigour derived from double heterozygosity of its parents.

Basing on field reports from Uganda and results of previous artificial inoculations (Musoli et al., 2001; Girma and Hindorf, 2001,) *C. arabica* can also be a source of resistance to control coffee wilt disease in *C. canephora*. Although *C. canephora* and *C. arabica* interspecific hybrids (Arabusta) haven been reported, we should anticipate some difficulties in the hybridizations because of the difference in ploidy levels of the two species. Crosses shall be carried out between *C. arabica* and *C. canephora* artificial tetraploids. Cytological analysis shall be performed on root cells of the F1 seedlings to verify true tetraploid individuals before they are planted out in the field. The true tetraploid F1 progenies will then be planted and evaluated for resistance to CWD and other agronomic traits in on-station field trials. Individual genotypes with CWD resistance and *C. canephora* cup qualities shall be selected and vegetatively multiplied for evaluation in on-farm trials. Clones that will perform well at this level shall be selected and released to farmers as clonal varieties.

Grafting

Some of the resistant clones identified in this study and others, which will be identified in future CWD tests, will be evaluated as CWD resistant rootstock for grafting scions of our commercial

clones (susceptible). The grafted materials will be evaluated for (i) compatibility between the scion and root stalk (ii) CWD effects on the scion. Since we do not know the plant mechanism used for resistance against the pathogen, we can not be sure that the scion will be free from CWD pathogen. In case these two concerns are satisfactory, grafting will be adopting for large scale multiplication of current commercial varieties on CWD resistant rootstocks.

Rootstock of other *Coffea* species will also be studied for this purpose. Successful interspecific grafting involving *C. canephora* and *Coffea liberica* spp. has been reported in previous studies (Couturon, 1993). Bearing in mind that, Bertrand et al (2001) reported depressing effects of *C. liberica* ssp. rootstock on yield and quality of *C. arabica* scion varieties, other agronomic properties of grafted varieties such as yield and quality shall also be studied.

Integrated coffee wilt management

As activities proposed above continue, and bearing in mind that none of them will give a new variety in less then five years, a short term option will be to continue providing farmers with planting materials of the lesser susceptible commercial clones. From this study it was established that commercial clones 257/53, 1s/6 and 1s/2 are very susceptible. These clones should be withdrawn from the commercial list. Only clones 223/32, 258s/24 and 1s/3 should be given out. These clones should be planted in newly cleared fields expected to be free of CWD infection. Wilting plants in these new plantings should be rouged out as according to observations in this study.

Conservation of C. canephora genetic resources

Bearing in mind the on going degradation of natural biodiversity reserves, it is important that *C. canephora* diversity observed in this study is protected. Since genetic differentiation among *C. canephora* populations is high, samples of a few genotypes will be obtained from many populations to capture enough natural genetic variability for conservation and utilization in future breeding programs. Ugandan *C. canephora* samples will be collected from Itwara, Kalangala and sites in Kibale in addition to other relict forests and isolated cultivated regions not included in this study. Because of threats from coffee wilt disease and other unforeseen natural disasters on *C. canephora* genetic resources, efforts will be made to find collaborator or an

international germplasm collection and conservation in multiple conservation centres in different countries to preserve.

5.1.7 Areas o f further studies

Spatial and temporal analysis

As noted in here, data collection for analyzing spatial and temporal spread of CWD was initiated late, when the disease incidence was high and the disease had spread in all parts of the fields. Consequently information on the starting of the disease and its initial pattern could not be discerned. Further studies could be conducted to generate this information. Information derived from the proposed study is important for laying strategies to control CWD by avoiding initial infections.

