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Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa.

Fifth annual report covering period 1/11/2005 to 31/10/2006

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Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa.

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COWIDI INCO Project YEAR 5

SUMMARY

Coffee wilt disease is the main constraint of Robusta coffee growing in the countries participating in the project. It is continuing to spread towards Equator province in DRC and is killing new coffee trees in Uganda in regions already affected. In Tanzania, CWD has affected new districts despite the proposed preventive measures, grubbing up and burning coffee trees displaying the first symptoms. In Ethiopia, the disease on *C. arabica* seems to be spreading and there are grounds for fearing that the genetic resources conserved at Jima could disappear. The seriousness and threat of CWD for coffee growing are real.

Preventive measures developed recently in Tanzania, or at the outset of the epidemic in Uganda, do not seem to be enough to stop the disease from spreading.

Variety resistance is considered the best option for an effective control of the coffee wilt disease and therefore the main objective of this project remains, to develop a global strategy to fighting the disease, based on durable tolerance/resistance, that is adapted to small holder agro-system and to the economic conditions prevailing in Africa.

In year 5, the work carried out built on results obtained in year 4. i.e:

- 1- Study the speciation of *Fusarium xylarioides* within the African Coffee wilt disease.
- 2- Assess the aggressiveness of isolates and characterize interactions
- 3- Identify coffee trees bearing resistance factors and analyse inheritance of resistance to CWD.
- 4- Analysis of genetic diversity of *Coffea canephora* from Uganda and DRC.
- 5- Analysis of spatial and temporal spread of the CWD in the field
- 6- Assess correlations between field resistance and artificial inoculation tests

↑ fin Résumé eothallic fungus. This explains the abundance of the perfect form in the reality four biological species/sterility groups (BS/SG group), and it is *G. xylarioides* complex. This result is congruent with microsatellite A RFLP. The exact status of the different BS/SGs as subspecies or

2- The contemporary canephora strain CAB003 is specific of the *Coffea canephora* and the arabica strain CAB007 is specific of *Coffea arabica*. In opposite the historical strains collected in CAR, Ivory Coast and Guinean are not specific of one Coffea species. The hypothesis is once again put forward, that the contemporary population present on *C. canephora* in DRC, Uganda and Tanzania could be derived from a strong foundation effect that counter-selected a specific isolate of the *C. canephora* species. That result clearly shows that the contemporary strains of *Fusarium xylarioides* have an evolutionary potential that could evolve towards the acquisition of complementary virulence that might prove pathogenic on the other species of *Coffea*.

3-Observations on field resistance have revealed different levels of susceptibility, with highly variable latency periods (1 to 24 months), suggesting the establishment of different quantitative defence mechanisms, and interactions with environmental conditions, and have led to the identification of two *C. canephora* genotypes (J1/1 and Q3/4) that are totally resistant to CWD

In addition, heritability of CWD resistance has been calculated at 50-60% mortality, (i) on full-sib progenies in a half diallel in the field, exposed to a natural inoculum, and (ii) on cuttings and progenies derived from open pollination, inoculated artificially under controlled conditions. Broad sense heritability of 0.3 was observed in the different trials analysed. The general combining abilities (GCA) are most significant for disease tolerance. It will therefore be possible to integrate the two resistant genotypes, J1/1 and Q3/4, with the wild Kibale and Itwara origins in breeding programmes to create tolerant varieties. However, these clones are going to be assessed right away in zones with high CWD pressure and in different agro-ecological contexts.

4- The analysis of *C. canephora* genetic diversity in Uganda focused on different wild coffee tree populations derived from surveys in the primary forests of Kibale and Itwara, a population of feral coffee trees from the Kalangala islands and populations of locally cultivated coffee trees, compared to the genetic groups described for this species (Dussert, 2003). The study with microsatellite markers made it possible to separate the populations into three groups. The populations of wild Ugandan coffee trees form a new genetic group, which is different from the groups known to date. The genetic diversity of those wild populations reveals a new source of genes likely to be used in future programmes to improve existing commercial varieties, especially if they harbour genes of resistance to CWD. Faced with the threat of CWD, and the discovery of diseased wild coffee trees, effective conservation of those resources "*in situ*" is not guaranteed and should be completed by placing them in an "*ex situ*" conservation collection in different national or international conservation centres.

In DRC, an analysis of the genetic diversity of a set of coffee trees from the Kiyaka station, whose genetic origin has not been formally identified, has revealed, with the help of the RAPD technique, quite substantial genetic diversity. This suggests a pool of genes that could be used in future selection programmes.

5- The spatial dynamics of the disease, between April 2001 and March 2006, studied in an experimental plot at Kituza in Uganda, is described by non-linear regressions and the logistical model fits those dynamics well. The spatial evolution of the disease has been analysed with geostatistical tools. The semi-variograms indicate the beginning of an epidemic in foci, i.e. there are spatial correlations on the scale of the plots studied. The size of the foci increases, and they merge to form a continuous set of diseased coffee trees interspersed with groups of resistant coffee trees. From the diseased trees, the spread can be in all directions, infecting neighbouring trees up to a distance of around 10 m. In order to effectively control the disease, especially when up rooting, it is necessary to up root the nearest neighbours of the infected tree, in a radius of at least ten metres. This up-rooting operation is economically feasible if the attack rate in the plot is under 10%.

COWIDI Scientific annual report

Year 5: November 2005 – October 2006

Introduction

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The consideration of the status of *C. arabica* is essential, because to develop resistance strategies it's necessary to cumulate résistance genes to the two forms of the pathogen. At present time, every form of *Fusarium xylarioides* is species specific. But some indicators draw to think that the evolutionary potential of the fungus can drive to emergence of new virulence with no specific species reaction

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WP1: Pathogen diversity

Task 1 (CORI-UNIKIN with participation of CIRAD-CABI): Survey and collection of anamorphic and teleomorphic forms of the fungus on various parts of trees, possibly on alternative hosts, in infested regions. Maintenance and dispatch to European labs.

During this period, partners continued to enrich the collection of *Fusarium xylarioides*. In Uganda, *F.xylarioides* has not been isolated from *C.arabica*.

Task 2: (CABI) Identification, storage, and exchange of isolates. Use a designed facility to ensure the successful maintenance of the pathogen strains. All data used to characterize the strains to be maintained on a database.

An extensive collection of nearly 300 strains of various *Fusarium* species were deposited at the CABI E-UK Centre, UCL and CIRAD for secure, long term storage. Most of these are the anamorphic form, *F. xylarioides*, were isolated from coffee plants affected by CWD, with identifications confirmed by CABI mycologists. The collection also includes other recognised pathogens of coffee such as *F. stilboides*, *F. lateritium*, *F. solani* and *F. oxysporum*, which were included in a number of the characterisation studies completed at CABI and elsewhere.

The input of data into the electronic database, including that for newly acquired strains continued. The database now holds comprehensive information relating to nearly 300 strains held by CABI, UCL, CIRAD and African partners.

Task 3 (CORI-UNIKIN CIRAD-UCL): Evaluation of the variability in isolate aggressiveness using standard inoculation tests.

The comparison of aggressiveness between MAT1 and MAT2 strains carry out in UNIKIN don't allow to conclude on a differential aggressiveness between the mating type.

Task 4: (CIRAD, CORI, UNIKIN UCL) Description of the fungal life cycle, asexual and sexual phases.

Perithecia production varied with the clone and climatic conditions. Under field conditions in Uganda, during the dry period (December – March) perithecia are not formed, but appear 2 or 3 days after rain. However, off-season rains can also trigger formation of perithecia in the field. At Kituza, off-season rains are not rare, which means in a coffee field there is normally a lot of inoculum.

Task 5 (CIRAD-CABI-UCL): Evaluation of genetic diversity using a range of techniques including PCR and microsatellites.

At UCL, previous work involved the use of *MAT*-PCR to characterise 20 *C. canephora* associated strains of *F. xylarioides* from Equator Province (DRC) as mating type *MAT-1*. Crossing results have shown preliminary *MAT*-PCR results to be correct, with all 20 strains from Equator Province found to be mating type *MAT-1*. Also described in previous reports was the method for *in vitro* production of perithecia between compatible *C. arabica*-associated strains (Biological Species 1, BS1) and *C. canephora*-associated strains (BS2). BS1 and BS2 by definition are reproductively isolated from one another and other populations. Recently a third category has been identified, BS3, in a cross between BBA 62457 (*C. excelsa* *MAT-2*) and ATCC 15664 (*Coffea* sp. *MAT-1*). The perfect state between these two strains was achieved and 10 ascospores were isolated. Of these 10 progeny two appear to be highly fertile, similar to the female parent BBA 62457 and the other eight resembled the male parent (the 10 progeny were backcrossed with both parents for *MAT* identification but due to time restraints and lack of funding the study could not be completed). A fourth group became apparent comprising four strains originating from Central and West Africa. All of these strains appeared to be incompatible with each other and members of the BS1-BS3. These four strains have been assigned to a residual sterility group (SG4). In conclusion it would appear that what is referred to as a single species is in fact at least four distinct reproductively isolated populations/biological species and should perhaps be referred to as the *G. xylarioides* complex. *MAT* sequencing results also confirmed the presence of four distinct clades corresponding to the BS/SG groups. Analysis of the left idiomorph flanking region revealed the presence of a 60bp region containing mating type-specific sequences common to both *G. xylarioides* and the *G. indica* complexes. Surprisingly, *C. arabica*-associated *G. xylarioides* *sensu lato* *MAT-1* strains IMI 375907 and BBA 62458 possess the sequence type for *MAT-2* strains in this region. It may be useful to note that the *G. xylarioides* *MAT* idiomorph primers are able to successfully amplify corresponding regions not only in strains belonging to the complex but also others in the *Gibberella fujikuroi* complex and may be used as a tool to identify undescribed cryptic species of *G. xylarioides* and closely related species.

At CIRAD, genetic variability was assessed among 182 single-spore derived strains collected from all the geographical zones affected by Coffee Wilt (DRC, Uganda, Tanzania, Ethiopia) and several different coffee species (*C. canephora*, *C. arabica*, *C. sp*). Included in these strains were several 'historical' strains collected during the first emergence in the 1950-1960s. Strains of *F. stilboides* and *F. decemcellulare* were also included in this study (materials and methods developed for DNA extraction, the 11 microsatellite markers and PCR amplification have all been described in previous reports). Analysis of the dendrogram revealed four distinct groups belonging to the species *F. xylarioides* and a 5th group made up of other species of *Fusarium* strains comprising strains as follows:

The Canephora strains group: Strains isolated from coffee trees belonging to the species *C. canephora*, in DRC, Uganda and Tanzania during the contemporary period between 1997 and 2002 (except RDC001, collected by Meyer in 1962 in DRC). The totality of that group is 100% uniform for the 11 microsatellite markers.

West African group: Two historical strains collected in Ivory Coast and Guinea, which are identical.

Central African group: Two strains collected during the 1st emergence of the disease. DSMZ 62457 from CAR was collected from *C. excelsa*. The origin of ATCC15664 is unknown.

The Arabica strains group: Strains collected between 1971 and 2002 from *C. arabica* in Ethiopia. These strains are identical.

Fusarium spp. group: Reference strains of different species of *Fusarium* and strains isolated from diseased coffee trees, present as saprophytes or secondary pathogens not responsible for Coffee Wilt. It was noted that strain ATCC 36325, mistakenly identified as being *F. xylarioides*, does not belong to this species.

Task 6 (CABI): Synthesis of the results of all above tasks in order to propose an explanation for the evolution of the fungus

The different techniques used by UCL, CABI and CIRAD to study pathogen diversity concord in their results and reveal 4 groups of *Fusarium xylarioides* strains. The lack of a sufficient sample of strains from this period of initial disease emergence has led to hypotheses being put forward as to the origin of the disease

The origin of the 'historical' strains still is unclear, as CWD does not appear to have been endemic in Guinea or Ivory Coast. Both regions were surveyed at the end of the 19th century and beginning of the 20th century for the collection of wild coffee trees when there were numerous transfers of wild material from forests to plantations. Coffee growing was the subject of intense activity and surveillance over that period. Therefore, if the disease was present at that time and causing problems it probably would have been reported. CWD was first reported in Ivory Coast in 1950 by Delassus and in Guinea by Conakry in 1958. A likely hypothesis is of an accidental introduction due to exchanges of planting material in the French-speaking zone from CAR. These two historical strains may be considered to be individuals representative of the original diversity in CAR. Strain MUCL 14186, collected in 1960, is a historical sample from the first emergence of the disease in DRC. The presence of the disease in RDC was considered to be an accidental introduction linked to planting material exchanges. When considering the historical strains, three groups stand out – one representing the Ivory Coast and Guinea populations, a second representing CAR and a third representing the disease in DRC (strain RDC001). If it is considered that the centre of origin of the disease is CAR and that the disease was due to accidental introductions during the first emergence between 1940-1960s in the other countries, then these historical strains can be considered to be strains representative of the original diversity of the *G. xylosteoides* population. The strains collected from *C. canephora* in DRC, Uganda and Tanzania were identical as were the *C. arabica* group collected in Ethiopia. The absence of diversity shown using the microsatellite markers studied raises the question of the role of the sexual cycle of this fungus. Abundant perithecia occur under natural conditions and this fungus is also heterothallic having two mating types MAT1 and MAT2. As those mechanisms are sources of recombination, the reason for the very low genetic diversity observed raises further questions

Promotion/dissemination outputs

Full details of promotion and dissemination outputs produced as a result of, or relating to, the work undertaken under Work Package 1 are available in the 'Individual Partner Final Report' produced by every partners. These outputs include journal publications, oral presentations, poster presentations, reports and other related publications and articles.

WP1 : Pathogen diversity : Milestones

Achievements

Task 1.

Collection of samples from the different CWD zones of the partner is virtually finished.
Long term keeping is operational.

Task 2

Development of an Excel electronic data base available for each partner

Task 3

Confirmation of the variability in isolate aggressiveness.

Task 4

Confirmation: *G. xylosporus* is a heterothallic fungus

In vitro crosses of *F. xylosporus* isolates, confirm that the progenies can indeed arise through sexual recombination.

F. xylosporus alone induce symptoms characteristics of CWD

Task 5

Genetics markers MAT1, MAT2, TEF, CL, H3 permit to separate *F. xylosporus* isolates and others *Fusarium* sp.

These results allow to distinguish 5 groups of strains within *Fusarium xylosporus* and the proposition of a *Giberella xylosporus* complex, and their placement within the *Giberella fujikiori* species complex.
Identification of *F. xylosporus* in Uganda primary forests.

Task 6

Confirmation of a fungus specialisation to the host species

Confirmation of sexual type that can origin recombination mechanism

Definition of the *Giberella xylosporus* complex

Clarification of the taxonomic structure within the *Giberella fujikiori* complex.

Still to be done

Task 1 to task 6

Ended

WP2 - Host/Pathogen interaction

Task 1 (CIRAD-UCL-CORI-UNIKIN) Identification of *Fusarium* isolates representing a wide range of host susceptibility/resistance by screening tests on seedlings both in Africa and in Europe using available germplasm.

Isolate ATCC36325 is not pathogenic, it does not belong to the species *F. xylarioides*. It was wrongfully described by Booth as being a female strain.

Historical isolates CBS74979 and CBS25852 confirmed their pathogenicity, inducing mortality on seedlings of *C. canephora*, and also proved to be pathogenic on the species *C. deweverei*, which is not the species from which those strains were collected.

The isolates ATCC15664 and DSMZ62457, isolated from *C. deweverei*, were pathogenic on both species, *C. canephora* and *C. liberica*.

These results show that the historical isolates tested had a broad host spectrum, and were able to induce mortality on young plants of the two species *C. canephora* and *C. deweverei* and isolate DSMZ62457 was pathogenic on the 3 species including *C. arabica*.

Isolate CAB003 was specific to the species *C. canephora*, and CAB007 on *C. arabica*

The host specificity of isolate present on *C. canephora* seems to be confirmed in the growing zones where both species lived side by side in DRC and Uganda, only *C. canephora* trees are affected. In Ethiopia in the Jima zone, at the EARO research station in a severely contaminated zone, only *C. arabica* trees are affected, *C. canephora* trees are unaffected.

This results show that the historical isolates had a broader host spectrum than the contemporary population represented by isolate CAB003 or CAB007.

The hypothesis is that the contemporary population present on *C. canephora* in DRC, Uganda and Tanzania could be derived from a strong foundation effect that counter-selected a specific isolate of the *C. canephora* species. That result clearly shows that the contemporary strains of *Fusarium xylarioides* have an evolutionary potential that could evolve towards the acquisition of complementary virulence that might prove pathogenic on the other species of *Coffea*.

Task 2 (CORI-UNIKIN-CIRAD) Conduct similar field inoculations in Africa to validate the inoculation method

In Uganda, *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 in screen house at Kituza and climatic chamber at CIRAD.

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*.

It was observed that traits/indicators of resistance to coffee wilt disease (% of dead plants, % of defoliation, AUDPC) 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.

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 and are convenient for preselection test.

Task 4 Combine all these observations to propose a hypothesis on the nature of the resistance

This result indicates that the "historical" *Fusarium* population, particularly DSMZ, displays quite a broad host spectrum, compared to the contemporary population, which seems restricted to specific reactions. We put forward the hypothesis that the contemporary population could have arisen from a strong foundation effect that counter-selected an isolate specific to the species *C. canephora*. This result also clearly shows that the contemporary strains of *Fusarium xylarioides* display a potential to evolve towards the acquisition of complementary virulence that could prove pathogenic on other *Coffea* species. The question is raised as to the existence of races within the species *Fusarium xylarioides*. For the moment, we do not have available the plant material needed to detect races, as it requires genotypes propagated by vegetative multiplication, or obtained by controlled pollination.

WP2 : Host/Pathogen interaction: Milestones

Achievements
Task 1 Confirmation of host specificity of contemporary canephora and arabica strains. No host specificity on DSMZ 62457
Task 2 Confirmation of correlation between field assessment resistance and artificial inoculation.
Task 4 Idem to WP1, task 6 Hypothesis of a fungus specialisation to the host species

Still to be done
Task 1 to 4 Ended

WP3 - Breeding for resistance

WP3 – Breeding for resistance

Task 1: Identify sources of resistance through field assessment

UGANDA

The ranking of 20 clones in the field trial at Kituza is similar to the previous years. Clone J/1/1 is resistant to coffee wilt disease and clone Q/3/4 had only 4.2% mortality. These clones are being propagated for further evaluation in other agro ecological conditions. The disease progress curves and the latent period are different and suggest that resistance is controlled by many genes.

Assessment of half sib progenies and rooted cuttings of nganda and erecta genotypes varied from 5% to 95% of mortality. Erecta progenies seems more resistant than nganda.

Task 2: Collect seeds and cuttings from representative genotypes of available germplasm

UGANDA

Transfert of plant material

Under agreements to transfer plant material, 22 unselected accessions from wild populations in Ivory Coast have been transferred to Uganda.

Genetic diversity of ugandan wild coffee

Representative genotypes of wild coffee from primary forests were analyzed using 24 microsatellite markers. The accessions were collected from 5 populations in Kibale forest and 6 populations of Itwara forest and from 4 populations in Kalangala islands forest (feral population). Representative genotypes of the known Guinean and Congolese *canephora* groups were included as controls. 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.

DRC

Analysis of the genetic diversity of 32 *canephora* coffee trees with RAPD and ISSR markers revealed quite extensive genetic diversity. This is the first work to have been undertaken on plant material originating from DRC. The preliminary results are promising, since they indicate the possibility of identifying agronomic or disease resistance traits that can be used in a selection scheme.

Task 3: Conduct screening tests in both Africa and Europe using isolates with a wide range of aggressiveness.

UGANDA

There are significant genetic differences for resistance between different Ugandan *C. canephora* populations (Kalangala islands, Itwara and Kibale primary forests, Nganda and Erect phenotypes). 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.

Task 4 Prepare and establish multilocal field trials with tolerant varieties.

UGANDA

Among genotypes studied, clone J1/1 and Q3/4 was highly resistant. 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 Q3/4 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 R1/4 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

Task 5 Analysis of the inheritance of the resistance.

UGANDA

Analysis of disease data collected from *C. canephora* clones and half diallel progenies in field trials at Kituza generated the information on resistance and inheritance of the resistance to coffee wilt disease in *C. canephora*. The inheritance was calculated from the different experiment at a plant mortality of 50-65%, given that the resistance was quantitative. Broad sense heritability calculated from the disease data of the half diallel progenies was moderate (0.329 and from the clones in the field was also moderate (0.333). Narrow sense heritability calculated from the half diallel progenies was low (0.112) and narrow sense heritability estimated by regression of half sib progeny means onto parent means in the field and screen house were moderate (0.183 and 0.369 respectively).

Task 6 Propose a breeding strategy towards durable resistance

UGANDA

This findings show that CWD resistance is heritable and therefore progenies of crosses between susceptible and resistant progenitors can have better resistance than their susceptible parents. Thus resistance of Uganda current commercial clones, which are susceptible to CWD, can be improved by hybridising with the resistant genotypes. However because *C. canephora* is predominantly out breeding and genotypes are heterozygous, progenies of these crosses are expected to be heterogeneous, with variable levels of CWD resistance and other required agronomic traits. Individual progeny trees can be evaluated and before selecting superior genotypes for propagation as commercial clones.

Therefore crossing specific CWD resistant clones with the current commercial clones has been initiated so as to combine the CWD resistance with high yields (2.5 tons of clean coffee per hectare per annum), good bean qualities (18-22g hundred beans weight, over 90% retained by screen 18/64) and good cup qualities of the commercial clones. Progenies of these crosses will be evaluated in on-station field trials as individual trees for all required traits (yield, liquor and physical/bean qualities and resistance to CWD and other major coffee diseases) equally and superior genotypes will be selected for release to farmers as clonal varieties. It is anticipated that selection of new varieties through this process will take up 20 years.

WP3: Breeding for resistance: milestones

Achievements

Task 1

Identification of various levels of field resistance (clonal trial CORI)
 Identification of resistant clone J1/1 and Q3/4 (clonal trial CORI)
 Vegetative multiplication of "survivors" plants resistant after double artificial inoculation in a mother garden (CORI)
 Dispatching in various hot spots of J1/1 and Q3/4, to confirm resistance

Task 2

Genetic analysis using microsatellites markers of wild Ugandan coffee germplasm and RAPD markers in DRC
 Confirmation of a new genetic group of Ugandan C. canephora different from other known groups

Task 3

Identification of resistant progenies (CORI, CIRAD, UNIKIN)
 Screening of seeds and cuttings of wild coffee by artificial inoculation
 Vegetative multiplication of "survivors" for further evaluation in field trials

Task 4

Assessment of field resistance of some clones in various geographical areas, in progress.

Task 5

Evidence of inheritance of CWD resistance.

Task 6

Confirmation on the existence of a Ugandan group of forest coffee trees.
 Hypothesis of different genetic factors controlling the CWD resistance.

Still to be done

Task 1 to 6

Ended

WP4 - The disease: epidemiology

Task 3. CORI, UNIKIN): Define the conditions conducive to the appearance of the sexual phase and its importance in the spread of the disease.

Perithecia production varied with clones. Susceptible clone 1s/2 supported more perithecia than the rest of the clones. Perithecia were rarely produced at the top grid, while perithicia were formed in abundance at the bottom grid in all clones.

Under field conditions during the dry period (December – March) perithecia are not formed, but appear 2 or 3 days after rain. However, off-season rains can also trigger formation of perithecia in the field. At Kituza, off-season rains are not rare, which means in a coffee field there is normally a lot of inoculum. Similarly, discarded dry seedlings or inoculated plants do not form perithecia unless watered. It is significant to note here that the recommendation for farmers to uproot infected plants at the earliest symptoms is meant to eliminate all sources of infection. However, many farmers do not uproot but cut plants down leaving stumps. Perithecia therefore continue to be formed on the remaining stumps and infection is spread to plants within the field or at distances away

Task 4 (CORI,UNIKIN): Evaluation of the duration of survival form of the pathogen.

Different experiments imply that the pathogen can persist in soil and become source of inoculum. The duration of survival in the soil stay unclear.

Stored infected wood pieces continued to reveal presence of *F. xylarioides* for 6 months.

Task 5 Elaboration of a simpifie model of the epidemic

UGANDA

Coffee wilt disease development in naturally infected *Coffea canephora* fields at the Coffee Research Institute 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.

Achievements

Task 3 & 4

Confirmation of the survival of the fungus during 6 months in the infected wood.
Confirmation of the survival of the fungus in the soil, duration unknown.