Heritability of CWD resistance

Information generated in here on heritability of CWD resistance is not sufficient. The number of genes involved in CWD resistance is not known and it is not known whether the resistance is controlled by dominant or recessive genes. The resistance mechanisms in different genotypes are also not known. All this information will be required for develop a more effective strategy for breeding CWD resistant varieties. Studies on progenies (F1 and backcross) from crosses between CWD resistant and susceptible genotypes would yield further information such mechanisms. But bearing in mind that *C. canephora* is an obligate out breeder and as noted in these studies, *C. canephora* parent genotypes are always very heterozygous. It is then not possible to attain some of these objectives without homozygous parents. Therefore double haploids could be generated from cultures of both female and male gametes to improve on the accuracy of such studies. Although double haploids in C. canephora are known to have low vigour (Lashermes et al. 1994), their use in breeding for resistance against CWD is vital. These double haploids would be analysed in field trials for CWD resistance/susceptibility. Analysis of progenies generated from crosses of susceptible and resistant double haploid parents should provide more information on inheritance of CWD resistance and genetic control of the resistance.

As known from many breeding programmes, incorporation of CWD resistance genes into commercial clones that should also have required quality traits could takes a long time and at times it is a gamble. Mapping studies could be initiated using the double haploid CWD

susceptible/resistant parents and their progenies to identify molecular markers and or QTLs associated with CWD resistance to assist in breeding resistant varieties and isolation of resistance genes for creating bacterial artificial chromosome (BAC) libraries.

Assessing for CWD resistance among previously known C. canephora diversity groups

Following differential reactions of *F. xylarioides* strains with their host species plus information from analysis of their DNA (CAB003 and DSMZ) (Bieysse, 2005) and from reproductive tests (Lepoint, 2006), we can deduce that either the coffee wilt disease scourge that destroyed trees of *C. canephora* and *C. liberica* spp. in West and Central African countries during the 1920s to 1960s is different from the current scourge or the two disease types were present by then, since Meiffren reported differential responses within genotypes to CWD in different localities within the same region. As reported by Saccas (1956) and Meiffrein (1961), resistant *C. canephora* genotypes from the DRC were used to control CWD in Central African Republic, Ivory Coast and within DRC, after failing to identify resistance among the genotypes in these countries. The resistant genotypes used in this re-planting belong to the SG2 genetic diversity group while genotypes from the Guinean group constituted the susceptible varieties which were grown in the Ivory Coast. But currently the disease is affecting *C. canephora* genotypes in the DRC that belong to the SG2. Tests carried out on *C. canephora* genotypes from the DRC (Bieysse, 2005) and Guinean group using *F. xylarioides* strains CAB003 and DSMZ indicated that some individual genotypes are resistant to these strains. But these tests did not specify diversity groups of Congolese genotypes (whether B, C, SG1 or SG2). It is then apparent that the interaction of *C. canephora* genetic diversity groups Guinean, Congolese B, C, SG1 and SG2 CWD pathogen is still not clear. Representative genotypes from these diversity groups, together with controls from the Ugandan diversity group should be systematically tested with *F. xylarioides* strains DSMZ62457 and CAB003 to provide more information. Other historical strains originating from trees of both *C. canephora* and *C. liberica* spp. in different countries (Ivory Coast, Guinea, CAR and Cameroon) should be included in these tests.

And through these resistance tests we shall deduce whether genetic diversity exhibited by microsatellite markers corroborates diversity in resistance to coffee wilt disease and it would help to grade the importance of these groups in combating coffee wilt disease through genetic resistance.

Through these tests, we anticipate to identify genotypes resistant to CWD. Resistant genotypes originating from different genetic diversity groups identified through these tests shall be planted in mother gardens and multiplied as clones (Figure 5.1). The clones shall be planted in on-station field trials and will be assessed for other agronomic traits. Resistant genotypes that shall perform well in the on-station field tests shall be cloned and planted in on-farm adaptation and adoption trials, where good performing (high yield, good cup and bean qualities, resistant to major disease of C. canephora in Uganda) clones shall be selected for released to farmers for cultivation as clonal varieties.

Open pollinated progenies and specific cross progenies derived by crosses among resistant parents from within these populations (Guinean, Congolese groups) and resistant parents from Ugandan populations shall also be evaluated for yield, quality and resistance to CWD and other major *C. canephora* diseases. Parents of progenies that will perform satisfactorily shall be planted in polycross seed gardens to provide seeds for farmers (Figure 5.1). This is anticipated to be a cheaper and faster source of planting material than clones. Secondly, it is anticipated that crosses involving parents from Ugandan populations and genotypes from other diversity populations will give rise to vigorous hybrids that would be benefiting from the heterozygosity between the parents.

5.2 Conclusion

These studies generated substantial information which will be useful in strategies for developing wilt resistant varieties to control the coffee wilt disease in *C. canephora*. It is now known that once coffee wilt disease starts affecting *C. canephora* trees in the field, it will continue killing all susceptible trees. Fields composed of susceptible trees can attain 100% loss. It is now established that infected *C. canephora* trees take one to sixteen months, from the time of the first symptoms, to death. Also that coffee wilt disease spreads from infected trees to cause new infections on up to 3 trees away.

There is high genetic diversity among and between *C. canephora* populations. Ugandan populations are significantly different from previously known C. canephora diversity groups and therefore they make a new group within the species. There is also high diversity within Ugandan *C. canephora*.

It is now known that CWD resistance/susceptibility susceptibility in *C. canephora* is genetically controlled. Inheritance of CWD resistance is not properly understood but it is hypothesized that resistance is controlled by many and resistance genes are differently available among *C. canephora* genotypes and populations and impart resistance through different mechanism.

Two CWD resistant *C. canephora* clones were identified which can be used in wilt control in the near future. In the medium term other resistant individuals genotypes identified through screening tests shall be multiplied as clones.

Knowledge on interaction of different CWD pathogen with its *Coffea* sp. host was generated. It is now known that *F. xylarioides* strain from *C. arabica* is not lethal to *C. canephora and F. xylarioides* strain from *C. liberica* var. *dewevrei* is lethal to *C. canephora* but it is less aggressive than the strain from within *C. canephora*. It was then logically observed that CWD epidemic of the 1920s to 1960s was either different from the current scourge or the two diseases existed together during the early scourge.

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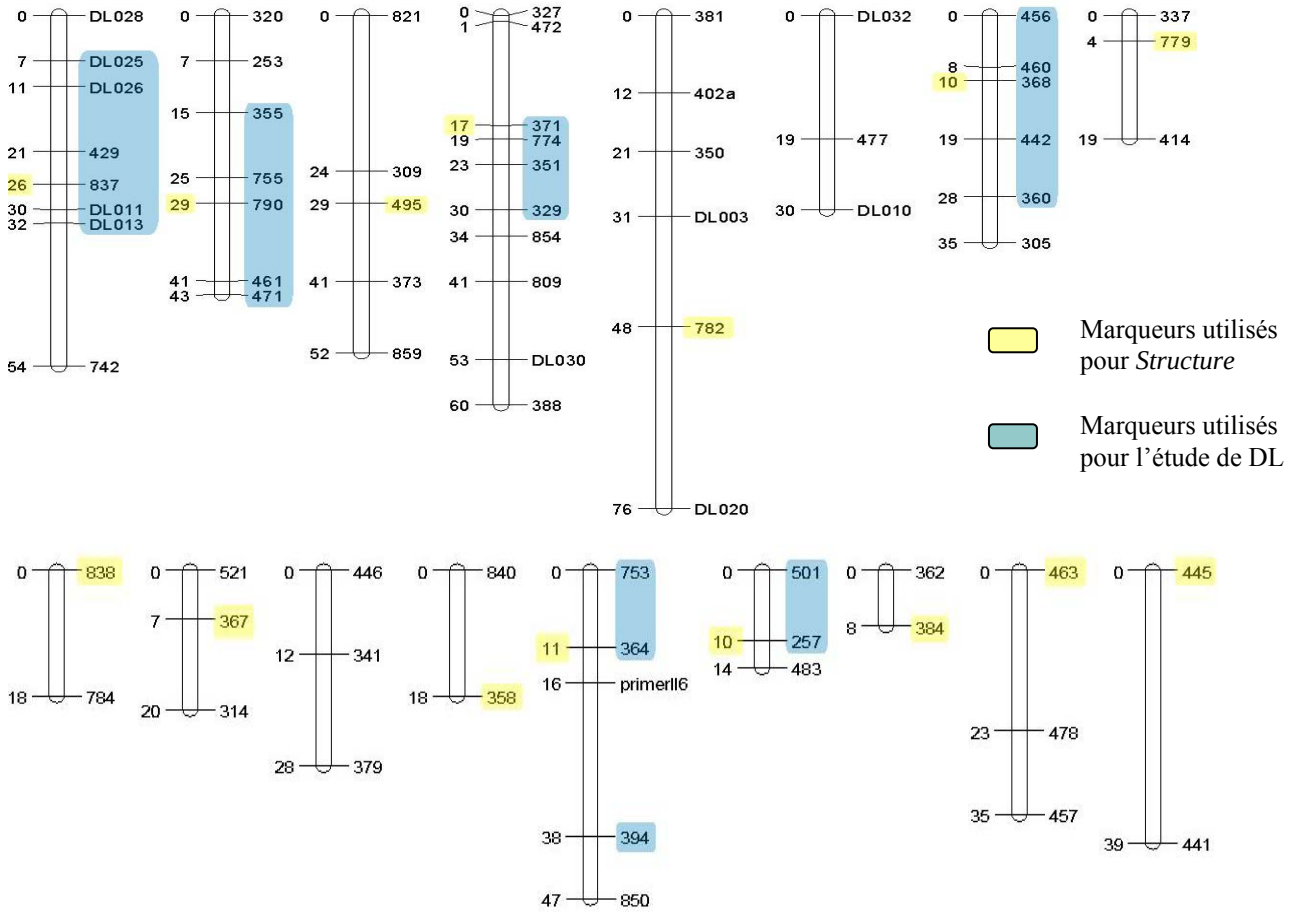
APPENDIX:

1.1 Map of *Coffea canephora* genome (CIRAD) and location of microsatellite markers used in studies on genetic diversity of Ugandan *C. canephora* populations.



Résumé

La trachéomycose du caféier, due à *Fusarium xylarioides*, représente une menace pour la production de café en Afrique. Cette maladie vasculaire signalée pour la première fois en 1927 en République Centre Africaine sur *Coffea liberica* var. *deweivri* provoque la mort de l'arbre dans un laps de temps de 1 à 24 mois après l'apparition des premiers symptômes. Progressivement la maladie est apparue sur *C. canephora* en Côte d'Ivoire (1947), République du Congo (1949), Guinée (1958). En 1957, elle était signalée sur *C. arabica* en Ethiopie. La mise en oeuvre à grande échelle de campagnes d'arrachage et la diffusion de variétés résistantes de *C. canephora* Pierre a permis d'éradiquer la maladie en Afrique Centrale et en Afrique de l'ouest vers la fin des années 50. Cependant au début des années 80, la maladie a ré-émergé en RDC et s'est étendue progressivement à l'Ouganda en 1993, puis à la Tanzanie (2000). En Ouganda la maladie affectait 90% des plantations avec un pourcentage moyen de plants morts de l'ordre de 44%. Aucun traitement phytosanitaire ne permet de contrôler la maladie, aucune variété commerciale résistante n'est disponible et il est déconseillé de replanter dans un sol infecté pendant plusieurs années.

1- L'évolution spatiale de la maladie indique un début d'épidémie en foyers qui augmentent en taille et fusionnent pour former un ensemble continu de caféiers malades, ponctué de groupes de caféiers résistants. A partir d'arbres malades, la dispersion peut se faire dans toutes les directions dans la parcelle et infecter les arbres voisins jusqu'à une distance d'environ 10 m

2- L'analyse de la diversité génétique de *C. canephora* en Ouganda, (populations sauvages et cultivées localement) a permis de séparer trois groupes. Les populations de caféiers sauvages d'Ouganda constituent un nouveau groupe génétique. La diversité génétique de ces populations sauvages met en évidence une source nouvelle de gènes susceptibles d'être exploités dans les futurs programmes d'amélioration des variétés commerciales existantes, surtout si elles recèlent des gènes de résistance à la trachéomycose.