Task 5

Description of the role of rainfall in the appearance of visible symptoms
A statistical analysis and graphic representation method has been adopted
The first results raise the hypothesis of disease spread from tree to tree

Still to be done

Task 1 to 6

Ended

Outline Plan Year 5

Tasks	Partners	
1-1 Survey and isolate collection	CORI UNIKIN	Ended Ended
1-2 Identification, storage and exchanges of isolates	CABI	Ended
1-3 Evaluation of aggressiveness	UNIKIN-CIRAD	Ended
1-4 Description of the fungus cycle	UCL-UNIKIN-CORI	In progress
1-5 Evaluation of genetic diversity by RAPD and microsatellites	CABI CIRAD	Ended Ended
1-6 Synthesis of the results	CABI	
2-1 Identification of isolates representative of genetic diversity and aggressiveness	CIRAD CORI UNIKIN	Ended Ended Ended
2-2 Field inoculations in Africa	CORI CORI UNIKIN	Planting "survivors" coffee plants in infected soils. Ended Dispatching of resistant clones in different areas. Ended Dispatching of seeds from resistant clones in different areas. Ended
2-3 Cyto-histological study, types of reaction	CIRAD	Ended
2-4 Hypothesis on the nature of resistance	CIRAD-CORI	Ended
3-1 Identification of sources of resistance in the field and wild forest	CORI UNIKIN	Ended Ended
3-2 Collect and dispatch seeds and cuttings	CORI UNIKIN	Ended Ended
3-3 Screening tests on seedlings and cuttings in Africa and Europe	CORI-UNIKIN-CIRAD	Ended
3-4 Multi-locational trials with tolerant varieties	CORI-UNIKIN	Ended
3-5 Analysis of the heritability of the resistance	CORI-CIRAD	Ended
3-6 Proposition for a breeding strategy towards durable resistance	CORI	Ended
4-1 Identification of sites representative of the epidemic	UNIKIN-CORI	Ended
4-2 Description of the spatio-temporal distribution of the disease in plantations	UNIKIN	Ended
4-3 Definition of the conditions conducive to the sexual phase	CORI-UCL	Ended
4-4 Evaluation of how and how long the pathogen survives	CORI-UCL	Ended
4-5 Elaboration of a simplified model and proposition of recommendations	UNIKIN	Ended
5-1 Organisation of workshop	CIRAD	Ended
5-2 Reporting and financial and scientific coordination	CIRAD	Ended
5-3 Missions	CIRAD	Ended
5-4 Final recommendation for a sustainable management of CWD resistance	CIRAD	Ended

MANAGEMENT ANNUAL REPORT

Organisation of the collaboration

In this fifth year, efforts have been focused on making use of the results acquired over the previous years and continuing with field trials and laboratory experiments.

A considerable effort has been made to sum up the situation, as part of thesis work undertaken during the project and finalized in December 2006, (thesis by Pascale Lepoint, UCL) and March 2007 (Thesis by Pascal Musoli, NARO). These two theses were defended outside the project period, but are a COWIDI project asset. This work involved strong, ongoing exchanges with all the partners.

In this final year, work or student hosting by the different partners were largely funded from participants' own resources, which very clearly shows the considerable commitment of the participants and their will to succeed.

Organization of the final meeting on 13 September in Montpellier with all the partners provided an opportunity to pool all the results and to present conclusions and the advances made by the project.

Meetings

Workshop final

13/09/2006. CIRAD - UNIKIN - NARO – UCL – CABI. Montpellier. Le meeting a regroupé l'ensemble des chercheurs ayant participés au projet.

Exchanges - Trainings

UCL

Thesis formation, "Etude de la variation de *G. xylophagoides* au cours de son cycle sexuel et/ou parosexuel" third year, Mrs Pascale Lepoint.

CIRAD – CORI

Thesis formation, third year, Montpellier University, M. Pascal Musoli.

Thesis: Use of genetic resources for resistance to control wilt disease, *Fusarium xylophagoides* of *Coffea canephora*.

Training in CIRAD: april 2006 to December 2006,
 January 2007 to March 2007.

Problems

Inexcusable delays in the submission of financial and scientific reports, which consequently prevented the release of funding.

OUTPUT

CIRAD

Internal report

BIEYSSE, D. (2006). Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. Fifth Annual Report, 1 November 2005 - 31 October 2006. INCO-DEV contract ICA4-CT-2001-10006. May 2007. Montpellier, CIRAD-BIOS.

Publications

RUTHERFORD, M.A., HAKIZA G., ABEBE M., ADUGNA G., KILAMBO D., BIEYSSE D. (2006) Epidemiology and variability of the coffee wilt pathogen. pp 42-44 in: Sweetmore,A., Kimmins, F. and Silverside, P. (eds). *Perspectives on Pests II: Achievements of research under UK Department for International Development Crop Protection Programme 2000–05*. Natural Resources International Limited, Aylesford, UK. 260+xvi pages. ISBN: 0-9546452-7-8

Participation to congress

PINARD Fabrice. CORNET Coffee Wilt meeting. Nairobi , Kenya. December 2005.

Bieysse, D., Lepoint, P., Hakiza, G., Kalonji, A., Tshilenge, P., Janzac, P., Roussel, V., Maraite, H. and Rutherford, M.. Coffee Wilt Disease: A Major Constraint To Coffee Production In Africa. European Fusarium Seminar 19-22 september 2006. Wageningen. The Netherlands

Bieysse, D¹., Lepoint, P²., Hakiza, G³., Kalonji, A⁴., Tshilenge, P⁴., Munaut, F⁵., Janzac, P¹., Roussel, V¹. Maraite, H^{2,5}. and Rutherford, M. Coffee Wilt Disease: a major constraint to coffee production in Africa. 9th European Fusarium Seminar (EFS9), 19-22 September 2006, Wageningen, The Netherlands.

Others

Transfert of wild coffee from Côte d'Ivoire to Uganda with quarantine period in CIRAD.

UCL

Internal Reports

LEPOINT, P. (2005). Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. Annual report for Work Package 1-, November 2005-31 October 2006. INCO-DEV contract ICA4-CT-2001-10006. April 2006.

Publications

LEPOINT, P. C. E., MUNAUT, F. T. J. and MARAITE, H. M. M (2005) *Gibberella xylospora* sensu lato from *Coffea canephora*: a new mating population in the *G. fujikuroi* species complex. *Applied and Environmental Microbiology* **71** (12), 8466-8471.

Thesis

Lepoint, P.C.E. December 2006. Speciation within the African coffee wilt pathogen. Thesis. Université catholique de Louvain. 208pp.

Journal articles submitted or in preparation

Lepoint, P.C.E. Characterization of the *MAT1-1* and *MAT1-2* idiomorphs in the *Gibberella xylospora* and *Gibberella indica* Species Complexes (*In preparation for Fungal Genetics and Biology*).

Lepoint, P.C.E., C.A. Decock, F.T.J. Munaut, and H.M.M. Maraite. Speciation within the *Gibberella xylospora* and *Gibberella indica* Species Complexes (*In preparation for Fungal Genetics and Biology*).

Lepoint, P.C.E., C.A. Decock, F.T.J. Munaut, and H.M.M. Maraite. Neotypification of *Gibberella xylospora* (*Fusarium xylospora*) (*In preparation for Taxon*).

Others

Deposit of referenced strains in the mycotheque MUCL

CORI

Internal Reports

HAKIZA, G. (2006). Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. Annual report for Work Package 1 and 2 November 2005-31 October 2006. INCO-DEV contract ICA4-CT-2001-10006. May 2007. Kitusa, Uganda, Coffee Research Institute.

MUSOLI, P. (2006). Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. Annual report for Work Package 3 and 4, November 2005-31 October 2006. INCO-DEV contract ICA4-CT-2001-10006. May 2007. Kitusa, Uganda, Coffee Research Institute.

Thesis

MUSOLI Chungason Pascal, 2007. Recherche de sources de résistance à la trachéomycose du caféier *Coffea canephora* Pierre, due à *Fusarium xylospora* Steyaert en Ouganda. **Docteur en Sciences, de l'Ecole Nationale Supérieure Agronomique de Montpellier**

Journal articles submitted

P. Musoli , P. Cubry , P. Aluka , C. Billot , M. Dufour , F. De Bellis , D. Pot, D. Kyetere , J. Ochugo, D. Bieysse , A. Charrier, T. Leroy . Genetic differentiation of wild and cultivated populations: diversity of *Coffea canephora* in Uganda. Submitted to Molecular Ecology.

Pascal C. Musoli, Christian Cilas, David Pot, Agnes Nabaggala, Saleh Nakendo, James Pande, André Charrier, Daniel Bieysse, Thierry Leroy. Inheritance of resistance to coffee wilt disease (*Fusarium xylospora* Steyaert) in *Coffea canephora* Pierre. Submitted to New phytologist Journal

Pascal C. Musoli, C. Cilas, A. Charrier, A. Kangire, G.M. ten Hoopen, C. Kabole, J. Ogwang¹ and D. Bieysse. Spatial and temporal analysis of Coffee Wilt Disease caused by *Fusarium xylospora* Steyaert in *Coffea canephora*. Submitted to European Journal of Phytopathology

Oral presentations

Pascal Musoli, Pauline Aluka, Philippe Cubry, Magali Dufour, Fabien de Bellis, James Ogwang, Denis Kyetere, Thierry Leroy, Daniel Bieysse, André Charrier. Fighting coffee wilt disease: Uganda wild *C. canephora* genetic diversity and its usefulness. ASIC 2006. 21st International Conference on Coffee Science, Montpellier, France

Others

Carry out of a mother garden with new resistant plant material in Kituza and different part of Uganda.
 Identification of resistant commercial clones
 Evaluation of field resistance and agronomic factors in different agroecological conditions of the identified plant material.

UNIKIN

Internal Reports

KALONJI A., TSHILENGE P. (2005). Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. November 2005-31 October 2006. INCO-DEV contract ICA4-CT-2001-10006. May 2007.

Congress-Meetings

Regional Coffee Wilt Programme- Annual Review & Planning Meeting : Mainstreaming the Prevention & Management of Coffee Wilt Disease in Government Policy in Africa. 8th- 9th December 2005, CABI-ARC, Nairobi, Kenya

4th African fine coffee Conference and exhibition, 15-17 february 2007. Addis Ababa, Ethiopia.

Final meeting on INCO Projects Coffee Wilt Disease (COWIDI), 9-10 septembre 2006, Montpellier (France).

Association Scientifique International du Café (ASSIC) '21st International Conférence on Coffee Science. 11-15 september 2006- Montpellier- France.

CABI

Oral presentations

RUTHERFORD, M., J. CROZIER, A. BUDDIE, J. INESON, S. LEA and J. FLOOD. (2005). Coffee Wilt Disease. Presented at the Regional Coffee Wilt Stakeholders Workshop, Nairobi, Kenya. 8-9 December 2005.

RUTHERFORD, M. (2006). CABI E-UK inputs to EU INCO-DEV funded coffee wilt disease project. Final project review workshop, CIRAD, Montpellier, 13 Sept. 2006 (presented by M. A. Rutherford, CABI E-UK).

Publications

RUTHERFORD, M. (2006). Current knowledge of coffee wilt disease, a major constraint to coffee production in Africa. *Phytopathology* **96**, 663-666

GIRMA, A.S., FLOOD, J., HINDORF, H., BIEYSSE, D., SIMONS, S. and RUTHERFORD, M. (2006). Tracheomycosis (*Gibberella xylospora*) - a menace to world coffee production: evidenced by cross inoculation of historic and current strains of the pathogen. *Proceedings of the 21st International Scientific Conference on Coffee Science (ASIC)*, 11-15 September 2006, Montpellier, France. pp 1268-1276.

RUTHERFORD, M.A. (2006) Epidemiology and variability of the coffee wilt pathogen. pp 42-44 in: Sweetmore,A., Kimmins, F. and Silverside, P. (eds). *Perspectives on Pests II: Achievements of research under UK Department for International Development Crop Protection Programme 2000-05*. Natural Resources International Limited, Aylesford, UK. 260+xvi pages. ISBN: 0-9546452-7-8

Internal Reports

RUTHERFORD, M. A. and CROZIER, J. (2006). Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease (CWD). Final Partner Report for CAB International, 2002-2006. INCO-DEV contract ICA4-CT-2001-10006. Ascot, CAB International.

Others

Development of a Fusarium Xylarioides database
Long term preservation nitrogen liquid of 60 isolates (CABRI)

Annexe 2**Contract number : ICA4-CT-2001-10006****Year 5****Data sheet
for annual report**

(To be compiled by the co-ordinator at 12-monthly intervals from start of contract. Figures to be up-dated cumulatively throughout project lifetime)

1. Dissemination activities

Totals (cumulative)

Number of communications in conferences (published)	15
Number of communications in other media (internet, video,)	1
Number of publications in refereed journals (published)	4
Number of articles/books (published)	2
Number of other publications	11

2. Training

Number of PhDs	2
Number of MScs	
Number of visiting scientists	6
Number of exchanges of scientists (stays longer than 3 months)	1

3. Achieved results

Number of patent applications	
Number of patents granted	
Number of companies created	
Number of new prototypes/products developed	
Number of new tests/methods developed	
Number of new norms/standards developed	
Number of new softwares/codes developed	
Number of production processes	

4. Industrial aspects

Industrial contacts	no
Financial contribution by industry	no
Industrial partners : - Large	no
- SME 1	no

S. Comments

Other achievements (use separate page if necessary)

' Less than 500 employees.

CIRAD

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

Fifth Annual Report (November 2005 to October 2006)

**CIRAD- BIOS
UMR BGPI
TA A 54/K
Campus International Baillarguet
34 398 Montpellier Cedex 5
France**

WP1: Pathogen diversity

Task 5 . Evaluation of genetic diversity by the microsatellite approach

Material and methods

Fungal strains and cultures

A collection of 182 single-spore strains collected from all the geographical zones affected by Coffee Wilt (DRC, Uganda, Tanzania, Ethiopia), established from different coffee species (*C. canephora*, *C. arabica*, *C. sp.*), including strains collected during the first emergence in the 1950-1960s, has been studied. The other *Fusarium* strains used in the study (*F. stilboides*, *F. decemcellulare*) came from partners or different international fungal libraries and strains not belonging to *F. xylarioides* isolated from infected material during the project.

The material and method developed for DNA extraction, the description of the 11 microsatellite markers and the PCR amplification and electrophoresis protocols have been described in previous reports.

Molecular data analysis

A dissimilarity matrix was calculated according to the Sokal-Michener index: $D(i,j)=u / (m+u)$ ("u"= the number of identical characters between individuals "i" and "j", and "m"= the number of different characters between the same individuals). The DARWIN 4.0 program (CIRAD, France) was used to calculate a distance matrix and plot a dendrogram by the "weighted Neighbour-Joining" method. To determine the robustness of the dendrogram, the data underwent bootstrap resampling with 100 removals and replacements.

Results

An analysis of the dendrogram revealed a distinction between 4 groups belonging to the species *F. xylarioides* and a 5th group made up of other species of *Fusarium*.

The Canephora strains group

The strains belonging to this group were taken from coffee trees belonging to the species *C. canephora*, in DRC, Uganda and Tanzania during the contemporary period between 1997 and 2002, except for strain RDC001, collected by Meyer in 1962 in DRC. The totality of that group is 100% uniform for the 11 microsatellite markers.

West African group

This group comprises two historical strains collected in Ivory Coast and Guinea. They are strictly identical.

Central African group

This group comprises two strains collected during the first emergence of the disease, of which one, DSMZ 62457, comes from CAR collected from *C. excelsa*. For ATCC15664, the host and zone of origin are unknown.

The Arabica strains group

This comprises strains collected between 1971 and 2002 from *C. arabica* in Ethiopia. These strains are strictly identical.

The Fusarium sp. group

This group comprises the reference strains of different species of *Fusarium* and strains isolated from diseased coffee trees, present as saprophytes or secondary pathogens, not responsible for Coffee Wilt. Note strain ATCC 36325, mistakenly identified as being *Fusarium xylarioides* don't belong to this species.

Discussion

The polymorphism revealed with the microsatellite markers used is very weak for all the groups.

Genetic diversity of the historical strains

Central African group

Strains ATCC 15664 (unknown host and geographical origin) and DSMZ 62457 collected from *C. excelsa* in CAR are strictly identical. Isolate DSMZ 62457 is considered as representative of the original diversity of *C. liberica* in CAR.

West African group

The 2 "historical strains" collected in Guinea from *C. canephora* and in Ivory Coast from *Coffea* sp., are strictly identical, but differ from the previous 2 historical strains and from the contemporary strains of the Canephora group and Arabica group. What is the origin of these strains? Coffee Wilt does not appear to have been endemic in Guinea or Ivory Coast. Those two regions were surveyed as early as the end of the 19th/beginning of the 20th century for the collection of wild coffee trees and there were numerous transfers of wild material from forests to backyard plantations in the first half of the 20th century. Coffee growing was the subject of intense activity and surveillance over that period. If the disease had been present, it would probably have been reported. It was reported for the first time in 1950 by Delassus in Ivory Coast. It was reported for the first time in Guinea (Conakry) in 1958. For Ivory Coast and Guinea, we put forward the hypothesis of an accidental introduction because exchanges of planting material in the French-speaking zone from CAR were substantial over that period. These 2 historical isolates can be considered as individuals representative of the original diversity in CAR. At the moment, the existence of the disease has not been formally established in those two countries, recent surveys do not mention it.

Strain MUCL 14186, collected in 1960, is a historical sample from the first emergence of the disease in DRC. This isolate stands out from the 4 historical isolates previously described. The presence of the disease in RDC was considered to be an accidental introduction linked to planting material exchanges (Fraselles).

Thus, if we consider the historical samples, 3 groups stand out. A group representing the Ivory Coast and Guinea populations, a group representing CAR and a group representing the disease in DRC (isolate RDC001). If it is considered that the centre of origin of the disease is CAR and that the disease was due to accidental introductions during the first emergence between 1940-1960s in the other countries, then these historical strains can be considered to be strains representative of the original diversity of the *Fusarium xylarioides* population.

Genetic diversity of contemporary populations

Arabica strain

Diversity is linked to geographical origin but especially to the host species from which the strain was collected. The strains collected from *C. arabica* in Ethiopia are perfectly homogeneous.

Canephora strain

The strains collected from *C. canephora* in DRC, Uganda and Tanzania are identical. In the case of the strains collected in DRC, particular attention needs to be paid to strain RDC001, as that "historical" strain is identical to the contemporary strains with the markers studied. Thus, the notion of re-emergence of the disease in DRC could be re-discussed. The hypothesis could be put forward that a population of the pathogen survived at an unperceivable level since the beginning of the 1960s, up to its re-emergence in the 1970s, for reasons yet to be defined. A clone of that strain would be responsible for the re-emergence and current spread of the disease on *C. canephora* in DRC, Uganda, Tanzania.

The absence of diversity for the 11 satellite markers studied raises the question of the role of the highly present sexual cycle in the life cycle of the fungus. Abundant perithecia occur under natural conditions. Moreover, this fungus is heterothallic and has mating types MAT1 and MAT2. As those mechanisms are sources of recombination, the reason for the very low genetic diversity observed will have to be studied.

Conclusion

The dendrogram identifies 4 groups containing isolates belonging to the species *F. xylarioides*. The 5th group is made up of individuals not belonging to the species *F. xylarioides*.

This result backs up the conclusions obtained in the study conducted at UCL with markers Tef, cel, H3, which made it possible to define 4 groups of strains making up the *Giberella xylarioides* complex.

The two approaches are different, but lead to the same result, which makes it particularly relevant.

These results raise two questions to which no answer can really be given at the moment:

Is there a common ancestor and what is the original disease focus?

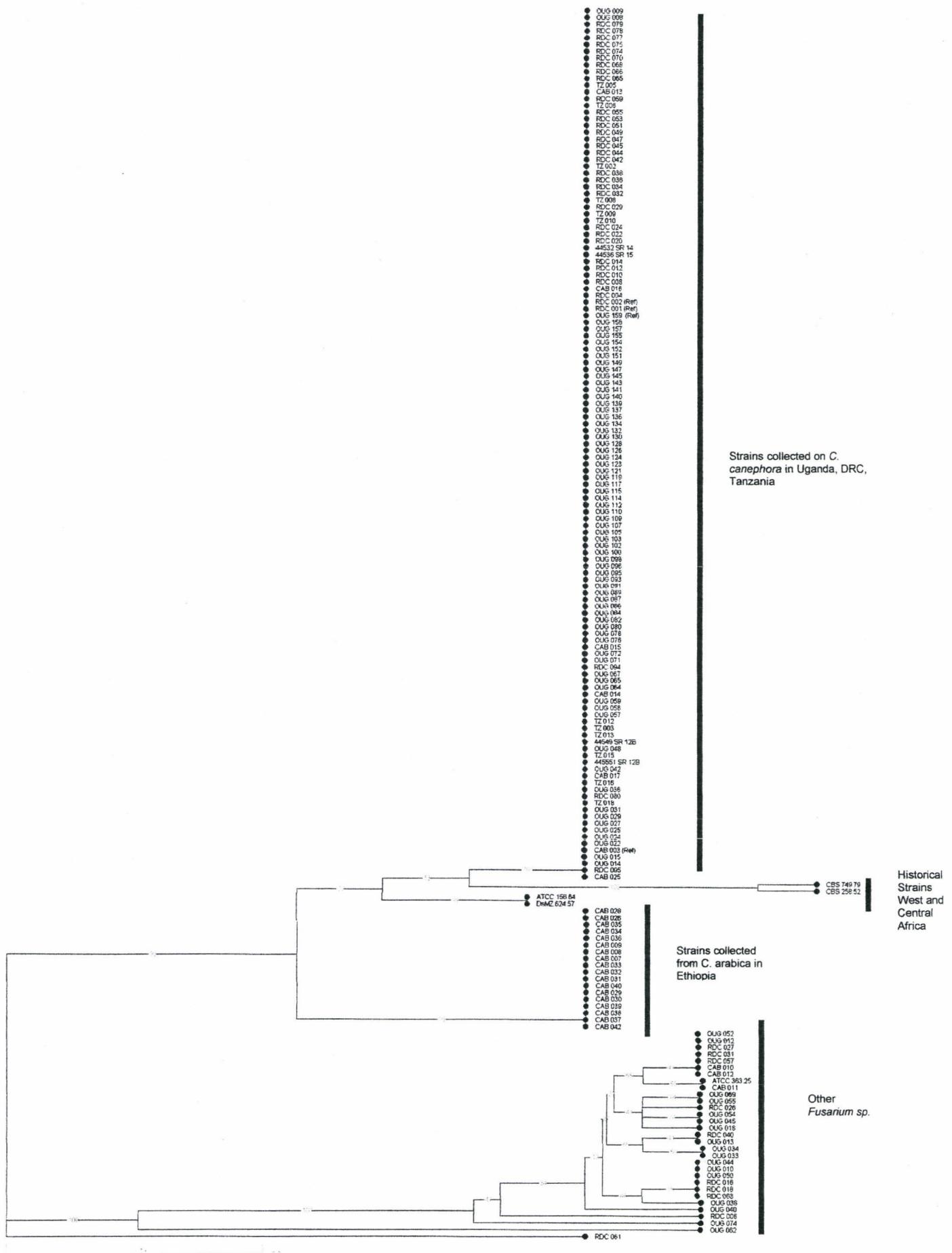


Fig 1. Unrooted Neighbour-Joining dendrogram obtained after calculation of distances according to the Sokal-Michener index from the polymorphism observed with 11 microsatellite markers.

Annex 1. Data on the isolates studied

Isolate	<i>Fusarium</i> species	Host species	Geographical origin	Collection year	Pathogenicity
OUG 008	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda	1997	+
OUG 009	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Hoima / Kiryangobe	2001	
OUG 010	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Hoima / Kiryangobe	2001	
OUG 012	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Hoima / Burindi	2001	-
OUG 013	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Hoima / Burindi	2001	-
OUG 014	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza	2001	
OUG 015	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza	2001	
OUG 018	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Kituza	2001	-
OUG 022	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Luwero	2001	+
OUG 024	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Luwero	2001	
OUG 025	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Luwero	2001	
OUG 027	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Luwero	2001	
OUG 029	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2001	
OUG 031	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2001	+
OUG 033	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Massaka	2001	+/-
OUG 034	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Massaka	2001	
OUG 036	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	+
OUG 038	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	
OUG 040	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	-
OUG 042	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	
OUG 044	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	
OUG 045	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	
OUG 048	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	
OUG 050	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2001	-
OUG 052	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 054	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Mubende	2001	-
OUG 055	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 057	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	+
OUG 058	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 059	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 062	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 064	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 065	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 067	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 069	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Mubende	2001	
OUG 071	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Jinja	2001	
OUG 072	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Jinja	2001	+
OUG 074	Other <i>Fusarium</i>	<i>C. canephora</i>	Uganda: Jinja	2001	-
OUG 076	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Jinja	2001	
OUG 078	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Jinja	2001	
OUG 080	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kiganda (Mubende)	2002	
OUG 082	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kiganda (Mubende)	2002	
OUG 084	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kiganda (Mubende)	2002	
OUG 086	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kiganda (Mubende)	2002	
OUG 087	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kiganda (Mubende)	2002	
OUG 089	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kiganda (Mubende)	2002	
OUG 091	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 093	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 095	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 096	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 098	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 100	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 102	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 103	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 105	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 107	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 109	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 110	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 112	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 114	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 115	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 117	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 119	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Mukono	2002	
OUG 121	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2002	
OUG 123	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2002	
OUG 124	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kyenjojo	2002	
OUG 126	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 1 s/2	2002	+
OUG 128	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 1 s/3	2002	+
OUG 130	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 1 s/6	2002	+
OUG 132	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 223/	2002	+
OUG 134	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 257/	2002	+
OUG 136	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 258/	2002	+
OUG 137	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial 258/	2002	+
OUG 139	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial C 1/7	2002	+
OUG 140	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial C 1/7	2002	+
OUG 141	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial C 1/7	2002	+
OUG 143	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial B 1/1	2002	+
OUG 145	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial L/2/7	2002	+
OUG 147	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial Q/3/4	2002	+
OUG 149	<i>F. xylarioides</i>	<i>C. canephora</i>	Uganda: Kituza Clonal trial B/6/2	2002	+

OUG 151	F. xyloso	Coffea. sp.	Uganda: -	2002	+
OUG 152	F. xyloso	Coffea. sp.	L	2002	+
OUG 154	F. xyloso	Coffea. sp.	Uganda: -	2002	+
OUG 155	F. xyloso	Coffea. sp.	Uganda: -	2002	+
OUG 157	F. xyloso	Coffea. sp.	Uganda: -	2002	+
OUG 158	F. xyloso	Coffea. sp.	Uganda: -	2002	+
OUG 159 (Ref)	F. xyloso	C. canephora	Uganda: Kituza	2002	+
RDC 001 (Ref)	F. xyloso	C. canephora	Rep. Of Congo: Yangambi	2001	+/-
RDC 002 (Ref)	F. xyloso	C. canephora	Rep. Of Congo: Isiro (Haut Zaire)	2001	+
RDC 004	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 006	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 008	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 010	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 012	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 014	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 016	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 018	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 020	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 022	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 024	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 026	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 027	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 029	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 031	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 032	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 034	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 036	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 038	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 040	Other Fusarium	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 042	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 044	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 045	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 047	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 049	F. xyloso	C. canephora	Rep. Of Congo: Oicha - Ngadi	2002	
RDC 051	F. xyloso	C. canephora	Rep. Of Congo: Mutwanga _ Bulongo	2002	
RDC 053	F. xyloso	C. canephora	Rep. Of Congo: Mutwanga _ Bulongo	2002	
RDC 055	F. xyloso	C. canephora	Rep. Of Congo: Itendi	2002	
RDC 057	Other Fusarium	C. canephora	Rep. Of Congo: Njiyapanda - Usando	2002	
RDC 059	F. xyloso	C. canephora	Rep. Of Congo: Muhanji	2002	
RDC 061	Other Fusarium	C. canephora	Rep. Of Congo: Masoya - Mweye	2002	
RDC 063	Other Fusarium	C. canephora	Rep. Of Congo: Masoya - Mweye	2002	
RDC 065	F. xyloso	C. canephora	Rep. Of Congo: Isiro - Modimbo	2002	
RDC 066	F. xyloso	C. canephora	Rep. Of Congo: Isiro - Modimbo	2002	
RDC 068	F. xyloso	C. canephora	Rep. Of Congo: Isiro - Muugbere	2002	
RDC 070	F. xyloso	C. canephora	Rep. Of Congo: Isiro - Nesu	2002	
RDC 074	F. xyloso	C. canephora	Rep. Of Congo		
RDC 075	F. xyloso	C. canephora	Rep. Of Congo		
RDC 077	F. xyloso	C. canephora	Rep. Of Congo		
RDC 078	F. xyloso	C. canephora	Rep. Of Congo		
RDC 079	F. xyloso	C. canephora	Rep. Of Congo		
RDC 080	F. xyloso	C. canephora	Rep. Of Congo		
RDC 094	F. xyloso	C. canephora	Rep. Of Congo		
RDC 095	F. xyloso	C. canephora	Rep. Of Congo		
TZ 002	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Rwensiga	2003	
TZ 003	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Rwensiga	2003	
TZ 005	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Nyahishenge	2003	
TZ 006	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Nyahishenge	2003	
TZ 008	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Karukwanzi	2003	
TZ 009	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Karukwanzi	2003	
TZ 010	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Karukwanzi	2003	
TZ 012	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Rutunguru	2003	
TZ 013	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo Rutunguru	2003	
TZ 015	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo -	2003	
TZ 016	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo -	2003	
TZ 018	F. xyloso	C. canephora	Tanzania: Kaisho/Murengo -	2003	
CAB 003 (Ref)	F. xyloso	C. robusta	Uganda	2000	+
CAB 007	F. xyloso	C. arabica	Ethiopia	2002	
CAB 008	F. xyloso	C. arabica	Ethiopia		
CAB 009	F. xyloso	Coffea sp.	Ethiopia		
CAB 010	F. decemcellulare	C. arabica	Ethiopia		
CAB 011	F. stilboides	C. arabica	Ethiopia		
CAB 012	F. lateritium	C. arabica	Ethiopia		
CAB 013	F. xyloso	C. canephora	RDC	1995	
CAB 014	F. xyloso	C. canephora	RDC	1996	
CAB 015	F. xyloso	C. canephora	RDC	1997	
CAB 016	F. xyloso	C. canephora	RDC	1998	
CAB 017	F. xyloso	C. canephora	RDC	1999	
CAB 025	F. xyloso	C. arabica			
CAB 026	F. xyloso	C. arabica			
CAB 028	F. xyloso	C. arabica			
CAB 029	F. xyloso	C. arabica			

CAB 030	F. xylarioides	C. arabica	Ethiopia-Bechi		
CAB 031	F. xylarioides	C. arabica	Ethiopia-Bechi		
CAB 032	F. xylarioides	C. arabica	Ethiopia-Tsanu		
CAB 033	F. xylarioides	C. arabica	Ethiopia-Tsanu		
CAB 034	F. xylarioides	C. arabica	Ethiopia-Gagi Bechano		
CAB 035	F. xylarioides	C. arabica	Ethiopia-Burusa		
CAB 036	F. xylarioides	C. arabica	Ethiopia-Wesheka		
CAB 037	F. xylarioides	C. arabica	Ethiopia-Wesheka		
CAB 038	F. xylarioides	C. arabica	Ethiopia-Fugosardo		
CAB 039	F. xylarioides	C. arabica	Ethiopia-(Kebele) Shonga		
CAB 040	F. xylarioides	C. arabica	Ethiopia-Ermichi		
CAB 042	F. xylarioides	C. arabica	Ethiopia-Kicho		
44532 SR 14	F. xylarioides	C. canephora			
44536 SR 15	F. xylarioides	C. canephora			
44549 SR 12B	F. xylarioides	C. canephora			
445551 SR 12B	F. xylarioides	C. canephora			
DsMZ 624.57	F. xylarioides	C. excelsa	Central African Rep.	1960s	+
ATCC 156.64	F. xylarioides	-		1960s	+
ATCC 363.25	F. stilboides	C. excelsa	Africa	1960s	-
CBS 258.52	F. xylarioides Gib	Coffea sp.	Ivory Coast.	1951	+
CBS 749.79	F. xylarioides	C. robusta	Guinea	1963	+

WP2: Host/Pathogen interaction

Task 1 Identification of isolates of *Fusarium xylarioides* representative of genetic diversity and aggressiveness

Two studies were undertaken this year.

1- Checking of the pathogenicity of isolates newly identified in the primary forests of Uganda.

The sequencing of ITS fragments of isolates collected from the primary forests of Uganda very clearly indicated that they belong to the species *Fusarium xylarioides*. It appeared important to check their host spectrum, to possibly establish a link with the historical isolates whose host spectrum extends to at least two species of *Coffea*. The trials were partially completed, due to a lack of *Coffea liberica* material available in sufficient quantities.

2- Analysis of *Coffea sp. / Giberella Fusarium* complex interactions

The analysis of interactions between isolates belonging to the *Giberella xylarioides* complex defined by Pascale Lepoint and different species of *Coffea* (*canephora*, *liberica dewevrei*, *arabica*) was continued.

The analysis of interactions was completed with work carried out at CIRAD in association with Pascal Musoli on genotypes from Uganda. The results are presented in the CORI section.

1-1 Material and methods

1-1-1 Plant material

Evaluation of the pathogenicity of "wild isolates" and the analysis of interactions was carried out on open pollinated seeds of *C. canephora* harvested from individual identified trees. In the text they will be called "progenies".

The *C. canephora* and *liberica dewevrei* seeds came from French Guiana and the *C. arabica* seeds from Costa Rica. The seeds were germinated in a climatic chamber at 25°C in darkness and transplanted at the soldier stage in the greenhouse on a substrate. The temperature was 25°C +/- 3°C with a relative humidity of 80%.

For all the trials, except trials A and B, the young 3-month-old plants were inoculated with a calibrated suspension (1.10^6 conidia/ml) by wounding under the cotyledon leaves. There were generally 20 plants per inoculation. In some rare case, 16 plants. For trials A and B, inoculation was carried out by injection on 9 to 12-month-old plants.

After inoculation the plants were kept in a climatic chamber at 25°, with a 12 h/12 h photoperiod. Symptoms were observed 100 days after inoculation.

1-1-2 Fungus material

Verification of the pathogenicity of "primary forest" isolates

The isolates chosen corresponded to those which had been confirmed to belong to the species *Fusarium xylarioides* by comparing ITS1 and P4 sequences with reference strain CAB003 (year 4 report). The isolates, OUG163, OUG164, OUG165, OUG166 came from the forest of Kibale, and isolates OUG182 and OUG184 from the forest of Itwara. The reference isolate was CAB003

Specific host/ pathogen interaction between *Coffea* sp. and isolates of the *Giberella xylospora* complex.

Interaction was studied between isolates representative of the *Giberella xylospora* complex and the three host species *C. canephora*, *C. liberica* and *C. arabica*.

Table 1. Plant host and geographical origin of the strains

Isolate	Host	Geographical origin
CAB 003	<i>C. canephora</i>	Uganda
DSMZ 62457	<i>C. liberica dewevrei</i>	CAR
CBS 25852	<i>C. sp.</i>	Ivory Coast
CBS 74979	<i>C. canephora</i>	Guinea
ATCC 15664	<i>C. liberica dewevrei</i>	Africa
ATCC36325	?	Africa
CAB 007	<i>C. arabica</i>	Ethiopia

Due to problems with synchronous availability of seeds and the very rapid loss of viability in *C. liberica* seeds, the inoculation dates were staggered in time and often in an incomplete design.

Table 2. Trials and description of the interactions tested

Strain	Trial						
	A	B	C	D	E	F	G
CAB 003	Canephora	Canephora Arabica		Canephora Liberica	Canephora Liberica Arabica	Canephora Liberica	
CBS 25852	Canephora					Canephora Liberica	Canephora Liberica
CBS 74979	Canephora					Canephora Liberica	Canephora Liberica
DSMZ 62457	Canephora		Arabica	Canephora Liberica	Canephora Liberica Arabica		Canephora Liberica
ATCC 15664	Canephora						Canephora Liberica
ATCC 36325	Canephora						
CAB 007		Canephora Arabica	Arabica	Canephora Liberica			

2- Results

2-1 Pathogenicity of "wild strains »

Table 3. Percentage of dead *C. canephora* plants inoculated with "wild strains" of *F. xylarioides*

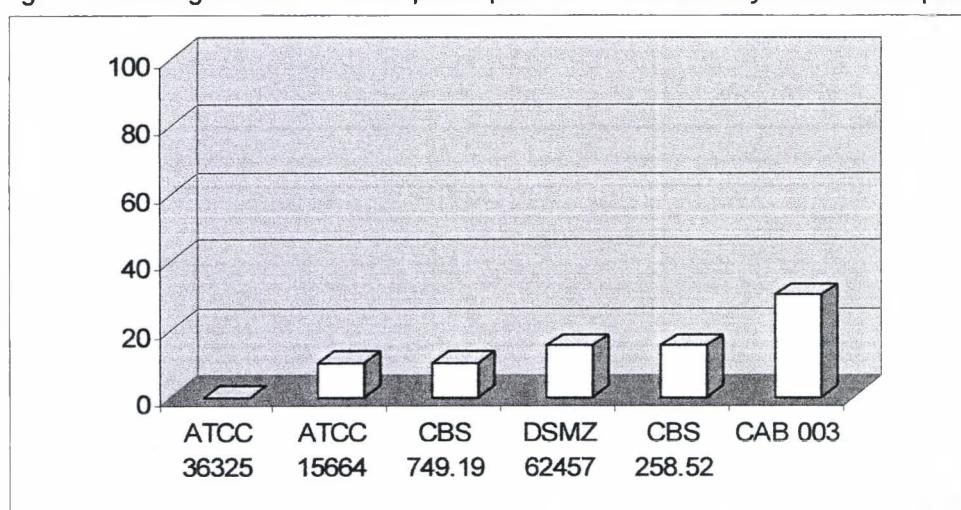
Isolate	Code	<i>Coffea</i> species	Nb. of plants	% of dead plants
OUG 163	1947	<i>C. canephora</i>	11	58
OUG 164			11	42
OUG 165			14	61
OUG 166			13	52
OUG 182			14	58
OUG 184			16	64
CAB003			14	78

The results indicate that all the wild isolates induced a mortality rate of between 42 and 64%. That rate was very similar to reference strain CAB003. These isolates indeed belong to the species *F. xylarioides*.

2-2 Analysis of *Coffea* sp. / *Giberella* *Fusarium* complex interactions

Trial A

Fig. 1. Percentage of dead *C. canephora* plants with *Giberella* *xylarioides* complex on *C. canephora*.

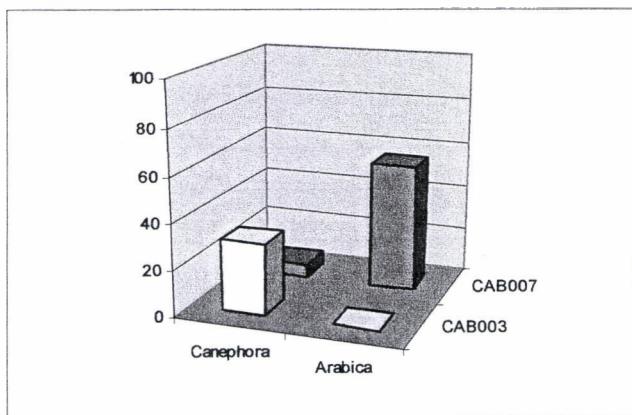


Twenty *Coffea canephora* plants were inoculated for each isolate. No symptom was induced with isolate ATCC 36325. On the other hand, all the other isolates of the *Giberella* *xylarioides* complex induced a mortality rate of between 10 and 30%. The infection process in the historical strains, despite being conserved for several decades, remained functional. It should be noted that the two strains ATCC15664 and DSMZ62457 induced mortality on the species *C. canephora* whereas they were isolated from the species *C. dewevrei*. The highest mortality percentage was observed with contemporary strain CAB003.

Trial B

This cross-inoculation trial on *C. canephora* and *C. arabica* plants is being used to test the host specificity of contemporary strains CAB003 (canephora strain) and CAB007 (arabica strain).

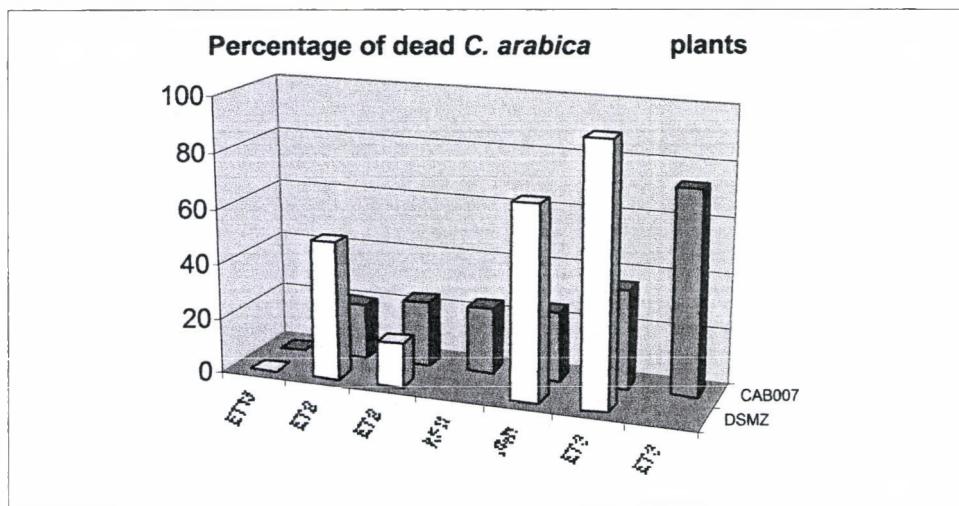
Fig. 2. Percentage of dead plants with *C. arabica* – *C. canephora* cross-inoculation



The *canephora* strain only induced mortality on *C. canephora*. The *arabica* strain induced mortality on *C. arabica* and one *C. canephora* plant died. Strict host specificity was not found, but it was suggested that the death of the latter plant may have been due to its weak growth.

Trial C

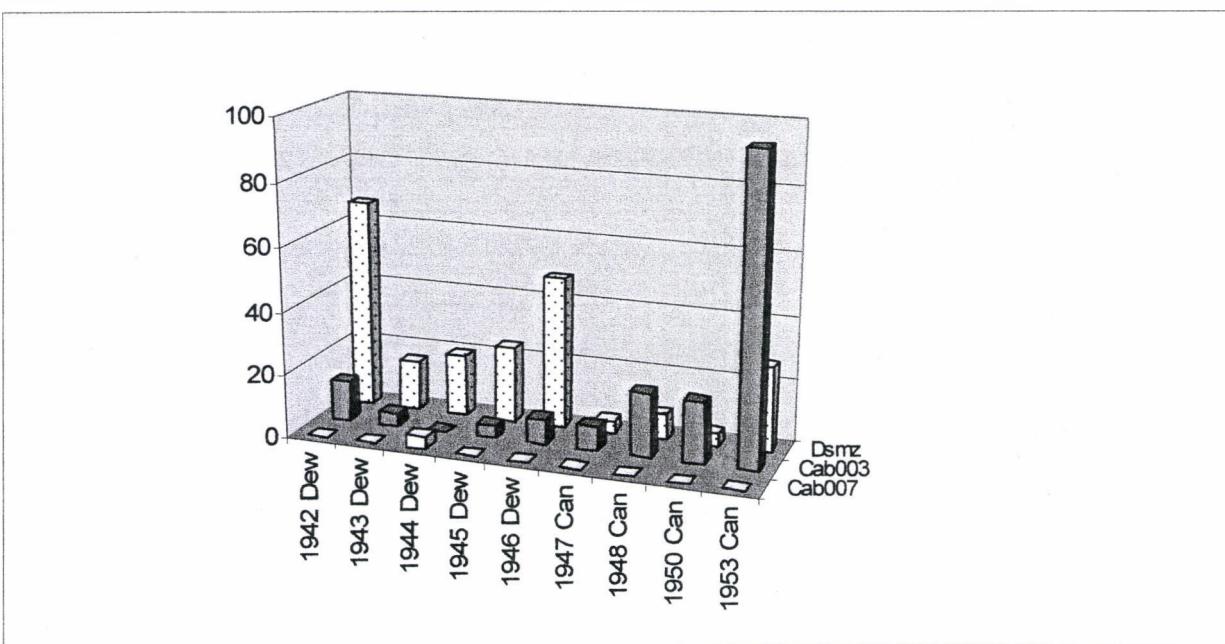
Fig. 3 Percentage of dead *C. arabica* plants with strains CAB007 and DSMZ62457



The mortality rate caused by the *arabica* strain CAB007 was between 0 and 74%. It was 0 to 94% with the *liberica* strain DSMZ62457. Origin E13 was resistant to both isolates. It is important to note that isolate DSMZ62457, which is not isolated from the species *C. arabica*, induced higher mortality rates than those recorded with specific strain CAB007.

Trial D

Fig.4. Percentage of infected plants inoculated with CAB003, CAB007 and DSMZ on *C. canephora*, *C. dewevrei*.



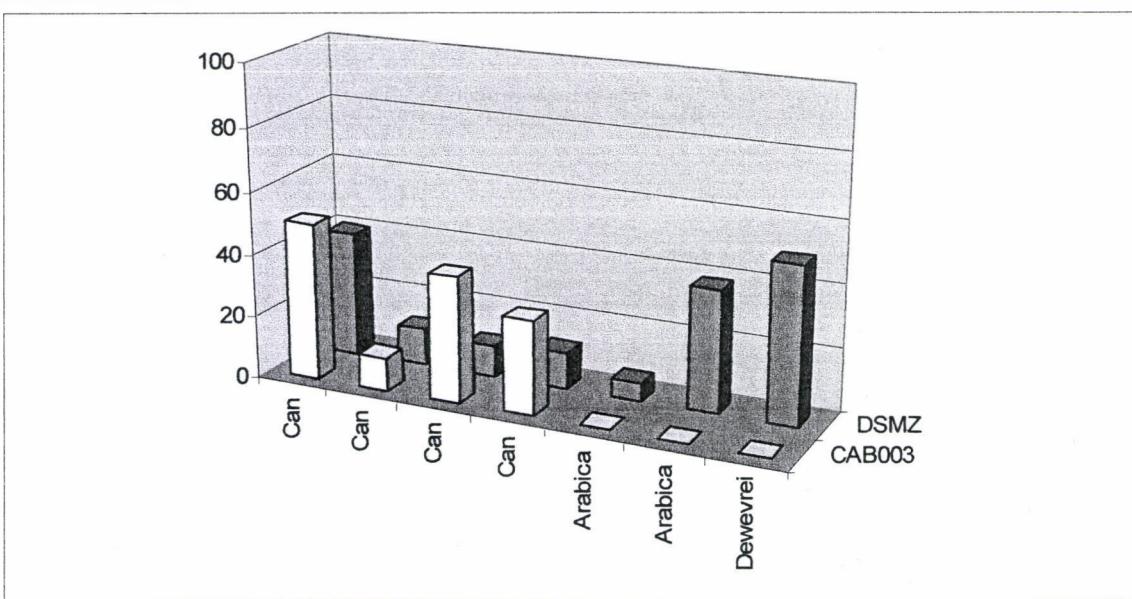
The *arabica* strain CAB007 did not induce any mortality on the species *C. canephora*. It killed one plant of the species *C. dewevrei* (physiological weakness?)

The *canephora* strain CAB003 was responsible for the death of 8 to 96% of the *C. canephora* plants and 4 to 12% of the *C. dewevrei* plants.

The *liberica* strain induced mortality on both species of coffee trees, with a much higher mortality percentage on the species *C. dewevrei*. The pathogenicity of that strain was expressed with greater intensity on the species of coffee trees from which it came.

Trial E

Fig. 5. Percentage of dead *C. canephora*, *C. liberica*, and *C. arabica* plants inoculated with CAB003 and DSMZ62457.

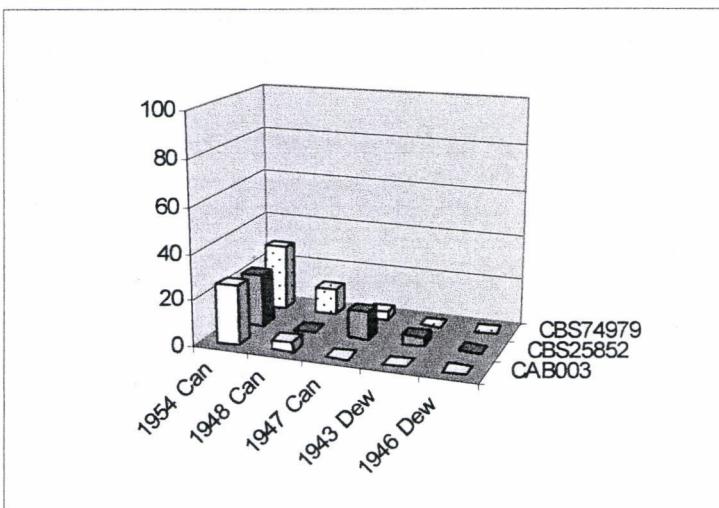


Isolate CAB003 only induced mortality on seedlings of the species *C. canephora*. No mortality was found on plants belonging to the species *C. arabica* and *C. dewevrei*.

Isolate DSMZ induced a mortality percentage varying from 6 to 50% respectively in the 3 species.

Trial F

Fig. 6. Percentage of dead plants of 2 *Coffea* sp. inoculated with strains CAB003 and CBS.



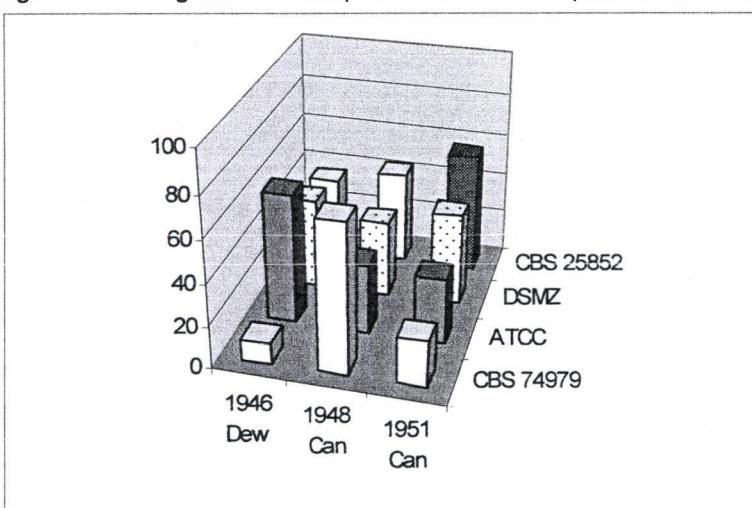
The 3 isolates induced the highest mortality percentage on progeny *C. canephora* 1954.

CAB003 only induced mortality on progenies belonging to *C. canephora* not on *C. dewevrei*.

The "historical strain" CBS 74 979 only induced mortality on progenies of *C. canephora*, no dead plants were found for *C. dewevrei*. Conversely, CBS 25 852 induced mortality on both species with 4% of dead plants for *C. dewevrei*.

Trial G

Fig. 7. Percentage of infected plants of 2 *Coffea* sp. inoculated with "historical strains"



This trial is used to assess the reactions of the 2 species *C. canephora* and *dewevrei* to the "historical isolates". Isolate CAB003 was not used for lack of a sufficient number of seedlings. It turns out that these historical isolates induced a mortality percentage on both species of *Coffea*.

3- Conclusion

Pathogenicity of wild isolates

The wild isolates collected from primary forest in Uganda induced the death of artificially inoculated seedlings and therefore belonged to the species *Fusarium xylarioides*. That result confirms those obtained by comparing the ITS sequences of the reference isolate and the wild strains.
Characterization of *Gibberella xylarioides* complex / *Coffea* spp interactions.