3- Les observations sur la résistance au champ ont mis en évidence différents niveaux de sensibilité avec des périodes de latence très variables (1 à 24 mois), ce qui suggère la mise en place de différents mécanismes de défense quantitatif et des interactions avec les conditions environnementales, et d'identifier deux génotypes de caféiers *C. canephora* (J1/1 et Q3/4) totalement résistants à la trachéomycose. Par ailleurs il semblerait que les isolats de *F. xylarioides* présentent une certaine spécificité d'hôte.

4- L'héritabilité de la résistance à la trachéomycose a été calculée à 50-60% de mortalité, (i) sur les descendances pleins frères dans un demi diallèle au champ, en présence d'un inoculum naturel d'une part, (ii) sur des boutures et des descendances issues de fécondations libres, inoculées artificiellement en conditions contrôlées. Une héritabilité au sens large de 0,3 est observée dans les différents essais analysés. Les aptitudes générales à la combinaison (AGC) sont les plus significatives pour la tolérance à la maladie. Il sera donc possible d'intégrer les deux génotypes résistants, J1/1 et Q3/4 avec les origines spontanées de Kibale et Itwara dans des programmes d'amélioration pour la création de variétés tolérantes. La culture de ces variétés devra être associée à des techniques culturales adaptées pour réduire la dissémination du champignon.

Mots clés : *Coffea canephora*, *Fusarium xylarioides*, diversité génétique, résistance, héritabilité, spécificité d'hôte, krigeage

Summary

Coffee wilt disease (CWD) caused by *Fusarium xylarioides* has been a menace to coffee production in Africa since 1920s. Use of phytosanitory control practices and re-planting with resistant varieties seemed to have eradicated CWD in Central and West African countries during 1960s and 1970s. However in 1980s, CWD was reported again in the Democratic Republic of Congo (DRC), from where it spread to Uganda and Tanzania. CWD was first reported in Uganda in 1993, where it rapidly spread on *C. canephora* in all areas of its cultivation. By 2002 CWD had affected 44.5% of *C. canephora* in Uganda.

This study was carried out to understand (i) spatial and temporal field evolution of CWD and (ii) genetic diversity of available *C. canephora* genetic resources that could be explored for resistance to control this disease, and assess (iii) prospects for CWD resistance in available *C. canephora* genetic resources and interaction of the host and the pathogen, and to (iv) determine genetic control of CWD resistance in *C. canephora* in Uganda. The study revealed that CWD spreads irregularly from trees of initial infections to neighbouring healthy trees, forming aggregated disease patterns and foci, which increase over time and coalesce into continuous stretches punctuated by spots of resistant hosts. The disease spreads from trees of initial infection to infect up to 3 healthy trees away in all directions. Ugandan *C. canephora* was fund to be genetically diverse from previously known diversity groups, thus it forms another diversity group within the species. There is high genetic diversity within Ugandan *C. canephora* populations and genotypes. CWD resistance among Ugandan *C. canephora* genotypes and populations was found to be quantitatively variable, suggesting that resistance is controlled by more than one gene. Two resistant clones, J/1/1 and Q/3/4, were identified. General combining ability for CWD resistance/susceptibility in *C. canephora* gent was significant and broad sense heritability of the resistance was medium. *F. xylarioides* strain CAB003 from *C. canephora* causes severe mortality to *C. canephora* followed by strain DSMZ62457 from *C. liberica* var. *dewevrei* (formerly type excelsa). *F. xylarioides* strain CAB007 from *C. arabica* is not lethal to *C. canephora*. Strain DSMZ62457 causes severe mortality to *C. liberica* var. *dewevrei* but strain CAB003 was not lethal to this species in this particular study.

Uprooting up to three healthy looking coffee trees neighbouring a diseased tree, when disease incidence is less than 10%, can minimise CWD incidence. There is sufficient genetic diversity in *C. canephora* for CWD resistance. CWD resistant individual genotypes can be selected for propagation as clones for replanting in wilt infected areas. *F. xylarioides* strain DSMZ6245 is a wide spectrum pathogen of *Coffea* species.

Key words: *Coffea canephora*, *Fusarium xylarioides*, Genetic diversity, resistance, inheritance, host specificity.