Isolate ATCC36325 is not pathogenic. That isolate does not belong to the species *F. xylarioides*. It was wrongfully described by Booth as being a female strain. This result confirms the conclusions reached by P. Lepoint and our own result obtained with microsatellite markers, excluding that species.

Historical isolates CBS74979 and CBS25852 confirmed their pathogenicity, inducing mortality on seedlings of the species *C. canephora*. They also proved to be pathogenic on the species *C. dewevrei*, which is not the species from which those strains were collected. The two isolates did not display any host specificity with regard to those two species, primarily in trial G.

In the same trial G, isolates ATCC15664 and DSMZ62457, although isolated from *C. dewevrei*, were pathogenic on both species, *C. canephora* and *C. liberica*.

These results show that the historical isolates tested had a broad host spectrum, and were able to induce mortality on young plants of the two species *C. canephora* and *C. dewevrei* and isolate DSMZ62457 was pathogenic on the 3 species (trials C, E).

Isolate CAB003 was specific to the species *C. canephora*. One exception was seen in trial D, where it induced mortality on young plants of *C. dewevrei*.

Although these are partial results, insofar as it was fairly complicated to implement a complete crossed inoculation for lack of *C. liberica* seeds, they show that the historical isolates had a broader host spectrum than the contemporary population represented by isolate CAB003.

Isolate CAB007 seemed specific to the species *C. arabica*, despite the 5% mortality rates obtained with a progeny of *C. dewevrei* and *C. canephora*. The specificity of isolate CAB007 for the species *C. arabica* seemed to be confirmed in the growing zones where both species lived side by side. In Ethiopia in the Jima zone, at the EARO research station in a severely contaminated zone, only *C. arabica* trees are affected, *C. canephora* trees are unaffected.

The interactions are summed up in the following table:

	CAB003	CAB007	DSMZ62457	CBS25852	CBS74979	ATCC15664	ATCC36325
<i>C. canephora</i>	+++	-	+	+	+	+	-
<i>C. liberica</i>	+		+++	+	+	+	-
<i>C. arabica</i>	-	++	++				-

The hypothesis is once again put forward, since it is extended to new exposures, that the contemporary population present on *C. canephora* in DRC, Uganda and Tanzania could be derived from a strong foundation effect that counter-selected a specific isolate of the *C. canephora* species. That result clearly shows that the contemporary strains of *Fusarium xylarioides* have an evolutionary potential that could evolve towards the acquisition of complementary virulence that might prove pathogenic on the other species of *Coffea*.

In addition, in the contemporary population of *F. xylarioides* present on *C. canephora* in RDC, Uganda and Tanzania, the question is raised of the existence of races. For the moment, we do not have the plant material needed to detect races, as genotypes are needed that are multiplied by vegetative propagation, or obtained by controlled pollinations.

WP3: Breeding for resistance

Task 2. Collect seeds from genotypes representative of the available germplasm.

As part of this project, an agreement between Ivory Coast and Uganda enabled an exchange of plant material. Below the list of 22 Ivorian origins produced from seeds and germinated under quarantine at CIRAD before being transferred to Uganda. Two plants per origin were transferred.

CIRAD Code	Ivory Coast Code	Number of plants sent	OBSERVATION
1768	121	2	
1770	392	2	
1771	464	2	
1772	A30	2	
1773	A03	2	
1776	KB3	2	
1780	KB8	2	
1783	KB12	2	
1786	02	2	
1789	02507	2	
1790	02569	2	
1791	02570	2	
1792	02571	2	
1793	02576	2	
1794	02593	2	
1795	02651	2	
1804	2504	2	
1805	2564	2	
1806	2596	2	
1808	311	2	
1811	178	2	
1814	32	2	
		2	

Task 5. Analyse the inheritance of resistance

The data gathered in Uganda by Mr Pascal Musoli for the study of resistance heritability were put to use during his stay at CIRAD in Montpellier, where he defended his thesis on 26 March 2007. That work is covered in two publications that have been submitted and a third that is currently being drafted.

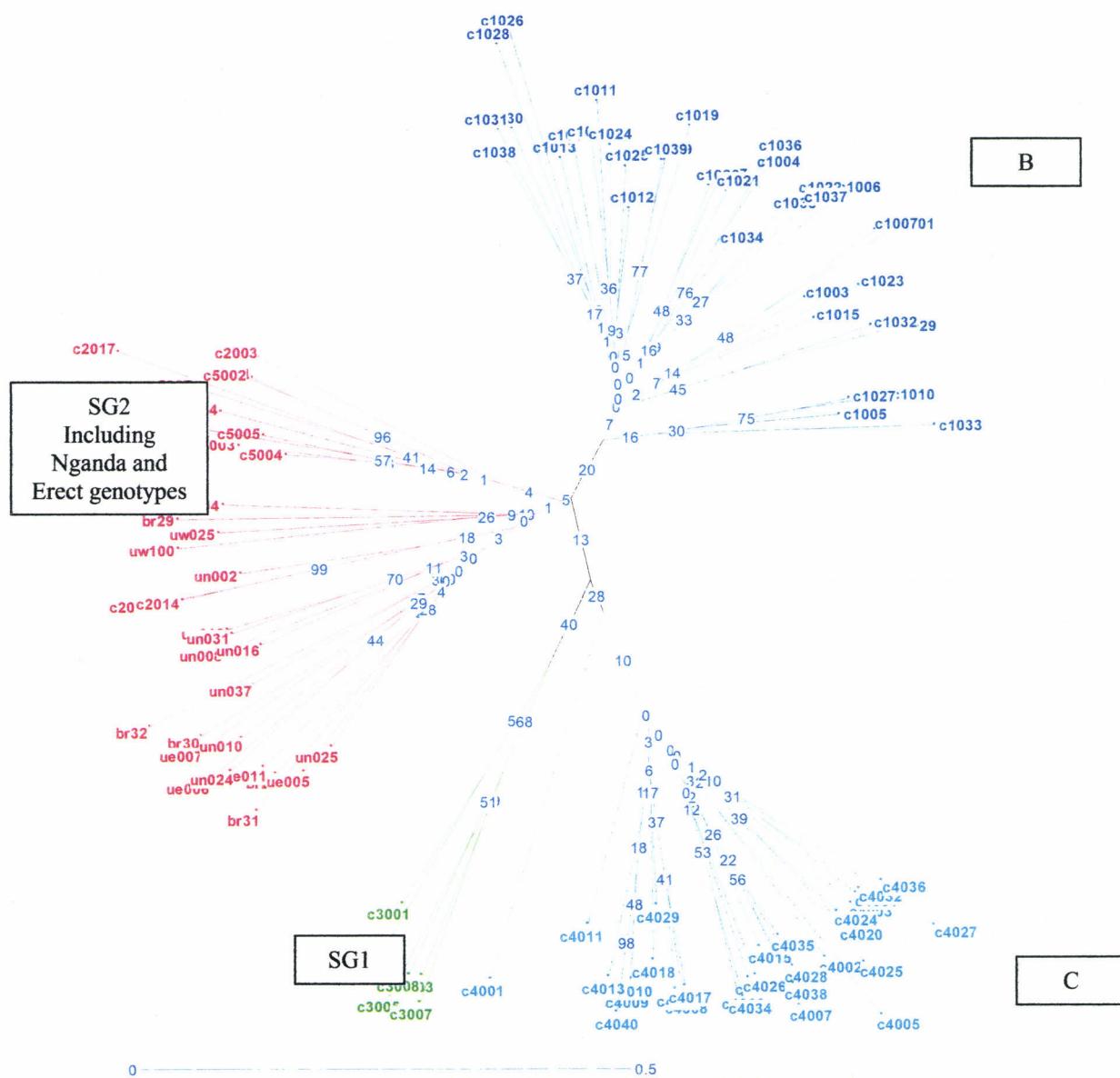
An analysis of the data made it possible to lay the foundations for characterizing *Coffea canephora* resistance to *Fusarium xylophagoides*.

Those joint analyses by CIRAD and NARO researchers are presented in the NARO section.

Diversity analysis within *Coffea canephora* species using molecular markers.

Part of the material collected in the frame of the INCO-COWIDI project continue to be integrated on a diversity analysis work developed in a thesis. The study concern predominantly accessions from the reference collection of Ivory Coast including several geographical origins from all the geographic area of *C. canephora*. The sample of about 350 individuals includes genotypes from Erect and Nganda types from Uganda in order to compare them to other geographic origins. This work follows the diversity analysis done on Uganda genotypes and will allow a better understanding of material migration and relation.

Preliminary results indicate that Erect and Nganda are grouped within the "SG2" diversity subgroup originated from central Africa. This group was originally composed by accessions from INEAC. Here is a graphical representation of genetic relationships between genotypes based on microsatellites data.



More has to be done by including genotypes from other geographical origin since this work is based on occasional prospecting on several geographic points but not on systematic sample from all the distribution area. In this frame diversity of several genotypes from Luki collection, obtain during the COWIDI project is join to the study and assess in relation with previous work.

UCL

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

Fifth Annual Report (November 2005 to October 2006)

**Université catholique de Louvain
Unité de Phytopathologie
Croix du Sud 2bte 3
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WP I: PATHOGEN DIVERSITY

1st - Important Note: *It was expected that, under the original contractual arrangement, UCL's inputs to the project would cease at the end of October 2005. As a result of a successful application to the EU for an extension of the project, we have been able to continue our research. However, given that the extension was not funded, research has relied on funds remaining from the original phase of the project and as a result has been somewhat limited.*

2nd - Important Note: *The results presented in this report and the previous, were developped in the share of the thesis of Mlle Pascale LEPOINT, funded by the project and the financial effort of UCL. The thesis "Speciation within the African coffee wilt pathogen" was defended on 22th December 2006.*

Task II: Identification, storage, and exchange of isolates

All reference strains used in UCL publications will be deposited in the BCCM/MUCL™ international culture collection before 31/07/2007.

Task IV : Description of the fungal life cycle, asexual and sexual phases

Crossing assays

In our annual report covering period 1/11/04 to 31/10/05, 20 *C. canephora*-associated strains originating from the Equator Province (DRC) were characterized by MAT-PCR as MAT-1 and crossed with *C. canephora* and *C. arabica* tester strains. Crossing results have been updated to confirm if preliminary MAT-PCR results are correct. Fertile perithecia oozing ascospores were obtained only in crosses with MAT-2 *C. canephora* mating type tester strain MUCL 44887. Of the 20 strains tested, three are completely sterile, seven are fertile only as males, and 10 are hermaphrodites. These results validate that the strains collected in the Equator Province are all MAT-1, which is most unusual, suggesting that no perithecia should be observed *in situ*.

In previous reports, UCL described the *in vitro* production of perithecia between compatible *G. xylophagoides* *C. arabica*-associated strains (**biological species 1 – BS 1**) and between compatible *C. canephora*-associated strains (**biological species 2 – BS 2**). Accordingly to the definition of a biological species (Mayr, 1940), BS 1 and BS 2 are reproductively isolated from each other and from other such populations.

Until recently, the teleomorph had never been formed in carrot agar crosses when compatible strains from the first epidemic (1950s) isolated from *Coffea* spp. in the Central African Republic (CAR), Guinea and Côte d'Ivoire were implicated in a cross. It was believed that fertility had been greatly

reduced, if not totally lost, through sub-optimal conservation conditions since initial isolation more than half a century ago. However, strain **BBA 62457** (*C. excelsa*, CAR?, 1971, **MAT-2**) constitutes an exception since this strain readily produces protoperithecia in individual cultures in addition to crosses, suggesting that its "female fertile" character (i.e., ability to produce perithecial initials) is still intact. The perfect state was obtained recently for the first time in a cross between ATCC 15664 (*Coffea* sp., Guinea?, 1964?, **MAT-1**) and BBA 62457 from which 10 ascospores have been isolated. Seeing as these two strains are reproductively isolated from strains belonging to the BS 1 and BS 2, ATCC 15664 and BBA 62457 have been assigned to a distinct biological species (**BS 3**).

Out of the ten viable progeny isolated from the **ATCC 15664 x BBA 62457** cross, two appear to be highly fertile and produce abundant stroma in culture similarly to female parent strain BBA 62457. The other eight strains sporulate to a lesser extent, do not form stromatic structures on PDA, and produce a pinkish pigment in the agar and aerial mycelium just as male parent ATCC 15664. The ten progeny strains were backcrossed with both parents for *MAT* identification, determination of fertility levels and the confirmation of the heterothallic origin of the ascospores. Results seem to indicate that four progeny are *MAT-1* and two are *MAT-2* whereas the remaining four show low levels of fertility that impede adequate *MAT* identification. Lack of funding and time did not allow us to confirm results obtained in crosses by *MAT*-PCR, nor to sequence the *MAT* gene for phylogenetic analysis.

These ten strains are valuable seeing as only two strains (ATCC 15664 and BBA 62457) have been identified to date from international culture collections as belonging to this biological species (**BS 3**). The identification of these supplementary, more fertile, strains belonging to BS 3 should enable the determination of a sufficiently fertile *MAT-1/MAT-2* mating type tester couple that can constitute reference strains with the aim of establishing the presence of additional biological species within what was previously termed *G. xylosporus*.

Four strains (**BBA 62455, CBS 25852, CBS 74979, MNHN 709**) dating back to the first coffee wilt epidemic (1950s) and originating from Central and West Africa appear to be incompatible between each other and with members of the three previously described biological species. These four strains have been subsequently assigned to a residual sterility group (**SG 4**).

Based on these results, it would appear that what was previously identified as a single species and called *G. xylosporus* entails in fact at least four reproductively isolated populations or biological species. It is thus more pertinent to speak of the *G. xylosporus* complex or *G. xylosporus* *sensu lato* when speaking of an unidentified isolate.

Morphological study

The teleomorph was characterized on the basis of *in vitro* crosses between reference strains originating from different geographical origins and *Coffea* spp. corresponding to the defined biological species (Table 1). Results were confronted with Heim and Saccas (1950) original description of the

perfect state as well as to data obtained from crosses implicating *G. indica* strains (a close wilt-inducing relative).

Herbarium samples received from the Museum National d'Histoire Naturelle de Paris (MNHN) that were collected in the 1950s in the Central African Republic (CAR, former Oubangui), Guinea and Côte d'Ivoire were also analyzed (data not shown). Seeing as these constitute dead specimens, no biological species could be defined in crossing assays. Moreover, DNA extractions were unsuccessful.

Table 1 Size of perithecia and ascospores obtained through *in vitro* crosses of members of the *G. xylosporus* complex and *G. indica* complex compared to reference descriptions by Heim & Saccas (1950) and Rai & Upadhyay (1982)

Species	Cross	Size (μm)		
		Perithecia		Ascospores ^a
		range	average	
<i>G. xylosporus</i> complex				
Ref	<i>Coffea</i> spp. sample	200-400 x 180-300	12-20 x 4-5 x 5-5.6	NS
BS 1	IMI 375909 x 389563	408 x 365	(10-)12-15(-16) x 4-5 x (4-)4.5-5.3(-6)	13.4 x 4.8 x 5.0
BS 2	MUCL 35223 x 43887	338 x 275	(11-)13-17(-20) x 4-5 x 4-5.6(-6)	15.3 x 4.8 x 4.9
BS 3	ATCC 15664 x BBA 62457	375 x 316	11-15 x 4-6 x 4.5-6	13.1 x 5.0 x 5.2
<i>G. indica</i> complex				
Ref	<i>C. cajan</i> sample	350-550	10-17 x 5-7	NS
BS 1*	IMI 271070 x 275452	325 x 283	(11-)11.5-15.6(16) x 4-6(-6.5) x 5-6(-8)	14 x 5.1 x 5.4

* The majority of ascospores are one septate and can present a slightly larger upper cell, explaining the three sets of measurements given for each cross analyzed. When available, data is indicated under the form (min-) centil 0,05 - centil 0,95 (-max).

NS: not specified

Task V. Evaluation of genetic diversity within *G. xylosporus*

Non-MAT genes: translation elongation factor 1- α (tef 1- α) - calmodulin (CL) - histone 3 (H3)

All of the sequencing results have been obtained for non-MAT genes (tef, CL, H3), assigning newly acquired strain **BBA 62455** (*C. canephora*, Guinea, <1964, MAT-2) with strains CBS 25852 (Coffea sp., Côte d'Ivoire, 1951, MAT-2), CBS 74979 (*C. canephora*, Guinea, 1963, MAT-2), and MNHN 709 (Coffea sp., CAR, 1950, MAT-1) corresponding to **BS 3**. Strain **BBA 62458** (*C. arabica*, Ethiopia, <1972, MAT-1) was identical in tef, CL and H3 sequences to all other *C. arabica*-associated strains and thus assimilated to **BS 1**.

MAT genes: Amplification of entire MAT1-1 idiomorph & part of the MAT1-2 idiomorph

Amplification of a small portion of the *MAT1-1* and *MAT1-2* idiomorphs was carried out initially with primers designed by Steenkamp et al. (2000) targeting respectively a restricted part of the *MAT1-1-1* (Gfmat 1a & 1b) and *MAT1-2-1* (Gfmat 2c & 2d) open reading frames (ORFs, Fig 1). Primers capable of amplifying respectively the entire *MAT1-1* idiomorph and the entire *MAT1-2-1* ORF of *G. xylospoides* strains were designed based on available sequences for related Fusaria. Available NCBI *F. oxysporum* (AB011379), *G. circinata* (AY21987), *G. subglutinans* (AY219878) and *F. guttiforme* (AY219875) sequences were aligned using ClustalW 1.82 (Pearson and Lipman, 1988) for the *MAT1-1* gene primer design. Primers Gxp5', Gxp8, Gxp9, Gxp11 and Gxp13 as well as internal primers used for sequencing were designed from highly conserved regions of the aligned sequences. Similarly, *F. oxysporum* (AB011378), *G. fujikuroi* (AF100926) and *G. circinata* (AF194869) sequences were aligned for *MAT1-2* Gxp21 primer design.

Amplification of the entire ***MAT1-1* idiomorph** in *G. xylospoides* strains can now be carried out via three distinct PCRs using primer couples Fo14 (Arie et al., 2000)/Gxp5', Gxp11/Gxp9, and Gxp8/Gxp13 (Fig 1A). A 4749 to 4803 base pair (bp) fragment was sequenced for *G. xylospoides* *MAT-1* strains **MUCL 35223, MUCL 44532, Gx3P22, IMI 375907, BBA 62458, ATCC 15664, MNHN 709** as well as for closely related *Fusarium* sp. strains **IMI 271070, NRRL 22540** and **NRRL 26064**.

Amplification of the entire ***MAT1-2-1* ORF** and a portion of the 3' flanking region was carried out with primer pair Fo14 (Arie et al., 2000) or Gfmat2d Steenkamp et al. (2000)/Gxp21 (Fig 1B.). A 1198 to 1382 bp fragment was sequenced for *G. xylospoides* *MAT-2* strains **OUG 008, MUCL 43887, MUCL 44549, IMI 204746, IMI 375908, IMI 379567, CBS 25852, CBS 74979, BBA 62455, BBA 62457** as well as for closely related *Fusarium* sp. strains **IMI 193652** and **IMI 275452**.

MAT sequencing results confirmed the presence of four distinct clades (phylogenetic species) corresponding to the four BS/SGs defined in crosses. Moreover, a close analysis of the left idiomorph flanking region (Fig 2), theoretically common to both mating types, revealed striking observations. The most unusual is the presence of a 60 bp region (marked by a red line) containing mating type-specific sequences common to both the *G. xylospoides* and the *G. indica* complexes. This region harbours very few species- and/or mating type-specific mutations. Surprisingly, *C. arabica*-associated *G. xylospoides* *sensu lato* *MAT-1* strains IMI 375907 and BBA 62458 possess the sequence type for *MAT-2* strains in this region.

The distinct PCR primer sets designed for the amplification of the *G. xylospoides* *sensu lato* *MAT* idiomorphs were successfully implemented to nine of the ten known *Gibberella fujikuroi* complex (GFC) mating population (MP A to H) tester pairs in addition to *F. oxysporum* strains (Fig 1).

Given that the designed *G. xylosporus* MAT idiomorph primers are able to successfully amplify the corresponding regions not only in strains belonging to this complex but equally in other members of the GFC, and that these genes appear to be phylogenetically pertinent in speciation studies; the use of the designed primers might enable the identification of other undescribed cryptic species within *G. xylosporus* as well as within other closely related species.

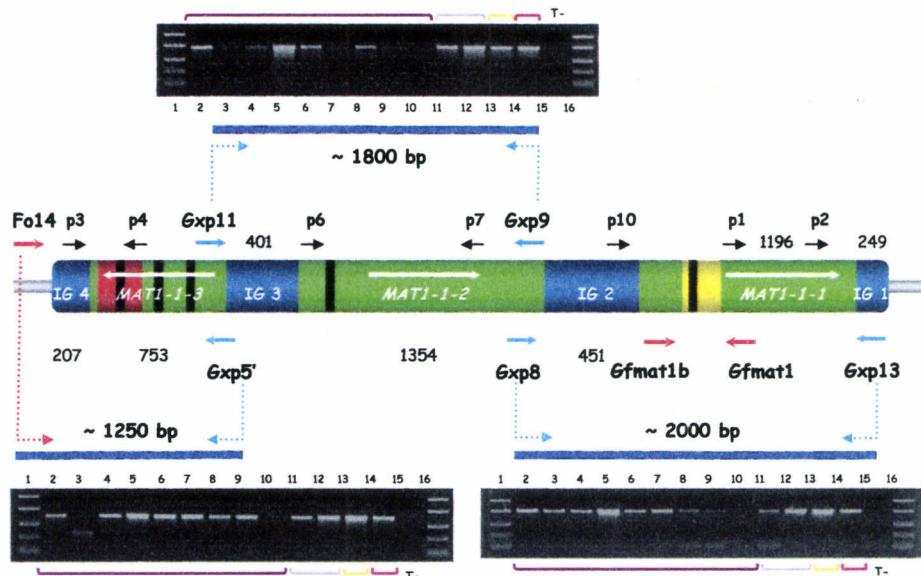
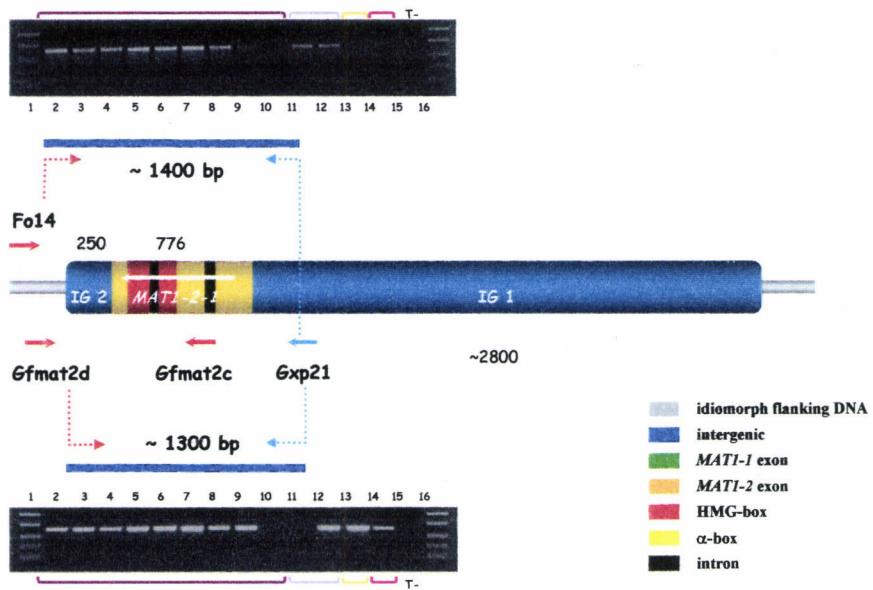
A. MATI-1**B. MATI-2**

Fig 1 Map of the mating type locus (*MAT*) adapted from Yun *et al.* (2000) for our study of *G. xylosporoides*. (A) *MATI-1* (~4605bp) and (B) *MATI-2* (~3824bp) idiomorphs drawn to proportion showing introns, exons, conserved domains, intergenic regions (arbitrarily designated IG 1, IG 2, IG 3 and IG 4) and flanking DNA. A white arrow indicates the direction of transcription of each of the four *MAT* ORFs. The centromere is situated on the left of the figure. PCR strategies for amplification of the *G. xylosporoides* and *G. indica sensu lato* *MAT* loci are described. Red arrows represent previously described primer sets, blue and short black arrows are respectively PCR and sequencing primers designed in this study. PCR gels obtained with designed primers using GFC and *F. oxysporum* strains are shown. Lanes 1 and 16 correspond to Fermentas Middle Range ladder indicating 100 bp, 400 bp, 850 bp, 2000 bp and 5000 bp. Lanes 2 to 10 correspond to GFC MPs A to I, lanes 11 and 12 are *F. oxysporum*, lane 13 represents *G. indica sensu lato*, lane 14 corresponds to *G. xylosporoides sensu lato*, and lane 15 for is a negative control (T-).

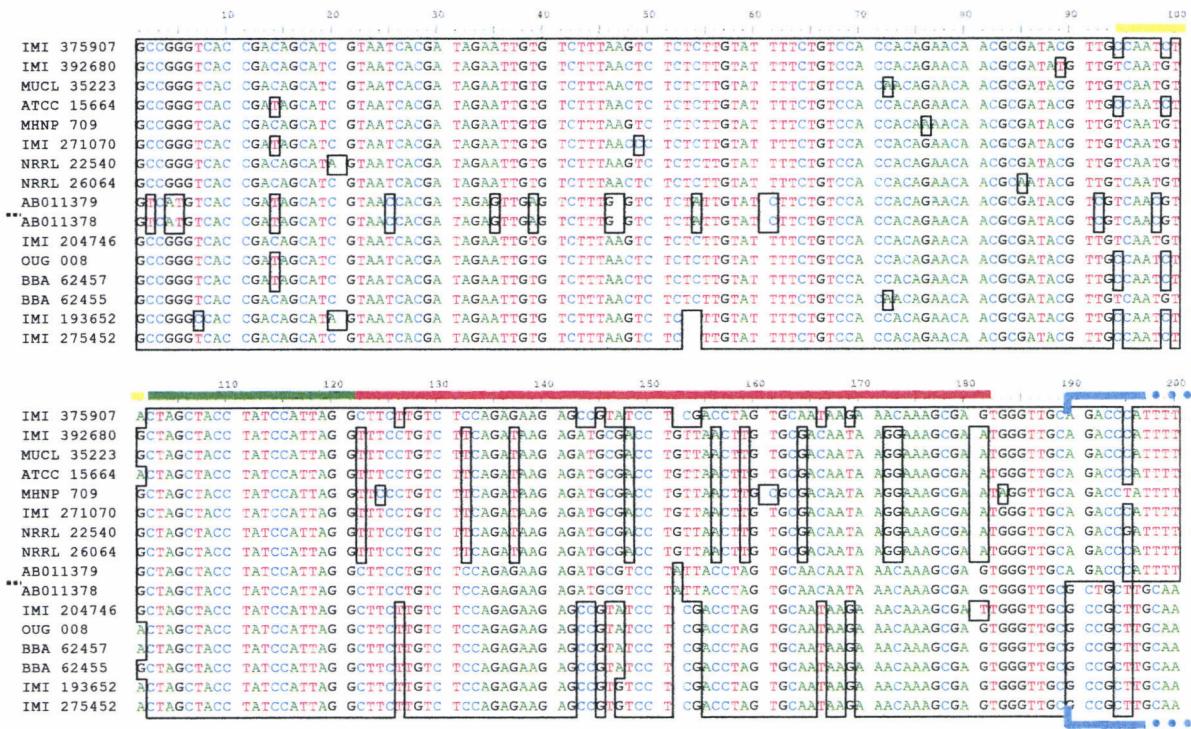


Fig 2 Sequence alignment of representative *MAT1-1* and *MAT1-2* flanking regions situated to the left of the idiomorph for *G. xylosporoides* and *G. indica* complex strains along with *F. oxysporum* (AB011378 and AB011379). *MAT-1* and *MAT-2* strains are separated by a dotted line and similarities in sequences are boxed. The beginning of the idiomorph is indicated by a blue line, the putative 60 bp *MAT*-specific region bordering it by a red line, a conserved 20 bp region is lined in green and an ambiguous 8 bp stretch in yellow.

NATIONAL AGRICULTURAL RESEARCH ORGANISATION (NARO)

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

Fifth Annual Report (November 2005 to October 2006)

Coffee Research Institute (CORI), Kituza
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UGANDA

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WP 1 and WP 2 by Dr. G. J. Hakiza and S. Olal.

The main focus of research remained on increasing/improving the status of knowledge on the Coffee Wilt Disease (CWD) and its pathogen. The series of studies designed to provide information on specific aspects of the disease or pathogen are elaborated below.

WP 1: Pathogen diversity

Task 1: Collection of *F. xylarioides* from robusta coffee (*Coffea canephora*)

Previous isolations of *F. xylarioides* were made from *Coffea canephora*, from different locations in Uganda, specific robusta clones, *C. canephora* tetraploids from CORI, *C. liberica*, *C. excelsa* from Kawanda Coffee collection.

Monitoring CWD incidence on the various coffee species in the Kawanda Coffee Collection and the Botanical Gardens at Entebbe was maintained. No new incidence of the disease was observed and therefore no new isolates of *F. xylarioides* were made during the period from these sources.

F. xylarioides has not been reported or isolated from *Coffea arabica* from any part of Uganda during the reporting period.

WP 2: Host/Pathogen Interaction

Task 3: Isolate aggressiveness using standard inoculation tests (different inoculation methods).

Variety reaction to CWD under natural infection in the field and screen house conditions.

Different methods of inoculations (stem injection, stem wounding by cutting and root dipping in standard inoculum) have been evaluated at CORI and were reported in previous reports. Root dip method is currently the method in use at CORI for screening for CWD resistance.

An attempt was made to compare reaction of seedlings/cuttings inoculated with the CWD pathogen. This was done by recording wilt incidence on clones exposed to natural infection at 4 weekly intervals. Mean wilt incidence for a period of 3 years when wilt disease progress has more or less stabilised. The cut off point for recording in the screen house is 90 days when wilt disease progress has also slowed down. The table below illustrates the situation.

Table 1: Comparison of reactions of clones to CWD under screen house and natural infection under field conditions:

Clone	Screen house inoculation (Mean CWD incidence (%) after 90 days).	Mean CWD incidence (%) under field conditions Jan. 2002 – Nov. 2006
1s/2	88.7	93.3
1s/3	100	95.1
1s/6	94.3	92.7
258/24	94.3	98.4

Although no correlation analysis was done, the mean CWD incidences for the clones were very close at the cut off points when few or no new infections occurred. This could imply screen house inoculations are a good indication of adult plant reaction to the disease. This is further supported by the fact that, to date very few plants screened have developed CWD symptoms under field conditions.

WORK PACKAGE (WP) 3: BREEDING FOR RESISTANCE AGAINST COFFEE WILT DISEASE

Dr Pascal Musoli

I Introduction

Coffee wilt disease (CWD) continued to be a major threat to coffee production and productivity in Uganda during the reporting period and variety resistance was still considered an important control strategy. All breeding activities initiated in previous periods under Work Package 3 continued during this reporting period.

II Objectives

The objectives of WK3 remained unchanged i.e.

- a) Identify sources of resistance against CWD through tests on young seedlings and cuttings and field assessments
- b) Assess inheritance of CWD resistance among robusta coffee in Uganda
- c) Evaluate genetic diversity of Ugandan *C. canephora*
- d) Define a breeding strategy towards developing varieties with durable resistance to CWD.

III Activities and progress

Research activities during the reporting period remained structured along work package objectives.

a) Identifying sources of CWD resistance

Anticipated sources of CWD resistance remained to be local germplasm available in Uganda and germplasm from foreign sources, mainly other African countries with history of having controlled CWD using variety resistance. Activities for this objective during this reporting period focused on evaluating materials from the following sources for resistance:

- 1) On-station robusta collections and their intraspecific hybrids
- 2) Wild forest robusta coffee.

These activities were carried out:

- i) In the screen house on young rooted cuttings and seedlings
- ii) In the field on young and mature coffee trees.

i) Evaluation for CWD resistance in the screen house using tests on young rooted cuttings and seedlings.

Screen house activities were carried out at CORI, Uganda. Half sib progenies and rooted cuttings of nganda and erecta genotypes in germplasm plots at Kawanda Agricultural Research Institute (KARI) and clones in a clonal evaluation trial at Kituza were tested for CWD resistance through artificial inoculation using root dip method. The plants were assessed weekly using a disease severity scale of 1-5, 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. Disease progress curves were plotted using the disease symptom severity data of each of the progenies to illustrate variations in temporal progression of coffee wilt disease among these progenies. Analysis of variance was performed on the disease data to test the significance of variations between the progenies.

CWD Resistance Nganda and erect progenies

Figure 1 shows temporal progression of the disease (percent plant mortality) among erecta progenies. This figure shows that the progenies were variably affected by the coffee wilt disease. The plant mortality progressed rapidly among progenies UE006, UE005 and UE016 to high final disease levels. Mortality progressed slowly among progenies UE012 and UE010. The mortality among these progenies at week 10 from the time of inoculations varied from 5% observed on progeny UE006 to 85% observed on progeny UE012.

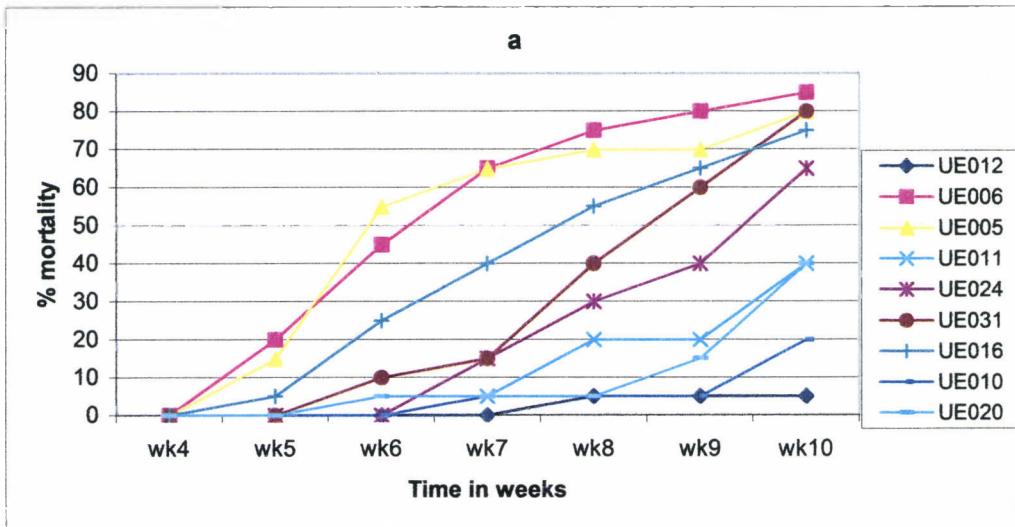


Figure 1: Progression of plant mortality among erecta half sib progenies from KARI.

Figure 2 shows temporal progression of the disease (percent plant mortality) among nganda progenies. This figure shows that nganda progenies were also variably affected by the coffee wilt disease. Plant mortality progressed rapidly among most of the nganda progenies to high final disease levels. However mortality progressed moderately among progenies UN004, UN015 and UN018 to moderate final disease levels.

Differences in progression of plant mortality between erecta and nganda progenies are almost unnoticeable. These results show that generally both erecta and nganda progenies are susceptible to coffee wilt disease, however there are some progenies among the erect that are more resistance than majority of nganda progenies. It is also evident that resistance varied between genotypes in each group.

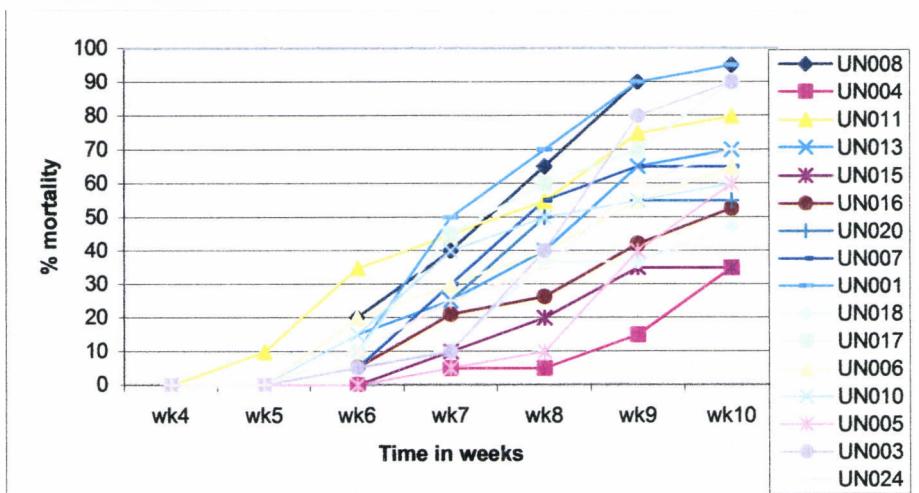


Figure 2: Progression of plant mortality among nganda half sib progenies from KARI

Analysis of variance performed on the 1-5 disease symptom severity data found significant ($p<0.0001$) genetic differences among nganda and erecta progenies (tables 1).

Table 1: Analysis of variance for CWD on nganda and erecta half sib progenies at week 10 after artificial inoculation

Source	df	ms	ss	f value	pr	C.V
Erecta						
Progeny	8	126.7	15.8	13.8	<0.0001	27.3
Error	168	192.2	1.1			
Nganda						
Progeny	15	69.8	4.7	4.4	<0.0001	23.9
Error	294	311.3	1.1			

When the progeny disease symptom severity means were ranked using Student-Keuls mean separation test, the erecta progenies were ranked into 4 overlapping resistance classes and the nganda progenies were grouped into 3 resistance classes (Table 2)

Table 2: Mean CWD symptom severity among erecta and nganda half sib progenies at week 10 after artificial inoculation

Nganda		erecta	
Progeny	Mean	Progeny	Mean
UN001	4.89a	UE006	4.75a
UN003	4.85a	UE005	4.7a
UN008	4.85a	UE031	4.63a
UN017	4.84a	UE016	4.45ab
UN011	4.74a	UE024	4.32ab
UN013	4.60ab	UE011	3.7bc
UN024	4.53ab	UE020	3.60bc
UN006	4.45ab	UE010	3.00c
UN010	4.25abc	UE012	2.11d
UN007	4.2abc		
UN020	4.00abc		
UN016	3.89abc		
UN018	3.89abc		
UN005	3.85abc		
UN015	3.59bc		
UN004	3.35c		

CWD Resistance Progenies of clones in the clonal trial at Kituza

Variation among progenies of clones in the field trial at Kituza was more noticeable (figure 3). The disease progression varied greatly between these progenies. It progressed very rapidly among susceptible progenies C/1/7, Q/6/1, B/6/2 and B/1/1. The progression on progeny B/2/1 was slow, while progression on majority of the progenies, such as 1s/2, E/3/2, G/3/7, was medium. The final disease levels at week 10 from the time of inoculation, also varied between progenies. Generally all progenies with rapid disease progression had highest levels of mortality. Mortality among these progenies ranged from 5% observed on progeny B/2/1 to 95% observed on progeny C/1/7.

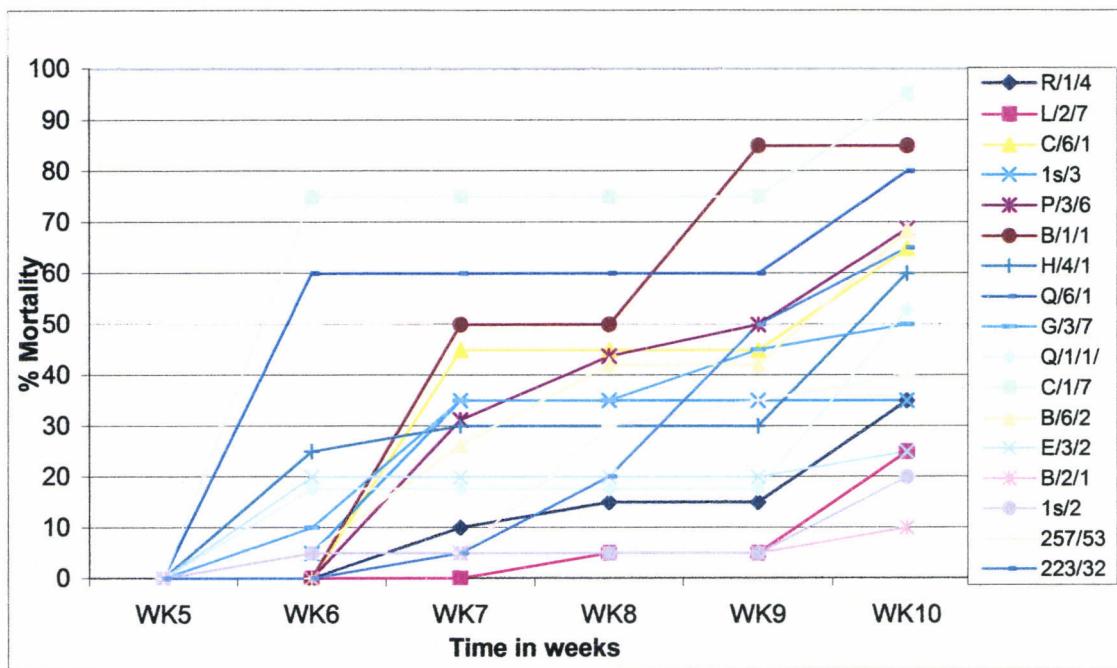


Figure 3: Progression of plant mortality among half sib progenies of clones in Kituza field trial

CWD Resistance Progenies of clones in the clonal trial at Kituza

Variation in disease progression on rooted cuttings was more erratic. Mortality among cuttings 223/23 and 1s:2, which had 0% mortality by week 7, rapidly progressed to 90% and 100% respectively by week 10 (Figure 4). Clones Q/3/4 and J/1/1 are known to be very resistant under field conditions. These results show that both field and screen house assessments tend to give a similar trend in resistance, the latter (screen house) seem to cause more disease. This may be due to the high spore concentration in the inoculum and the physiological stress caused by root dipping inoculation method. This method involved uprooting and cleaning roots of the plant before inoculation, a practice that causes damage to the root system of the plant. The field represents the actual situation. Clone B/2/1 is known to be moderately susceptible under field conditions and clone 257/53 is highly susceptible under field conditions but in the screen house they appeared to be resistance. This differential response could be due to different resistance mechanisms used against disease penetration by these clones.

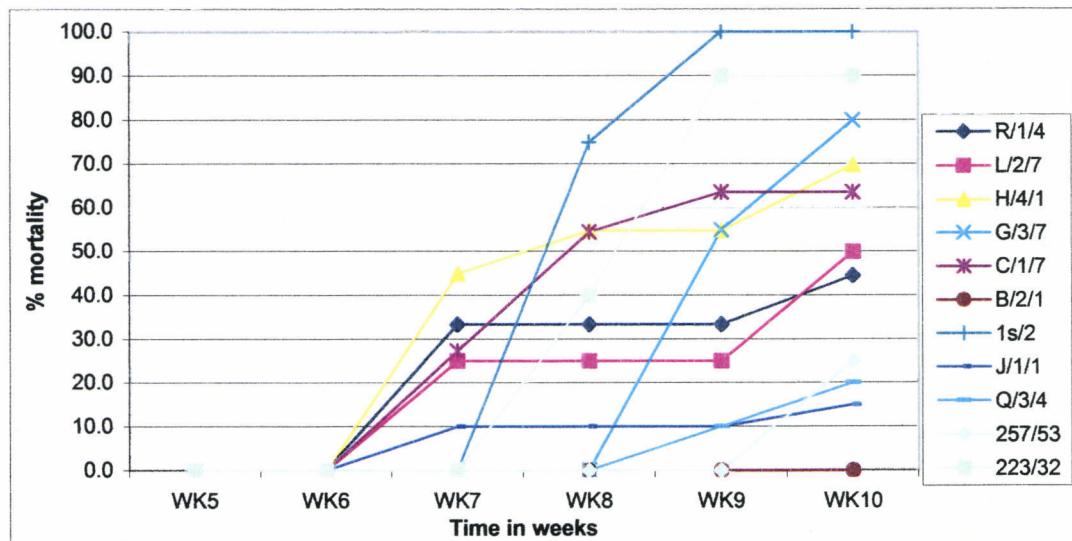


Figure 4: Progression of plant mortality among rooted cuttings of clones in Kituza field trial

CWD Resistance Progenies of wild genotypes from forests and progenies from Kalangala islands

Half sib progenies of wild robusta coffee genotypes from Kibale National Park (forest) and Itwara forest were inoculated together with half sib progenies from Kalangala Islands in Lake Victoria. Plant mortality varied between sources (figure 5). A comparative disease progression among these progenies together with progenies of nganda and erecta populations described above is given in figure 5. The disease progressed at varying rates to varying levels for different sources. It progressed rapidly among nganda and erecta progenies to 67% and 54% plant mortality respectively by week 10 from the time of inoculation. The progression on Kibale progenies was moderate (39%). Plant mortality progressed slowly among Itwara and Kalangala progenies to 19% and 15% respectively. There were differences between progenies within sources for disease progression and final level (Table 3). Mortality among progenies within sources varied from 0% to 90% in Kibale, 0% to 28.6% in Itwara and 0% to 45% for Kalangala. These results indicate that generally genotypes in Kalangala and Itwara have more resistance to coffee wilt disease than genotypes from Kibale. Presence of progenies with zero percent mortality among Kibale, Itwara and Kalangala points to the significance of these genotypes as source of resistance genes for developing wilt resistant varieties.

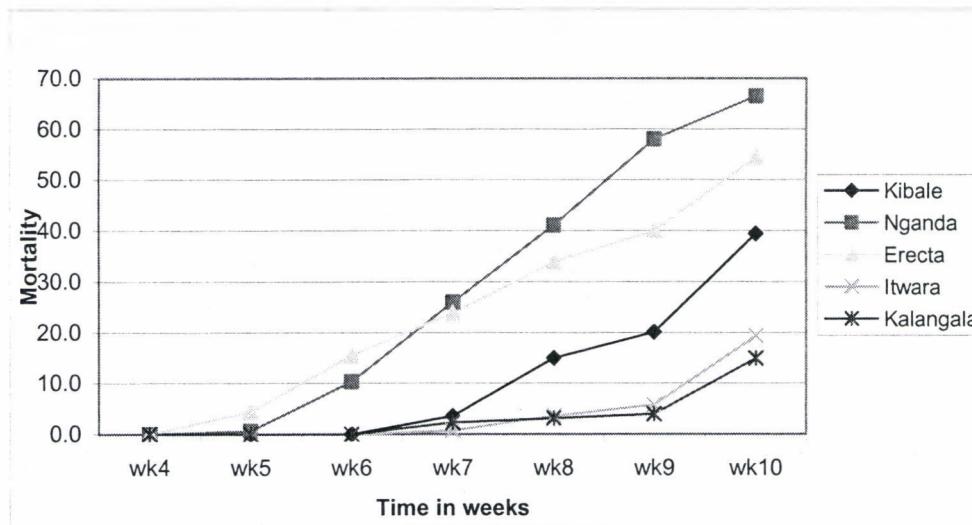


Figure 5: Progression of plant mortality among half sib progenies from different populations

Analysis of variance performed on the 1-5 disease symptom severity data at 10 weeks after inoculation found highly significant ($p<0.0001$) genetic difference between progenies of Kibale and Kalangala (Table 3). The difference between Itwara progenies were not significant at 5% probability but could be considered significant ($p=0.08$) at 8% probability. Mean separation tests performed on the disease symptom severity means within sources ranked the progenies in Kibale and Kalangala into 3 overlapping resistance classes overlaps (Table 4). There were no classes for Itwara, since difference between progenies means from this source are not significant.

Table 3: Analysis of variance table for CWD on *C. canephora* half sib progenies from different populations at week 10 after artificial inoculation

Source	df	ms	ss	f value	pr	C.V
Combined data						
Kibale						
Progeny	6	78.4	13.1	7.7	<0.0001	35.03
Error	63	107.5	1.7			
Itwara						
Progeny	9	38.2	4.2	1.7	0.08	79.9
Error	101	242.3	2.4			
Kalangala						
Progeny	22	113.3	5.1	2.8	<0.0001	78.6
Error	373	677.5	1.8			

Table 4: Mean disease symptom severity among half sib progenies of different *C. canephora* populations at week 10 after artificial inoculation

Kalangala		Itwara		Kibale	
Progeny	Mean	Progeny	Mean	Progeny	Mean
UW218	3.05a	UW154	3.00a	UW010	4.85a
UW204	2.95ab	CPT11 TR2	2.50a	UW004	4.50a
UW199	2.30abc	UW155	2.33a	UW009	4.16ab
UW191	2.07abc	UW146	2.14a	UW012	3.57abc
UW189	2.00abc	UW106	2.14a	UW005	2.86abc
UW205	1.95abc	UW123	2.10a	UW022	2.33bc
UW212	1.85abc	UW090	1.79a	UW008	1.67c
UW210	1.80abc	UW098	1.67a		
UW219	1.80abc	UW136	1.11a		
UW183	1.80abc	UW091	1.07a		
UW180	1.67abc				
UW181	1.50abc				
UW185	1.50abc				
UW215	1.45abc				
UW211	1.40abc				
UW201	1.31bc				
UW206	1.31bc				
UW182	1.30bc				
UW217	1.26bc				
UW203	1.25bc				
UW209	1.20bc				
UW194	1.17c				
UW198	1.00c				

ii) Evaluation for CWD resistance in multi-clonal field trials

During the reporting period, assessment continued on robusta coffee clonal trial at CORI. Data collection on yield and CWD incidence continued. Yield data is not presented in this report but results for CWD since 2001 up to March 31, 2006 are shown in table 5. The results reveal a general increase of plant mortality among the clones during the reporting period. Mortality on clone Q/3/4 remained very low (4.2%) and clone J/1/1 remained resistant (table 5).

Table 5: Percent CWD incidence on clones of *C. canephora* in a field trial at Kituza

Clone	April 01	April 02	April 03	April 04	April 05	March 06
J/1/1	0.0	0.0	0.0	0.0	0.0	0.0
Q/3/4	0.0	0.0	4.2	4.2	4.2	4.2
1S/3	12.5	12.5	12.5	12.5	33.3	33.3
R/1/4	11.1	27.8	33.3	33.3	33.3	33.3
C/6/1	12.5	16.7	16.7	20.8	29.2	50.0
Q/6/1	50.0	50.0	50.0	50.0	50.0	50.0
B/2/1	29.2	41.7	50.0	50.0	54.2	54.2
223/32	12.5	20.8	41.7	41.7	58.3	58.3
L/2/7	12.5	33.3	45.8	54.2	58.3	62.5
Q/1/1	41.7	50.0	50.0	50.0	66.7	66.7
B/1/1	29.2	37.5	50.0	66.7	70.8	75.0
257/53	29.2	62.5	70.8	75.0	83.3	83.3
G/3/7	25.0	37.5	58.3	70.8	79.2	83.3
P/5/1	54.2	70.8	75.0	79.2	87.5	87.5
E/3/2	20.8	70.8	87.5	87.5	87.5	87.5
1S/2	4.2	4.2	20.8	41.7	83.3	87.5
P/3/6	54.2	87.5	91.7	91.7	91.7	91.7
B/6/2	37.5	66.7	83.3	83.3	87.5	91.7
H/4/1	27.8	77.8	83.3	94.4	94.4	94.4
C/1/7	41.7	58.3	83.3	87.5	91.7	95.8
Mean	25.3	41.3	50.4	54.7	62.2	64.5

Analysis of variance performed on assessment data on the 1-5 disease symptom severity data of the first and last assessment dates found highly significant ($p<0.0001$) genetic differences between clones (table 6). Mean separation tests performed on the symptom severity data ranked the clones into 7 overlap resistance classes (Table 7). These results too show variable CWD genetic resistance among *C. canephora* clones.

Table 6: Analysis of variance for coffee wilt disease on *C. canephora* clones in field clonal trial at kituza

Date	Source	df	ss	ms	f-value	Pr>f
27/4/2001	Clone	19	235.56	12.4	4.89	p<.0001
	Replicate	3	1.85	0.62	0.24	0.866
	Error	421	1067.87	2.54		
	Coefficient of variation	76.1				
31/3/2006	Clone	19	573.88	30.2	13.01	p<.0001
	Replicate	3	12.52	4.17	1.8	0.147
	Error	421	977.59	2.32		
	Coefficient of variation	41.82				

Table 7: Mean CWD on *C. canephora* clones affected by coffee wilt disease in clonal field trial at Kitusa

Clones	27/4/2001	12/10/2001	28/3/2002	12/9/2002	21/2/2003	8/8/2003	23/1/2004	9/7/2004	24/12/2004	10/6/2005	25/11/2005	31/3/2006
C/1/7	2.75abc	3.00abcd	3.33abcd	3.58abc	4.33ab	4.46abc	4.5ab	4.54ab	4.67ab	4.67ab	4.83a	4.83a
B/6/2	2.75abc	3.42abc	3.92abc	4.0abc	4.33ab	4.33abcd	4.33ab	4.54ab	4.54abc	4.62ab	4.75a	4.79a
H/4/1	2.61abcd	3.72ab	4.17ab	4.33ab	4.33ab	4.33abcd	4.72a	4.78a	4.78a	4.78a	4.78a	4.78ab
P/3/6	3.46a	4.29a	4.54a	4.67a	4.67a	4.67a	4.67a	4.67a	4.67ab	4.67ab	4.67ab	4.67ab
1S/2	1.17cd	1.21e	1.21fg	1.33ef	1.96de	2.25efg	2.79cde	3.29abcd	4.25abc	4.46abc	4.54abc	4.54abc
E/3/2	2.08abcd	3.12abcd	3.92abc	4.2ab	4.33ab	4.5ab	4.5ab	4.5ab	4.5abc	4.5abc	4.5abcd	4.5abcd
P/5/1	3.33a	3.67ab	3.83abc	3.83abc	3.83abc	4.17abcd	4.29abc	4.5ab	4.5abc	4.5abc	4.5abcd	4.5abcd
G/3/7	2.13abcd	2.37bcde	2.54bcdefg	2.87bcde	3.33abcd	3.67abcde	3.83abcd	4.0abc	4.12abc	4.25abc	4.33abcd	4.42abcd
257/53	2.42abcd	3.29abcd	3.5abcd	3.75abc	3.83abc	3.83abcde	4.0abc	4.0abc	4.33abc	4.33abc	4.33abcd	4.33abcd
B/1/1	2.17abcd	2.21bcde	2.5cdefg	2.87bcde	3.04abcd	3.58abcde	3.67abcd	3.87abc	3.96abc	3.83abcd	4.0abcde	4.0abcde
Q/1/1	2.67abcd	2.67bcde	3.0abcde	3.08bcd	3.08abcd	3.08abcdef	3.17abcde	3.25abcd	3.58abcd	3.67abcde	3.67abcdef	3.67abcdef
L/2/7	1.71bcd	2.17bcde	2.33cdefg	2.33cdef	2.83bcd	2.83cdef	3.12abcde	3.17abcd	3.33abcd	3.37abcde	3.5abcdef	3.5abcdef
223/32	1.63bcd	1.92cde	2.12defg	2.46cdef	2.67bcd	2.75def	2.79cde	2.83cd	3.0cd	3.37abcde	3.37abcdef	3.37abcdef
B/2/1	2.25abcd	2.42bcde	2.79bcdef	3.0bcd	3.0abcd	3.12abcdef	3.12abcde	3.17abcd	3.17bcd	3.17bcde	3.21bcdef	3.25bcdef
C/6/1	1.5bcd	1.67de	1.67efg	1.67def	1.67de	1.67fg	1.87ef	2.0de	2.17d	2.5de	3.04cdef	3.13cdef
Q/6/1	3.0ab	3.00abcd	3.0abcde	3.0bcd	3.0abcd	3.0bcdef	3.0bcde	3.0bcd	3.0cd	3.0cde	3.0def	3.0def
1S/3	1.5bcd	1.67de	1.67efg	1.67def	1.71de	1.71fg	1.71ef	2.0de	2.5d	2.58de	2.62ef	2.67ef
R/1/4	1.44bcd	1.67de	2.11defg	2.33cdef	2.33cde	2.33efg	2.33df	2.33de	2.33d	2.33e	2.33f	2.33f
Q/3/4	1.00d	1.00e	1.04g	1.04f	1.17e	1.17g	1.17f	1.17e	1.17e	1.17f	1.2g	1.25g
J/1/1	1.00d	1.00e	1.0g	1.0f	1.0e	1.0g	1.0f	1.0e	1.0e	1.0f	1.0g	1.0g

Importation and screening exotic germplasm for resistance against CWD in Uganda

CORI still experienced difficulty in getting a partner country to provide foreign materials. The only 69 seedlings of 14 clones received from Ivory Coast through CIRAD in the previous reporting periods and planted out in an isolated field at CORI were maintained in the field in preparation for evaluation.

b) Inheritance of the CWD resistance

Inheritance of resistance to CWD was estimated from disease symptom severity data (1-5 scale) collected from diallel progenies in a field trial at CORI and the clonal trial described above.

Estimation from diallel progenies in the field

Analysis of the progenies and their parents in the field trial at Kituza shows that both the progenies and their parents were differently affected by coffee wilt disease. Mortality among the parents of the diallel progenies ranged from 33-100% (Table 8). Analysis of variance performed on the disease symptom severity data at different assessment dates found significant differences between the parents (Table 9, ANOVA of first and last assessments as example). Mortality among the diallel progenies ranged from 42-100% (Table 8). Analysis of variance performed on the progeny data also found significant genetic differences between the progenies (Table 9). It was observed that progenies of crosses involving highly susceptible parents were generally more susceptible than progenies derived from crosses involving less susceptible parents

Analysis for inheritance of CWD resistance using disease symptom severity data on diallel progenies and a model adapted for half diallel analysis of the Diogene software, found significant general combining ability (GCA) for CWD susceptibility/resistance throughout the assessment period, implying there is additive genetic relationship between parents and offspring for CWD resistance (Table 10). Specific combining ability was only significant for the first assessment. Broad sense heritability estimated from the same progenies at a disease level of 55-65% plant mortality was moderate (27-33%) and corresponding narrow sense heritability was low (less than 0.15, Table 10).

Broad sense heritability estimated from clones in the clonal trial at Kituza, 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.

Table 8: Percent mortality among *C. canephora* parent clones and their diallel progenies used for estimating inheritance of CWD resistance

Parent	Assessment dates							
	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	100.0
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 9: Analysis of variances for disease symptom severity caused by CWD among *C. canephora* diallel progenies used for estimating inheritance f CWD resistance

Date	Defoliation severity (1-5 scale)	% plant mortality	Replicate x family								
			Replicate			Family/Parents					
			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
3-Jun	3.7	65.7	1	1.14	28.64ns	34	2.53	0.001****	34	1.352	7.90ns
3-Dec	3.9	71.2	1	0.056	80.79ns	34	2.245	0.015***	34	1.102	28.32ns
4-Jun	4.0	73.8	1	0.001	97.46ns	34	2.036	0.084**	34	1.073	32.06ns
4-Dec	4.1	76.9	1	0.033	84.97ns	34	1.998	0.113**	34	0.909	57.24ns
5-Jun	4.1	78.3	1	0.046	82.57ns	34	1.863	0.319**	34	0.859	65.32ns
5-Nov	4.2	79.8	1	0.314	58.25ns	34	1.649	1.485*	34	0.878	62.33ns
6-Mar	4.2	79.8	1	0.331	57.26ns	34	1.624	1.768*	34	1.026	38.71ns

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

Table 10: Variance components and heritability estimates from diallel analysis of the diallel progenies

Date	%								% morality	
	mortality	SCA	GCA	V_d	V_a	V_g	V_e	V_p	h²_b	h²_n
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)
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)
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)
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)
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)
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)
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)
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)

* 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; h²_b is narrow sense heritability; h²_n is broad sense heritability. Figure in parenthesis are confidence interval

c) Evaluation of genetic diversity of Ugandan robusta coffee

During this reporting period analysis of genomic DNA of Ugandan *C. canephora* genotypes from Kibale forest (wild), Itwara forest (wild), Kalangala Islands in Lake Victoria (feral), erecta (cultivated with erect phenotype) and nganda (cultivated with spreading phenotype) was completed. Half sib progenies of the mother trees sampled from these sources were analyzed for CWD resistance given above. The diversity of Ugandan genotypes was compared with that of previously known diversity groups within the species (table 11). There is significant observed heterozygosity across sources, confirming the out breeding nature of the species. There was variation in gene diversity across all sources. Within Ugandan populations, wild Kibale and Itwara had lower gene diversity than cultivated populations, including Kalangala, indicating crossings occurred for cultivated genotypes. Mean number of alleles is high in Ugandan sources, compared to other sources. There are private alleles among sources, although the private alleles are harbored by only about 10% of the individuals. Among Ugandan sources, wild sources and Kalangala have the highest number of private alleles. If we consider all sources, wild Guinean, Itwara and Kalangala regions have the highest number of private alleles. However, not a lot of loci have specific alleles in most sources, indicating they have a strong similarity in their genetic base.

Table 11: Diversity statistics

Source	Observed heterozygosity & (p value)	Gene diversity & (p value)	No. of alleles	Private alleles (Ugandan sources)	Private alleles (all sources)
Itwara	0.396 (0.044)	0.586 (0.041)	193 (8.04)	37	14
Kibale	0.288 (0.043)	0.531 (0.051)	177 (7.38)	19	4
Kalangala	0.405 (0.045)	0.628 (0.049)	206 (8.58)	34	17
Nganda	0.407 (0.043)	0.623 (0.048)	194 (8.08)	14	7
Erect	0.397 (0.045)	0.625 (0.048)	172 (7.17)	12	6
Guinean	0.35	0.50	179 (5.29)		24
SG2	0.41	0.69	229 (6.74)		12
SG1	0.27	0.37	242(7.12)		11
Congolese B	0.37	0.50	173 (5.09)		7
Congolese C	0.37	0.45	101 (2.97)		14

Gene diversity for Guinean and Congolese regions was adopted from CUBRY et al., 2005. Number of private alleles for Ugandan regions was detected with 24 markers among 196 individuals. Number of private alleles for all regions was detected with 18 markers on 232 individuals.

Diversity tree of 232 individuals representing the different sources is given in figure 6. The tree separates individuals of Guinean and Congolese groups into their previously described diversity groups, which are different from those of Ugandan individuals. The structure of the Ugandan population has four subgroups which include wild-Kibale, wild-Itwara, feral-Kalangala and erecta-nganda diversity subgroups. These results indicate that Ugandan materials are outside the previously known *C. canephora* genetic diversity and should therefore be considered as a new diversity group within the species.

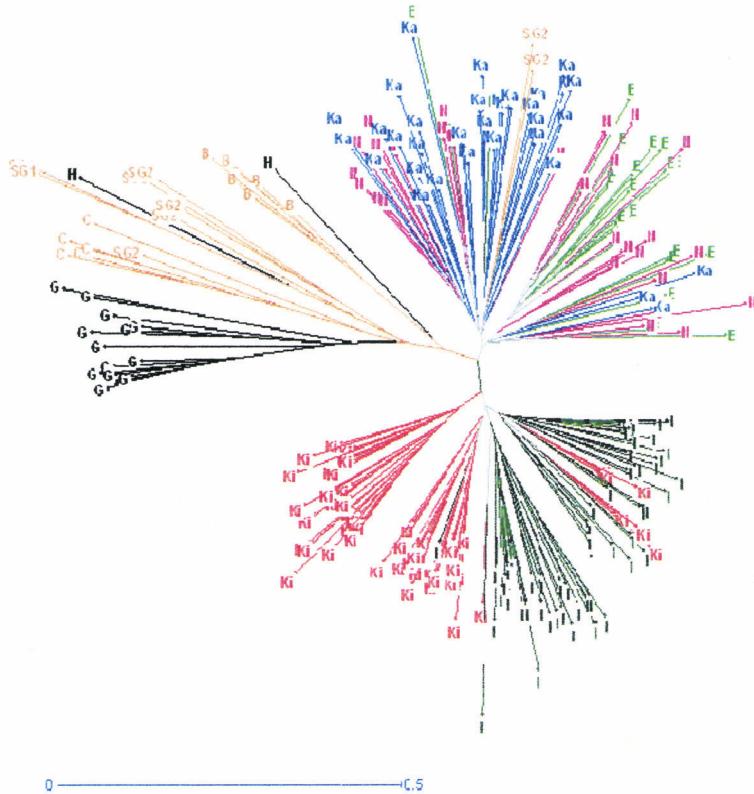


Figure 5: Phylogenetic tree of 232 individuals using weighted neighbor joining method among 18 microsatellite loci. G indicates Guinean; I indicate Itwara forest; KI indicates Kibale forest; N indicates Nganda; K erect and K kalangala islands

Genetic differentiation: F_{ST} coefficients revealed high (> 0.150) differentiation, whether you consider all the ten sources or only the five Ugandan sources. Considering separately the three Ugandan sources constituted by different sites, Kibale has a highly significant F_{ST} coefficient, meaning that sites in this source are highly differentiated into populations. On the other hand, F_{ST} coefficients are not significant within Itwara and Kalangala, meaning that the populations belonging to these sources are not differentiated. Results of the Mantel tests for Ugandan populations pointed out a significant correlation between genetic and geographic distances, indicating geographical distance was responsible for independent evolution of these populations

d) Conclusion

Screen house tests at CORI have continued to identify *C. canephora* genotypes resistant to CWD. These tests have revealed that levels of CWD resistance vary among robusta genotypes and that the resistance is controlled by many genes that are not equally distributed among the genotypes. Field evaluations confirm that resistance levels vary among genotypes. Studies on genetic diversity revealed that Ugandan genotypes are different from known *C. canephora* groups and therefore form a new diversity group

within the species. These studies also confirmed that Ugandan *C. canephora* populations are differentiated into genetic sub groups which are based on geographical distances. The studies found Kalangala, Kibale, Itwara populations genetically distinct from nganda and erecta as a group, meaning nganda and erecta are genetically very similar although they have different phenotypes. Tests performed on progenies from these sources revealed that the populations have variable levels of CWD resistance.

WP 4: Epidemiologie

Task 2: Description of the spatio-temporal spread of the coffee wilt disease

On-farm activities

Wilt incidence increased with time at all the farms but varied with farms probably due to different environmental conditions or field management at each farm. No evident reasons for variation including age of coffee, site of farm whether on flat, level, or steep slope, pruning practices, cropping system, presence or absence of shade and how the diseased trees were managed, can be highlighted.

On-station activities at Kituza

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.

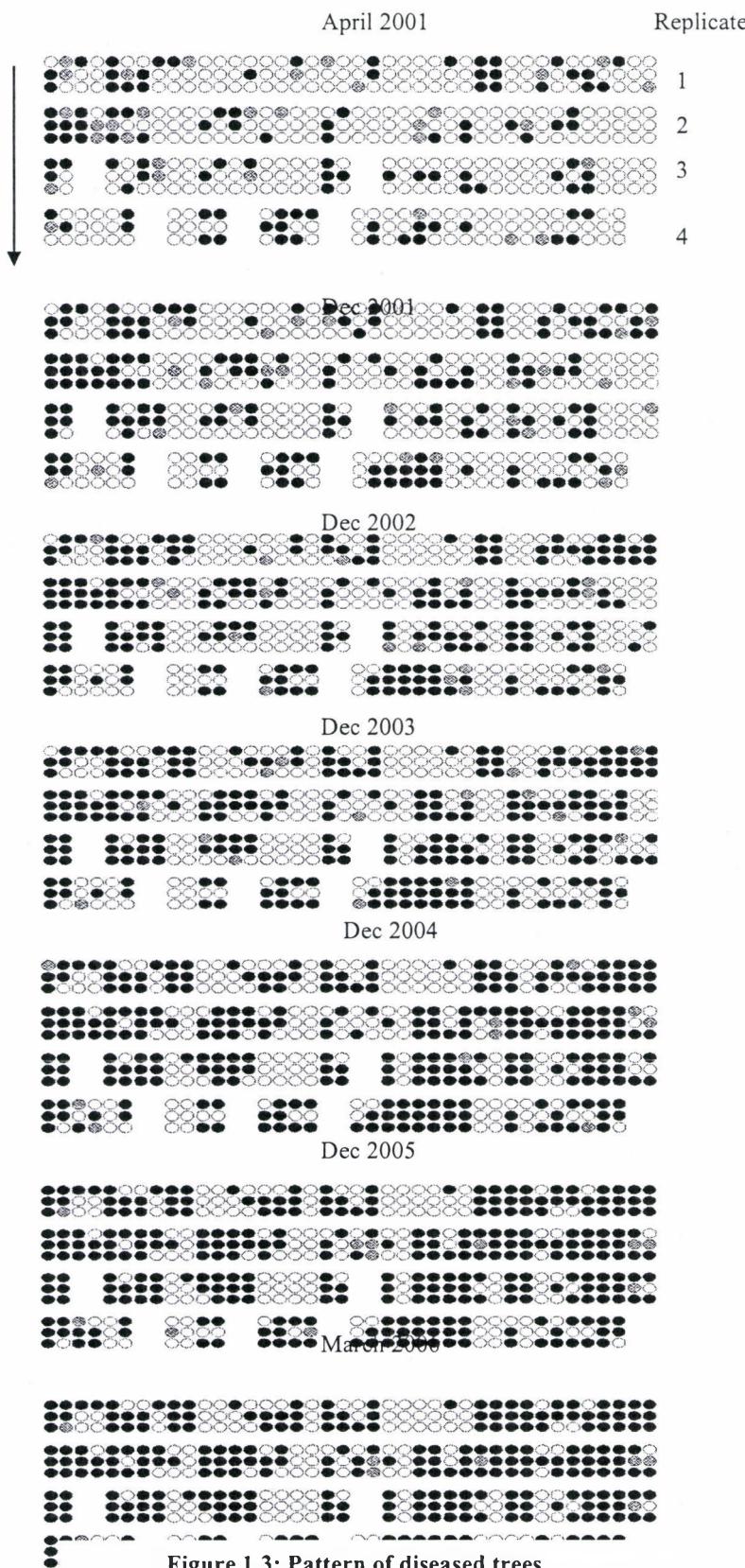


Figure 1.3: Pattern of diseased trees

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

Task 3 Evaluation of the duration of survival form of the pathogen

Survival/inoculum potential of *F. xylarioides* in soil over time

More seedlings were infected in soil freshly sampled than in soil stored over a period of time from the three sites. Seedlings (3 out of 10) planted in freshly sampled forest soil developed wilt symptoms 76 – 83 days and died within 6 – 8 days from first symptoms. No infection occurred in collected from Blocks 36 and 38. Subsequent monthly plantings from the 3 sites on stored soil continued to reveal more wilting from Forest soil up to the 5th month of storage. Soil from Block 36 was infective only in the first and second month of storage, while block 38 had no infection by end of 2005. (Mean latent periods for seedlings planted in freshly picked soil were 52 days. Latent period varied from 60 – 106 days (2 – 4 months) from planting in stored soil depending on length of storage). This strongly implies that the pathogen can persist in soil and become source of inoculum. Nursery operators normally collect fertile soil from the forests. If this soil is not properly sterilized, coffee wilt disease could be initiated or spread to various destinations.

Role of farm tools/machete or panga in transmission of CWD

Different trials have demonstrated that there is possibility of transmission of CWD through contaminated tools commonly used on the farm. The recommendation for sterilization of tools using alcohol, disinfectant or fire is valid and should be adhered to.

Survival of *F. xylarioides* in infected stem/wood pieces over time.

Stored infected wood pieces continued to reveal presence of *F. xylarioides* for 6 months. No growth occurred on agar media from 7 – 12 months. Wood pieces therefore are likely to remain infective for at least 6 months from the death of trees, whether buried in soil or standing in the fields. Uprooting and burning where feasible is still recommended to reduce inoculum.

Task 4 Evaluated in the field the ways and duration of survival of the pathogen.

Inoculum sources from outside the field

There was evidence of sources of infection from outside the coffee field. Infected trees distances away from the source of infection in the field, could imply outside sources of infection. At Kituza, part of the field adjacent to the forest is suspect, since forest coffee also had CWD and most of the trees died of the disease. More infection was observed on trees adjacent to the forest.

Rate of spread within the field depended partly on susceptibility of the clones and partly on other factors mentioned earlier. In the more susceptible clones spread from one plant to another was rapid. More infection and rapid disease progress was observed during the wet season (March – May, September – November) than during the dry season (December – February, June – August).

Description of the fungal life cycle, asexual and sexual phases

Perithecia production on naturally infected coffee trees in the field was monitored at 4 weekly intervals on clones 1s/2, 1s/3, 1s/6, 223/23 and 258/24. The grid positions on coffee trees monitored were:

Top	65 cm from ground level
Middle	35 cm from ground level
Bottom	5 cm from ground level.

Results obtained were in agreement with earlier observations. Perithecia production varied with clones. Clone 1s/2 supported more perithecia than the rest of the clones. Perithecia were rarely produced at the top grid, while perithicia were formed in abundance at the bottom grid in all clones.

Under field conditions during the dry period (December – March) perithecia are not formed, but appear 2 or 3 days after rain. However, off-season rains can also trigger formation of perithecia in the field. At Kituza, off-season rains are not rare, which means in a coffee field there is normally a lot of inoculum.

Similarly, discarded dry seedlings or inoculated plants do not form perithecia unless watered.

Table 2: Perithecia formation on selected clones under field condition at Kituza

Clones	Grid Positions		
	Top	Middle	Bottom
1s/2	+	+	+
1s/3	X	x	+
1s/6	X	x	+
223/23	X	x	+
258/24	X	x	+
Seedlings	Perithecia produced only around collar regions after death of seedlings.		

It is significant to note here that the recommendation for farmers to uproot infected plants at the earliest symptoms is meant to eliminate all sources of infection. However, many farmers do not uproot but cut plants down leaving stumps. Perithecia therefore continue to be formed on the remaining stumps and infection is spread to plants within the field or at distances away.

Description of the fungal life cycle

In all clones perithecia were rarely produced at the top grid, while these were formed in abundance at the bottom grid. Under field conditions during the dry period perithecia were not observed. Within 2 weeks of rainy days after the death of infected trees in the field perithecia formation began from the bases of the stems and progressed upwards along the stems. Off-season rains can also trigger formation of perithecia in the field. At Kituza, off-season rains are not rare, which means in a coffee field there is normally a lot

of inoculum. Similarly, discarded dry seedlings or inoculated plants do not form perithecia unless watered. Perithecia in seedlings were confined to the collar regions and did not extend along the stems.

The pathogen is found in soil though direct isolation from soil was not possible. It is assumed the pathogen (in form of ascospores, mycelial pieces or conidia?) enter the host tissue through the roots and establishes itself in the xylem vessels causing blockage and hence wilting of the plant only on the affected side. When the affected branch dries up, numerous perithecia are formed mostly on the lower part of the stem and a few cm below ground, where blue black staining is present. Infection can also occur though wounds on the lower part of the stems. These wounds are normally inflicted during weeding using hoes or machetes. Wounds higher up the plant do not result in full-blown wilt symptoms. In such cases, the affected branch develops symptoms above point of entry and eventually dries up, but the lower portion of the stem remains unaffected.

Inoculum from diseased stems can be spread by people as they transport firewood, grazing animals, and winds or washed into soil by rain to form soil inoculum or moved by run-off. During the rainy season, the host produces new shoots and is active which result in more infection. During dry season due to moisture stress, disease progress within the plant is slow.

As a control measure it is recommended that farmers uproot infected plants at the earliest symptoms in order to eliminate sources of infection. However, many farmers do not uproot but cut plants down leaving stumps. Perithecia therefore continue to be formed on the remaining stumps and infection is spread to plants within the field or at distances away.

Task 5: Elaboration of a simplified model of the epidemic and proposition of adapted recommendations

This study revealed that CWD spreads from infected *C. canephora* trees to cause new infections of up to three trees away, forming aggregated disease patterns.

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 result 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.

UNIKIN

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

Fifth Annual Report (November 2005 to October 2006)

**University de Kinshasa
B.P. 866, Kinshasa XI RDC**

WP1: PATHOGEN DIVERSITY

Task 4: Description of the fungal life cycle, asexual and sexual phases

Effet des types de croisement (mating type) sur le développement de la trachéomycose dans les conditions naturelles

1. Objectif

Les hypothèses émises sur les causes de la réémergence de la trachéomycose en République Démocratique du Congo, évoquent l'éventualité d'une variabilité génétique que le pathogène aurait connue au cours du temps. La conséquence est le développement de nouveaux pathotypes plus agressifs. Chez les champignons, la sexualité est une cause majeure de la variabilité génétique au sein d'une population fongique. Trois modes de reproduction prévalent chez les champignons filamenteux : l'hétérothallisme, l'homothallisme et la reproduction asexuée (Roger, 1953). Dans son mode de reproduction, qui est de type hétérothallique, le *Gibberella xylospora* (Heim et Saccas) conduirait, en fonction des souches-parents, à une variabilité génétique au sein de sa population (Maraite, Comm. Pers. 2006). En effet, les espèces hétérothalliques nécessitent un partenaire pour compléter le cycle sexuel tandis que chez les espèces homothalliques ce cycle peut être complété sans partenaire (Nasraoui & Lepoivre, 2003).

Les récents travaux conduits *in vitro* sur la sexualité de *G. xylospora* avec des souches congolaises, ougandaises et tanzaniennes, ont démontré chez cette espèce que le mode hétérothallique est contrôlé par un locus avec deux allèles, Mating type 1 (MAT-1) et Mating type 2 (MAT-2). Les isolats compatibles produisent de périthèces fertiles, montrant le suintement des ascospores au niveau de l'ostiole, endéans 2 à 8 semaines après croisement (Lepoint *et al.*, 2005).

Le présent essai a pour objectif d'étudier la sexualité de ces types de croisement dans les conditions naturelles et de déterminer leur influence sur le développement de la trachéomycose par le biais de différents symptômes observés.

2. Matériel et méthodes

2.1 Milieu expérimental

Le Jardin Expérimental de la Faculté des Sciences Agronomiques de l'Université de Kinshasa a servi de site pour le présent essai qui constitue la première répétition de l'étude. Les coordonnées climatiques du site sont les suivantes : 4°19' de latitude Sud et 15°18' de longitude Est et une altitude de 350 m. Le climat est du type AW₄ d'après la classification de Koppén. Le caractère climatique est chaud et humide avec deux saisons, une grande saison sèche de 4 mois, allant de

mi-mai à mi-septembre et une grande saison pluvieuse survenant entre février et mai. La température moyenne annuelle oscille autour de 24°,5°C et accuse de faibles variations. Le mois de juillet est le plus froid et le février est le mois le plus chaud. Les précipitations moyennes annuelles sont de 1500 mm reparties sur 9 mois. L'humidité relative est maximale en avril et en mai. Elle est minimale en septembre et octobre. L'évaporation est maximale à la fin de la saison sèche.

Les principales données climatiques caractéristiques de la période expérimentale sont enregistrées par la Station météorologique du Centre Régional d'Etudes Nucléaires de Kinshasa (CREN-K), située à environ 300 m du site expérimental. Elles sont présentées dans le tableau.

Tableau 1 : Données climatiques enregistrées au cours de la période de l'essai dans le Jardin Expérimental de l'UNIKIN.

Mois de l'année 2005	Température moyenne (°C)	Humidité Relative (%)	Précipitation (mm)
Juin	27,9	76,8	0,20
Juillet	27,5	80,0	0,20
Août	27,9	76,0	17,7
Septembre	27,9	76,0	20,2
Octobre	28,6	80,6	183,4
Novembre	28,6	81,3	248,7
Décembre	27,7	89,6	266,6

Source : Départ. de Physique des sols et hydrologie du Centre Régional d'Etudes Nucléaires de Kinshasa 2005).

2.2 Matériel végétal

Des plantules de cafier issues de la germination des graines récoltées sur un sujet sensible attaqué par la trachéomycose et bien identifié (*Coffea canephora* var Robusta : I1010203/OG) récolté à Beni ont été utilisées dans le présent essai. Les plantules ont été placées dans les sachets en polyéthylène de dimension 15 cm x 35 cm x 0,05 cm remplis de terreau forestier. A l'inoculation les plantules avaient 27 mois d'âge avec 4 à 5 paires de feuilles et un bois aouté en dessous de l'hypocotyle.

2.3 Souches utilisées

Deux types de croisement de *Gibberella xylospora* Heim et Saccas (anamorphe *Fusarium xylospora* Steyaert), Mating type 1 et Mating type 2, ont été utilisés. Ils ont été fournis par le Laboratoire de Phytopathologie de l'Université catholique de Louvain en Belgique, où ils sont déposés à la Mycothèque de cette Université (MUCL) sous la dénomination MUCL44532 et MUCL44536 respectivement pour le Mating type 1 (Mat-1) et Mating type 2 (Mat-2).

2.4 Technique d'inoculation

La méthode d'inoculation utilisée a été celle de blessure pratiquée dans la tige et l'insertion d'un inoculum sous forme de fragment de culture du pathogène de 5 mm de diamètre. Ce fragment est prélevé à l'aide d'une perce-neige sur milieu SNA en boites de Pétri maintenues à 27°C à l'obscurité dans l'étuve pendant 10 jours. L'endroit précis de prélèvement dans la boîte de Pétri est déterminé par la présence d'une forte densité en conidies observée à l'envers de la boîte de Pétri sous microscope. L'insertion de l'inoculum dans l'entaille sur la tige a été précédée par une désinfection superficielle avec de l'éthanol à 70% qui a été laissé s'évaporer pendant 5 minutes. Les points d'inoculation ont été pratiqués en différents endroits sur la tige des cafiers et constituent les traitements étudiés :

- Traitement 1 : Mating type 1 (Mat-1) inoculé à 1cm en dessous des feuilles cotylédonaires sur le plan de la première paire de vraies feuilles ;
- Traitement 2 : Mating type 2 (Mat-2) inoculé à 1cm en dessous des feuilles cotylédonaires sur le plan de la première paire de vraies feuilles ;
- Traitement 3 : Mating type 1 et Mating type 2 inoculés en position face- à face à 1cm en dessous des feuilles cotylédonaires ;
- Traitement 4 : Mating type 1 inoculé à 1 cm au-dessus de Mating type 2;
- Traitement 5 : Mating type 2 inoculé à 1 cm au-dessus de Mating type 1 ;
- Traitement 6 : Témoin non inoculé

2.5 Variables observées

L'expression de la maladie est évaluée par quatre variables se rapportant aux symptômes observés au rythme de 7 jours.

- Moment d'apparition des symptômes : il permet de déterminer la précocité de l'apparition de chaque symptôme ;
- Sévérité des symptômes : elle est exprimée par le pourcentage de plants présentant un symptôme donné par rapport aux plants observés;
- Abondance des périthèces : il s'agit du nombre des périthèces, établi selon l'échelle de cotation mise au point par Maraite (2002) : **0** = pas de périthèces ; **1** = jusqu'à 3 périthèces simples ou groupés ; **2** = plus de 4 à 10 périthèces ; **3**= plus de 10 périthèces ;

- Etat des périthèces : représenté par différentes manifestations des périthèces. Il est exprimé par l'échelle de cotation (Maraite, 2000) : **G** = Stroma avec périthèces à ostiole visible; **S** = Stroma sombre sans périthèces ; **E** = Périthèces vides et dégradés.

L'abondance et l'état de la maturation des périthèces sont étudiés sur la tige des sujets malades, dans un espace délimité par un cylindre de 1 cm de hauteur. A partir du point d'inoculation on marque, à l'aide d'un stylo à encre indélébile, cette aire sur tige dans les deux sens à partir des points d'inoculation. Les observations sont faites sous loupe binoculaire. Les périthèces individuels sont prélevés avec le scalpel à lame n°11 et montés dans une goutte du lactophénol bleu et leur identification est vérifiée au microscope, d'après la description de *Gibberella xylarioides* (Saccas, 1951; Booth 1971) et l'examen des asques et des ascospores.

2.6 Dispositif expérimental et analyse statistique

L'expérimentation a été conduite suivant un dispositif en blocs complètement randomisés de 6 unités expérimentales par bloc. Chaque unité expérimentale est constituée de 9 plants qui ont été tous observés. Les données sont analysées à l'aide des procédures aov (Analyse de la variance) et glm (Modèle Linéaire Généralisé) à l'aide du logiciel R, version 2.4.0 du 03/10/2006 (The R Foundation for Statistical Computing, <http://cran.r-project.org>). La comparaison multiple des moyennes a été faite par le test HSD de Tukey du même logiciel.

3. Résultats

Les observations faites sur les sujets inoculés ont montré, dans l'ordre chronologique, l'apparition des manifestations symptomatologiques suivantes : le brunissement des feuilles, le dessèchement des feuilles et la mortalité des plants. Le moment d'apparition de ces différents symptômes varie selon les traitements. Nous présentons dans les figures 1, 2 et 3 les résultats en rapport avec le moment d'apparition respectivement du brunissement, dessèchement et de la mortalité.

3.1 Moment d'apparition des symptômes (jour après inoculation)

Le temps écoulé entre le moment d'inoculation et l'apparition des symptômes a permis d'évaluer la précocité dans l'attaque de la trachéomycose induite par les deux types de croisements inoculés en différentes positions sur la tige.

3.1.1 Brunissement des feuilles

L'examen de la figure 1 montre que la période d'incubation, conduisant à l'apparition du brunissement n'est pas différente quel que soit le traitement ($p=0.660$).

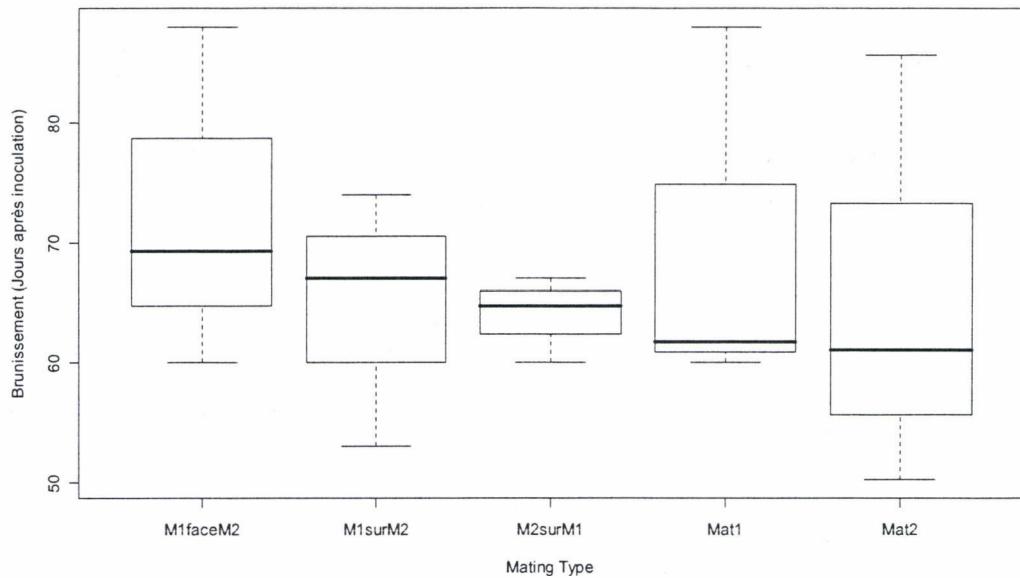


Fig. 1 : Moment d'apparition du brunissement enregistré sur des plantules de cafier inoculées avec les Mating type 1 et Mating type 2 du *G. xylophagoides* en différentes positions sur la tige.

3.1.2 Dessèchement des feuilles

La figure 2 révèle que les mating types, utilisés seuls ou en combinaison montrent des différences en ce qui concerne le dessèchement des feuilles ($p=5.817e-06$) avec les plants soumis au Mating Type 1 qui se dessèchent plus tardivement que les autres ($p=0.043$). En admettant le seuil de 20%, cet effet retard de MAT - 1 dans l'induction de ce symptôme pourrait être perçu et hiérarchisé dans quelques-unes de ses combinaisons comme suit : MAT-2 sur MAT - 1 ($p=0.06$), MAT - 1 face MAT - 2 ($p=0.18$) et MAT - 1 sur MAT - 2 ($p=0.20$).

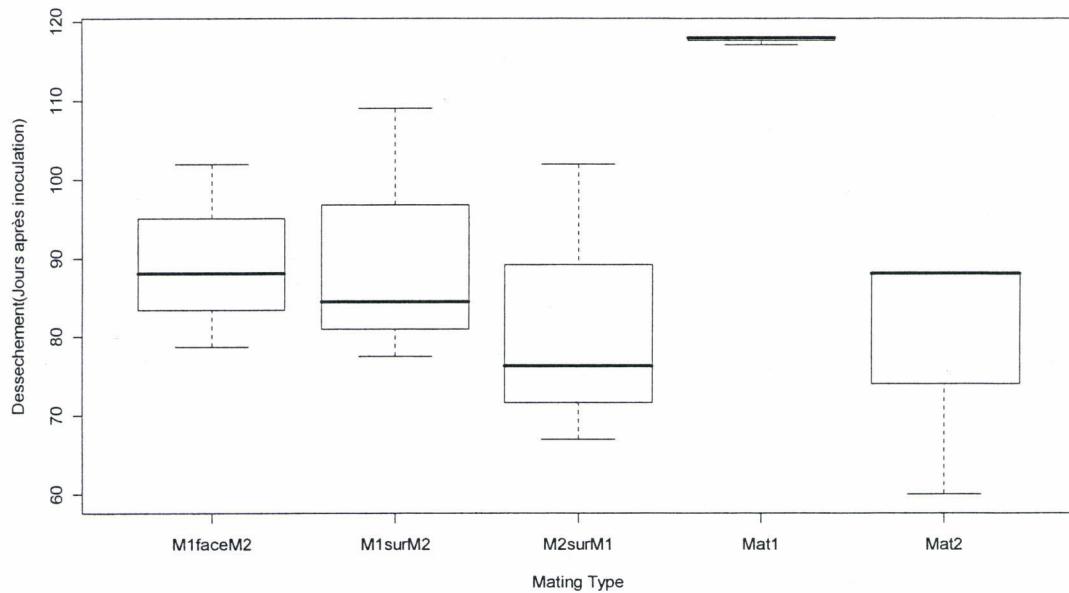


Fig. 2 : Moment d'apparition du dessèchement des feuilles de caféiers inoculés avec les Mating type 1 et Mating type 2 du *G. xylosporus* en différentes positions sur la tige.

3.1.3 Mortalité des plants

La figure 3 indique que la mortalité intervient à des périodes différentes ($p=1.199e-12$) selon le type de croisement. L'inoculation avec le MAT - 1 induit plus tardivement la mortalité. Dans la combinaison, l'influence de MAT - 2 est plus importante quelle que soit la position d'inoculation.

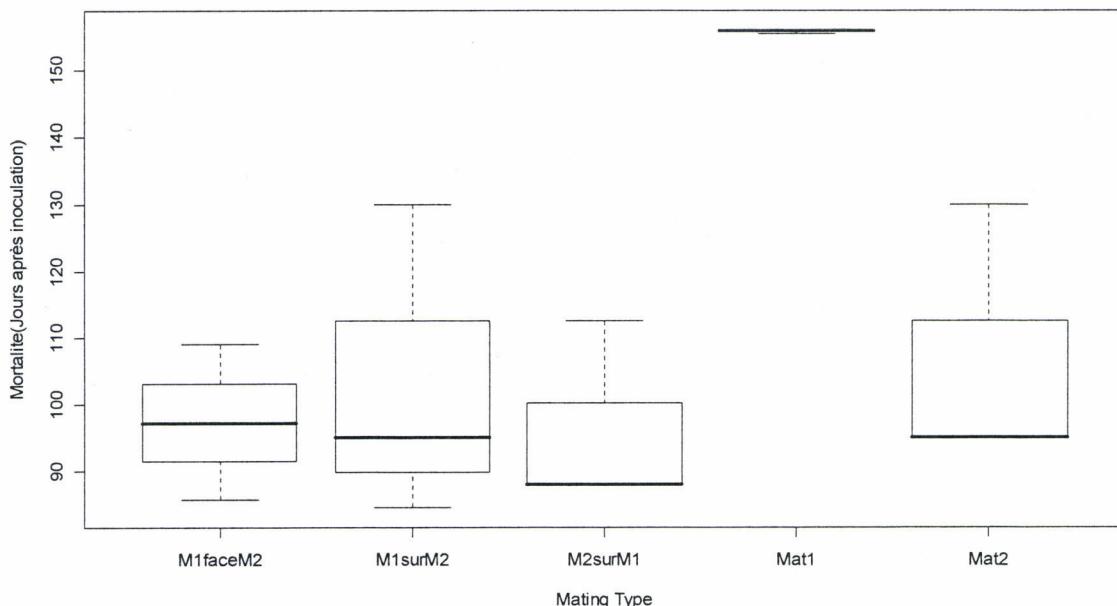


Fig. 3 : Moment d'apparition de la mortalité des plantules de caféier inoculées avec les Mating type 1 et Mating type 2 du *G. xylosporus* en différentes positions sur la tige.

3.2 Taux de différents symptômes

L'évaluation du niveau de différents symptômes est présentée dans les figures 4, 5 et 6.

3.2.1 Brunissement des feuilles

La figure 4 décèle une nette différence entre le niveau de brunissement des feuilles des plants non inoculés (Témoin) les plants inoculés. La comparaison de 2 types de croisement, utilisés isolément en inoculation ou en combinaisons ne révèle pas des différences dans la sévérité de brunissement des feuilles.

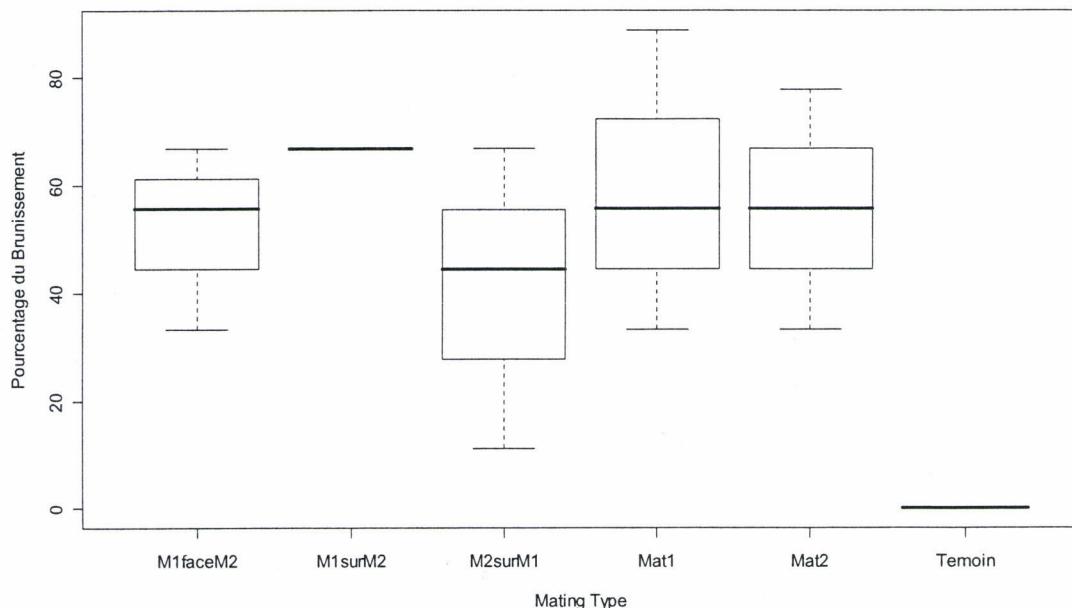


Fig. 4 : Sévérité du brunissement enregistrée sur des plantules de cafier inoculées avec les Mating type 1 et Mating type 2 du *G. xyloariooides* en différentes positions sur la tige.

3.2.2 Desséchement des feuilles

La figure 5 illustre que les plants ayant subi des inoculations avec les Mating types, seuls ou en combinaison, présentent des cas de dessèchement des feuilles, contrairement aux plants non inoculés ($p=0.02296$). Cependant les plants inoculés avec le MAT-1 semblent avoir moins de feuilles desséchées que ceux inoculés avec le MAT-2 ainsi que dans tous les cas où ce dernier intervient en combinaison. L'influence de MAT-2 est encore prépondérante comme dans le cas de taux brunissement des feuilles.

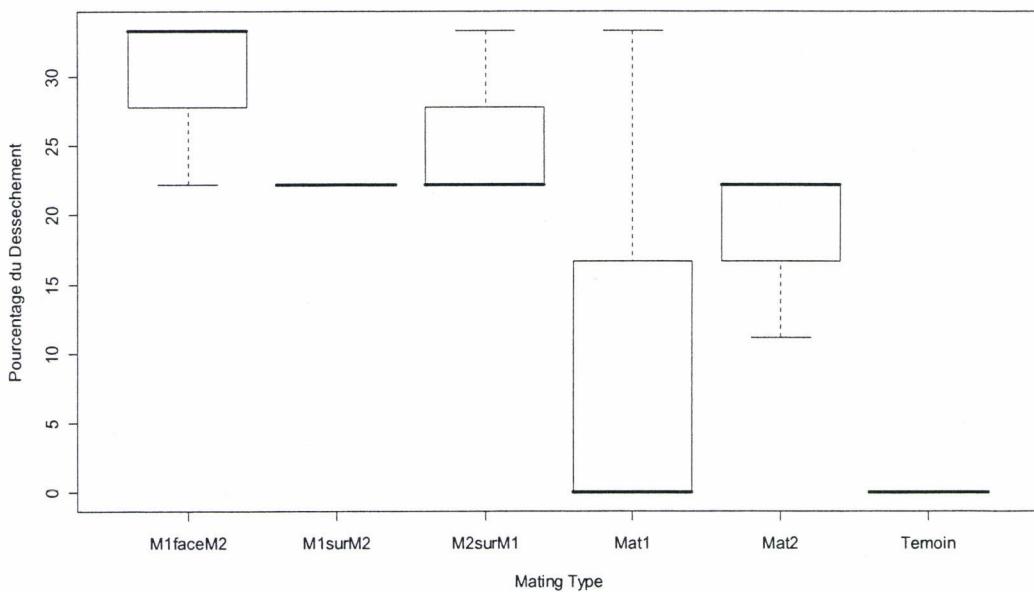


Fig. 5 : Sévérité du dessèchement enregistrée sur des plantules de caféier inoculées avec les Mating type 1 et Mating type 2 du *G. xyloariooides* en différentes positions sur la tige.

3.2.3 Taux de mortalité des plantes

Il se dégage de l'observation de la figure 6 que seuls les plants inoculés avec les 2 types de croisements ont connu de manière très significative ($p= 0.002982$) des cas de mortalité, contrairement ceux qui ne sont pas inoculés. Le Mating type 1 semble avoir une faible importance dans la mortalité des plants, tandis que le Mating type 2 aurait un effet contraire, seul ou dans toutes ses combinaisons où il intervient.

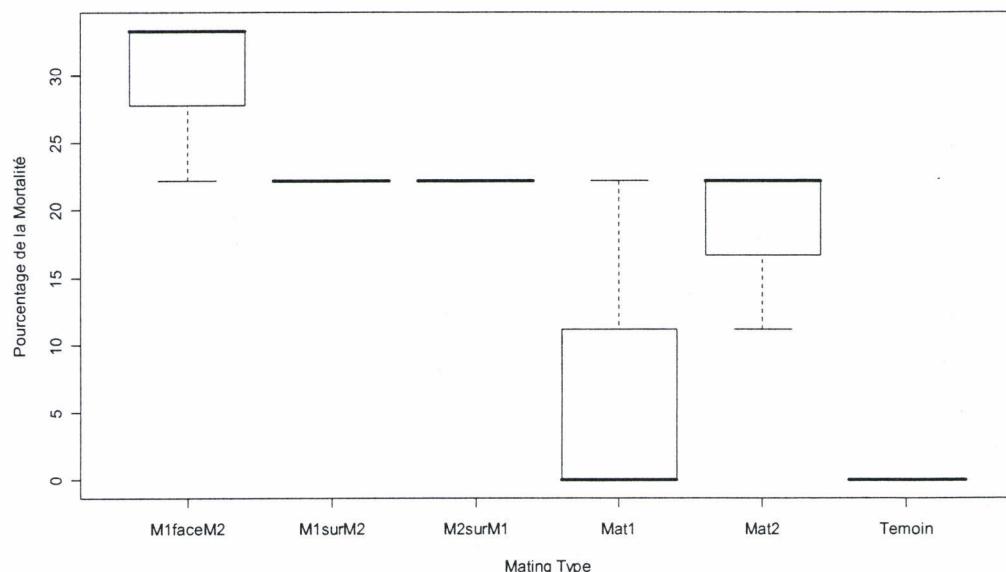


Fig. 6 : Mortalité enregistrée sur des plantules de caféier inoculées avec les Mating type 1 et Mating type 2 du *G. xyloariooides* en différentes positions sur la tige.

A l'issue des observations sur le moment d'apparition et la sévérité des symptômes, il convient de retenir l'effet plus marqué de Mating type 2, inoculé seul et en combinaison avec le Mating type 1. En effet, lorsque des différences sont enregistrées entre les deux types de croisement, le Mating type 2 entraîne plus précocement le dessèchement des feuilles (fig. 2) et la mortalité des plants (fig.3) que le Mating type 1. Il en est de même dans leurs combinaisons où la tendance est orientée vers le Mating type 2.

Les corrélations calculées révèlent des différentes valeurs entre les symptômes observés. Une corrélation positive a été trouvée entre le dessèchement et mortalité ($R^2 = 0.8304$). Par contre entre le brunissement et la mortalité, il n'y a pas eu de corrélation ($R^2 = 0.2212$), comme entre le brunissement et le dessèchement ($R^2 = 0.1174$).

3.3 Production des périthèces

Les résultats concernant la production et l'état des périthèces sont présentés dans le tableau 2.

Tableau 2 : Production des périthèces enregistrée après inoculation avec les Mating types 1 et 2 en différentes positions sur la tige des plantules de cafiers.

Position des points d'inoculation	Abondance	Fréquence (%) de différents états		
		S	G	E
MAT - 1	0	0	0	0
Mat 2	2	20	0	0
MAT - 1 face à MAT - 2	2	50	12,5	0
MAT - 1 sur Mat 2	3	33,3	16,6	0
MAT - 2 sur Mat1	3	16,6	0	16,6
Témoin	0	0	0	0

La comparaison de deux types de croisement montre que les périthèces sont plus abondants sur les plants inoculés avec le Mating type 2 (cote 2) que sur les inoculés avec le Mating 1 (cote 0). En combinaison, aucune tendance dans la position de l'un par rapport à l'autre n'est perceptible.

En ce qui concerne la fréquence de différents états des périthèces, il apparaît que l'état de périthèces bien formés (état G) est enregistré avec des combinaisons de deux types en position face-à-face (12,5 %) et lorsque le Mat-1 est au-dessus de Mat-2 (16,6%). Par contre sur les plantules inoculées avec le Mat-1 et tout comme sur celles qui n'ont pas été inoculées, aucun état de périthète n'est enregistré.

Par ailleurs il est remarqué l'absence d'une suite logique dans l'enregistrement de différents états de périthèces, à partir de stroma (état S) jusqu'à l'évidement des périthèces (stade E) en passant par leur maturité (état G). Sur les tiges des plants inoculés avec le Mat-2, le développement des périthèces (états G et E) à partir des 20 % des cas de stroma n'a pas été perçu. Il en est de même pour

les plants inoculés avec les différentes combinaisons : l'état E est absent sur les Mat-1 face au Mat-2 ainsi que Mat-1 au-dessus de Mat-2. L'état G à son tour n'est pas observé sur les plants inoculés avec la combinaison Mat-2 au-dessus de Mat-1. Ce constat pourrait suggérer que le rythme des observations, qui est hebdomadaire, soit raccourci. Il offrirait l'espoir saisir tous les stades du développement des périthèces, en particulier dans les cas de combinaisons de deux de croisements étudiés. Ainsi le moment de suintement des ascospores, signe de fertilité des périthèces, pourrait être détecté. Une autre suggestion va dans le sens de coupler toutes ces observations avec les données météorologiques *in situ* afin d'établir une meilleure causalité entre ces facteurs et la maturité des périthèces.

WP 3: Breeding for resistance

Screening tests for varietal evaluation

1. Objectif

L'essai sur la résistance variétale a été conduit à Beni dans le Nord-Kivu en conditions naturelles de la trachéomycose depuis le mois de mai 2006. Il a comme objectif le criblage de différents génotypes du caféier pour la résistance à la trachéomycose en vue de déterminer ceux qui présenteraient un bon niveau de résistance et qui peuvent être inclus dans le schéma de sélection pour leur diffusion auprès des planteurs.

2. Matériel et méthodes

2.1 Génotype végétal

Les plantules utilisées dans cet essai sont issues de la germination des graines récoltées individuellement sur 35 différents pieds de caféier Robusta dans Bloc-café de la Station de l'INERA Kiyaka. La récolte des graines a été effectuée sur de différentes lignes dans ce Bloc. Chaque ligne de plantation représente un matériel génétique différent, introduit dans un essai d'adaptation locale dans cette Station. A l'inoculation les plantules ont l'âge de 8 mois avec l'hypocotyle bien aouté. Elles sont identifiées dans le tableau 3.

Tableau 3 : Liste de génotypes inoculés pour la résistance à la trachéomycose en conditions naturelles d'expression de la maladie.

N° d'ordre	Identité de la variété	N° d'ordre	Identité de la variété
1	KR 16/13A	19	KR 20/51
2	KR19/1A	20	KR 19/11
3	KR 18/10A	21	KR 12/6A
4	KR A/6	22	KR 19/28
5	KR 17/55	23	KR 20/50
6	KR 19/1B	24	KR 20/10
7	KR 18/30	25	KR 17/47
8	KR 19/12	26	KR 8/10
9	KR 10/7A	27	KR19/55
10	KR 19/18A	28	KR 16/55
11	KR 19/18B	29	KR 1/1
12	KR 1/3	30	KR 6/6
13	KR 18/10	31	KR 16/13B
14	KR 8/8	32	KR 9/8
15	KR 10/7B	33	KR 3/5
16	KR 19/26	34	KR 2/5
17	KR 20/31	35	KR C/3
18	KR 19/31		

2.2 Souche utilisée

Les plantules sont inoculées chacune avec une souche récente de *Fusarium xylarioides*, dénommée Betonge, isolée à partir du matériel en provenance de cette localité (Betonge) dans la

Province Orientale. L'échantillon d'où elle est isolée a été récolté le 24/12/2005 et cultivé sur milieu SNA.

2.3 Méthode d'inoculation

L'inoculation a eu lieu le 25 mai 2006. Elle a été réalisée par la méthode d'insertion d'un fragment de culture gélosée du pathogène dans une entaille précédemment décrite (point 2/4).

2.4 Dispositif expérimental et analyse statistique

L'essai a été conduit suivant un diapositif en blocs complètement aléatoires. Chaque bloc, représentant une répétition comporte 35 parcelles correspondant génotypes étudiés. Dans chaque parcelle, il y a 20 plantules qui sont inoculées et font l'objet des observations



Fig.7 : Une vue de l'essai de résistance variétale conduit en milieu naturel à Beni (RDC)

Variables observées

Les différents symptômes de la trachéomycose du cafier ont été enregistrés suivant leur délai d'apparition et leur sévérité.

3. Résultats

Les données en rapport avec l'incidence et le taux de différents symptômes sont en cours de traitement. La figure 8, présente le taux de mortalité enregistré sur les différents génotypes à 5 mois après inoculation.

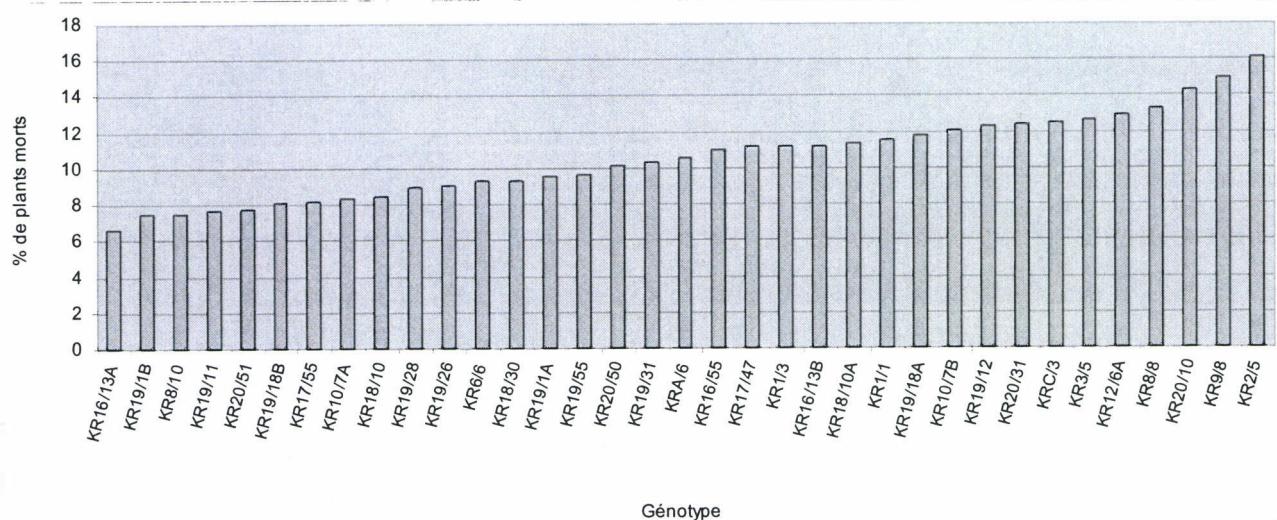


Fig. 8 : Niveau de résistance à la trachéomycose de différents génotypes de caféier robusta à 5 mois après inoculation artificielle avec une souche de *F. xyloarioïdes*

Au regard du pourcentage de mortalité enregistré, il apparaît qu'il existe des différences ($p<0,05$) dans le comportement des génotypes de caféier étudiés pour leur résistance à la trachéomycose. De différents groupes qui sont dégagés par le test de Tukey sont présentés dans le tableau 4.

Tableau 4 : Comportement variétal (% de mortalité de plants inoculés) de différents génotypes de cafier testés pour la résistance à la trachéomycose.

N° d'ordre	Génotype	% de plants morts	Groupe
1	KR16/13A	6,6329	a
2	KR19/1B	7,5329	a
3	KR8/10	7,5329	a
4	KR19/11	7,6329	a
5	KR20/51	7,7829	a b
6	KR19/18B	8,0829	a b
7	KR17/55	8,1829	a b
8	KR10/7A	8,3829	a b
9	KR18/10	8,4329	a b
10	KR19/28	8,9329	a b c
11	KR19/26	9,0829	a b c
12	KR6/6	9,2829	a b c
13	KR18/30	9,3329	a b c
14	KR19/1A	9,5329	a b c
15	KR19/55	9,6829	a b c
16	KR20/50	10,1829	a b c
17	KR19/31	10,3329	a b c
18	KRA/6	10,5829	a b c
19	KR16/55	11,0329	a b c
20	KR17/47	11,1829	a b c
21	KR1/3	11,2329	a b c
22	KR16/13B	11,2329	a b c
23	KR18/10A	11,3829	a b c
24	KR1/1	11,5329	a b c
25	KR19/18A	11,8329	a b c
26	KR10/7B	12,0829	a b c
27	KR19/12	12,3329	a b c
28	KR20/31	12,3829	a b c
29	KRC/3	12,4829	a b c
30	KR3/5	12,6329	a b c
31	KR12/6A	12,8829	a b c
32	KR8/8	13,2829	b c
33	KR20/10	14,2829	b c
34	KR9/8	14,9829	b c
35	KR2/5	16,0829	b c

L'analyse du tableau 4 montre que les génotypes étudiés se classent en 4 différents groupes représentés par des lettres différentes lettres. Le premier comprend les génotypes avec le pourcentage de mortalité des plants allant de 6,63 à 7,63 % (représenté par la figure 9) ; le deuxième de 7,78 à

8,43 % (représenté par la figure 10) ; le troisième de 8,93 à 12,88 % (représenté par la figure 11) et le quatrième de 13,28 à 16,08 % (représenté par la figure 12). Au vu de ces différents groupes, le premier et le quatrième sont les plus contrastés et peuvent suggérer le seuil de 8 % comme seuil supérieur des génotypes résistants et celui de 13 % comme seuil inférieur des génotypes sensibles. Les génotypes avec des valeurs intermédiaires peuvent encore subir des tests de confirmation de la résistance.



Fig. 9 : Une parcelle représentant un génotype résistant.



Fig. 11 : Une parcelle représentant un génotype sensible (KR 8/8).



Fig. 10 : Une parcelle représentant un génotype à confirmer pour la résistance (KR 817/47).



Fig. 12 : Une parcelle représentant un génotype sensible (KR 2/5).



Fig. 12 : Une parcelle représentant un génotype sensible (KR 2/5).

ANALYSE DE LA DIVERSITE GENETIQUE DES ACCESSIONS DE CAFEIERS ROBUSTA (*Coffea canephora* Pierre)

1. Objectif

L'évaluation de la variabilité génétique dans les populations de *C. arabica* en utilisant des marqueurs moléculaires tels que RAPD, ISSR, ALFP, et SSR a été sujet de plusieurs études (Aga *et al.*, 2005 ; Agwanda *et al.*., 1997 ; Anthony et autres., 2002 ; Chaparro *et al.*, 2004 ; Lachermes *et al.*, 1993 ; et Masumbuko & Bryngelsson, 2006). Des analyses semblables n'ont pas été menées chez le *C. canephora*, particulièrement pour le matériel génétique provenant d'Afrique. L'objectif principal de la présente étude est d'évaluer le niveau de la variation génétique parmi des accessions du café robusta de la République démocratique du Congo en utilisant des marqueurs de RAPD et d'ISSR. Une telle diversité offrira l'espoir d'établir une liaison avec la résistance testée en champ.

2 Matériel et méthodes

2.1 Ressources génétiques

Des graines récoltées sur 32 pieds-mères de caféier (*Coffea canephora* Pierre) var Robusta ont fait l'objet de la présente étude. Elles ont été récoltées dans le Bloc-Café de la Station de Recherche de l'Institut National pour l'Etude et la Recherche Agronomiques (INERA) à Kiyaka dans la province de Bandundu en RDC (tableau 5). Les graines ont été déparchées et gardées à 4°C avant l'extraction de l'ADN.

Tableau 5: Pieds-mères fournisseurs des graines et embryons de caféier Robusta en provenance de Kiyaka (RDC)

N° d'ordre	Identité des accessions	N° de la ligne de plantation	N° du pied-mère dans la ligne
1	KR 1/1	1	1
2	KR 1/3	1	3
3	KR 2/5	2	5
4	KR 8/8	8	8
5	KR 8/10	8	10
6	KR 9/8	9	8
7	KR 10/1	10	1
8	KR 10/8	10	8
9	KR 11/8	11	8
10	KR 12/1	12	1
11	KR 12/4	12	4
12	KR 12/6	12	6
13	KR 14/1	14	1
14	KR 14/10	14	10
15	KR 15/3	15	3
16	KR 15/4	15	4
17	KR 15/6	15	6
18	KR 15/12	15	12
19	KR 16/2	16	2
20	KR 16/5	16	5
21	KR 16/7	16	7
22	KR 17/48	17	48
23	KR 17/55	17	55
24	KR 19/11	19	11
25	KR 19/12	19	12
26	KR 19/18	19	18
27	KR 19/31	19	31
28	KR 20/3	20	3
29	KR 20/50	20	50
30	KR 20/51	20	51
31	KR A/1	A	1
32	KR B/4	B	4

Les accessions qui présentent un même numérateur représentent des descendants de demi-sœurs qui partageraient en commun un même parent maternel. Ces parents ont été sélectionnés environ 50 ans sur base de leur productivité et de la taille des fruits.

Un échantillon de 30 graines déparchées a été considéré pour de chaque accession. Les graines ont été débarrassées des débris, lavées et placées dans l'azote liquide pour l'extraction de l'ADN. En outre, 30 embryons ont été extraits d'un autre échantillon de graines de chaque accession à l'aide d'un scalpel. Pour favoriser cette extraction, les graines déparchées ont été imbibées dans l'eau de robinet pendant 3 semaines avec changement journalier de l'eau. Les embryons extraits ont été gardés à -20°C jusqu'au moment de l'extraction de l'ADN.

2.2 Extraction de l'ADN

L'ADN génomique des graines a été extrait d'une part des graines entières et d'autre part des embryons extraits des graines

Extraction de l'ADN à partir des graines

Le mode opératoire pour l'extraction de l'ADN génomique des graines a été inspiré de celui de Nkongolo *et al.* (2001) avec de légères modifications. Trente graines entières par matériel ont été broyées dans un mortier en présence de l'azote liquide jusqu'à l'obtention d'une poudre fine. La poudre ainsi obtenue a été suspendue dans 10 ml de tampon CTAB (100 mM Tris HCl pH 8,5, 20 mM EDTA, 1.4 M NaCl, 2% [v/v] CTAB) préchauffé à 60°C. Elle a été ensuite incubée dans un bain-marie à 60°C. Après incubation un volume équivalent (10 ml) de mélange chloroforme-octanol (24:1) a été ajouté aux échantillons pour être lavés trois fois. Après chaque lavage, les échantillons sont centrifugés à 13.000 tours par minutes (RPM) pendant 15 minutes à 20°C. Après le dernier lavage, un volume équivalent d'Isopropanol a été ajouté au surnageant afin de précipiter l'ADN. Les échantillons sont gardés une nuit à -20°C. Ils ont été ensuite centrifugés à 6000 RPM pendant 20 minutes à 4°C. Un volume de l'éthanol suffisant pour immerger le culot a été ajouté pour laver l'ADN pendant 10 minutes. Après séchage par aspiration de traces de l'éthanol sous vide, l'ADN a été dissout dans 250 µl de tampon TE (Tris-EDTA). La quantification de l'ADN a été effectuée avec le Fluorescent DNA Quantification Kit (Bio-Rad).

Le test de qualité a été réalisé dans le but de s'assurer que l'ADN n'est pas dégradé. Un volume de 5 µl d'ADN de chaque échantillon a été soumis à l'électrophorèse dans un gel d'agarose 1% contenant 0,5 µg/ml de bromure d'éthidium. Les produits de gel ont été interprétés avec le système Bio-Rad ChemiDoc XRS et analysés grâce au logiciel Discovery Series Quantity One.

Extraction de l'ADN à partir des embryons

A partir des embryons l'extraction d'ADN a été faite suivant même mode opératoire que celui de l'extraction à partir des graines. La principale modification a consisté en l'utilisation de PolyVinylPyrrolidone ou PVP (Sigma, Chemical Co, St Louis, USA) à 1% et de β-mercaptopropanol (Sigma, Chemical Co, St Louis, USA) 0,30% dans le tampon CTAB.

2.3 Amplification de l'ADN

Six amores RAPD et 7 amores ISSR, synthétisées par Invitrogen Life Technologies ont été choisies dans un test préliminaire d'amplification fait sur un échantillon de 4 accessions tirées au hasard du groupe du lot des accessions (tableau 5). Toutes les amores ont été standardisées dans une solution stock à 20 ng/ml. L'amplification de l'ADN a été effectuée suivant le mode opératoire décrit par Nkongolo (1999). Chaque réaction a été réalisée avec le kit Applied Biosystems, New Jersey-USA, dans un volume de 25 µl contenant 10,4µl d'eau; 2 µl d'ADN génomique; 2,1µl de tampon 10x PCR Buffer II; 4µl de MgCl₂; 0,5 de chaque nucléotide, 0,5 µl d'amorce et 4 µl de *Taq* polymérase. Pour chaque amorce, un témoin négatif, ne contenant pas d'ADN, a été inclus. Une goutte d'huile minérale a été ajoutée dans chaque réaction pour éviter l'évaporation dans le thermocycleur. Les échantillons ont été amplifiés dans le thermocycleur (Perkin Elmer, Norwalk, CT 06856, USA). Les cycles ont été programmés comme suit : une dénaturation initiale à 95°C pendant 5 minutes, suivie de 2 minutes d'incubation à 85°C, après laquelle la *Taq* polymérase a été ajoutée, ensuite 42 cycles de 30 secondes à 95°C, 90 minutes à 55°C et 30 secondes à 72°C; enfin une étape d'elongation finale à 72°C pendant 7 minutes, suivie d'une incubation à 4°C.

Les produits d'amplification ont été séparés par électrophorèse dans un gel d'agarose 1% (Invitrogen Life Technologies) dans le tampon 0,5 X Tris-Borate-EDTA (TBE) contenant 0,5 µg/ml de bromure d'éthidium. L'échelle 1 Kb Plus DNA Ladder (Invitrogen Life Technologies) a été utilisée comme référence. Elle possède des bandes de 100 à 12.000 paires de bases. Les produits de gel ont été interprétés avec le système Bio-Rad ChemiDoc XRS et analysés grâce au logiciel Discovery Series Quantity One.

2.4 Analyse des marqueurs

Deux groupes d'amores, ISSR et RAPD, ont été essayés sur un échantillon de 4 accessions en vue de sélectionner celles qui favorisent un grand polymorphisme. Seules les amores ayant produit de profils consistants d'amplification et intenses sont sélectionnées pour les analyses futures. La présence ou l'absence des bandes ont été notées par un système binaire de 1 ou 0 respectivement. Les bandes de faible intensité n'ont pas été prises en compte pour les analyses. Les données enregistrées ont été analysées avec le logiciel RAPDistance Program version 1.04 (Armstrong *et al*, 1994, cités par Nkongolo et Nsapato, 2003). Les distances génétiques ont été calculées avec le coefficient de similarité de Jaccard. Des dendrogrammes ont été construits en utilisant l'analyse de degré de parenté ou "neighbour-joining" (Saitou and Nei, 1987, cités par Nkongolo et Nsapato, 2003)

à partir des coefficients de similarité. Le degré de relation entre les matrices de similarité engendrées par les données de RAPD et de ISSR a été calculé avec le coefficient de corrélation de Pearson.

3 RESULTATS

3.1 Extraction et amplification de l'ADN

L'ADN génomique a été isolé à partir des échantillons des graines entières et des embryons a subi le test de qualité qui a révélé la non dégradation de celui-ci (fig.14).



Fig. 14 : Profil montrant la présence de l'ADN dans les échantillons utilisés pour le test de qualité

L'observation de la figure 14 révèle la présence de l'ADN dans tous les échantillons testés. Cette présence est illustrée par la bande de plus de 12000 pb présente dans chaque échantillon. Toutefois, si le test de qualité s'est révélé positif, il convient de cet ADN est en quantifiable. La figure 14 présente la quantité amplifiée d'ADN pour chaque accession utilisée comme échantillon, à côté de témoins positifs dont l'ADN est déjà quantifié.

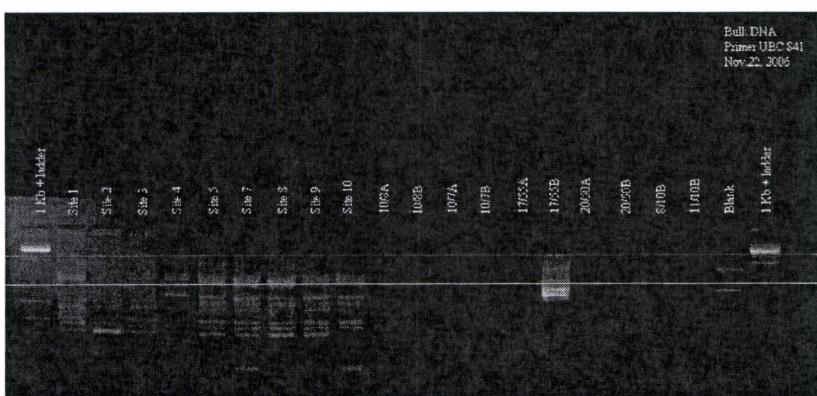


Fig. 15: Quantité d'ADN amplifié à partir des échantillons de graines entières et des embryons

Il apparaît de l'observation de la figure 15 les concentrations en ADN ont été beaucoup plus faibles dans les échantillons provenant des graines entières que dans ceux issus des embryons. En effet seul l'échantillon 1755B, issu de l'extraction des embryons a pu révéler une quantité d'ADN amplifié, illustré par les bandes situées entre 850 et 1500 pb. Le tableau 6 présente le rendement en ADN exprimé par ses différentes concentrations obtenues dans ADN des graines entières et dans les embryons pour toutes les accessions les accessions.

Tableau 6 : Rendement comparé en ADN des graines et des embryons destinés à la recherche de diversité parmi les différentes accessions de caféier Robusta de la RDC.

N° d'ordre	Identité des échantillons	Concentration (ng/μl)	
		des graines	des embryons
1	KR 1/1	50,12	138,27
2	KR 1/3	128,53	592,28
3	KR 2/5	83,62	331,5
4	KR 8/8	84,74	1168,05
5	KR 8/10	29,43	400,34
6	KR 9/8	187,49	341,50
7	KR 10/1	27,30	243,62
8	KR 10/8	135,25	297,14
9	KR 11/8	24,02	154,60
10	KR 12/1	28,42	826,37
11	KR 12/4	220,60	583,95
12	KR 12/6	65,73	447,93
13	KR 14/1	18,01	464,95
14	KR 14/10	95,88	161,95
15	KR 15/3	170,62	481,91
16	KR 15/4	72,38	949,79
17	KR 15/6	133,32	422,16
18	KR 15/12	100,16	196,80
19	KR 16/2	26,52	160,67
20	KR 16/5	219,48	377,24
21	KR 16/7	31,44	98,52
22	KR 17/48	41,73	764,30
23	KR 17/55	51,65	759,64
24	KR 19/11	37,21	798,47
25	KR 19/12	43,74	1001,40
26	KR 19/18	30,88	629,25
27	KR 19/31	37,57	717,45
28	KR 20/3	24,21	763,05
29	KR 20/50	30,01	604,28
30	KR 20/51	55,31	188,70
31	KR A/1	108,93	502,95
32	KR B/4	42,44	450,55

Le rendement en ADN a varié de 24,02 à 220,60 ng/μl pour les graines alors que dans les embryons elle a été échelonnée entre 98,52 et 1168,05 ng/μl. Ainsi pour la poursuite des travaux, il sera retenu les échantillons en provenance des embryons.

3.2 Optimisation de la concentration standard d'ADN

La figure 16 illustre les polymorphismes produits avec de différentes concentrations d'ADN testées pour les réactions PCR.

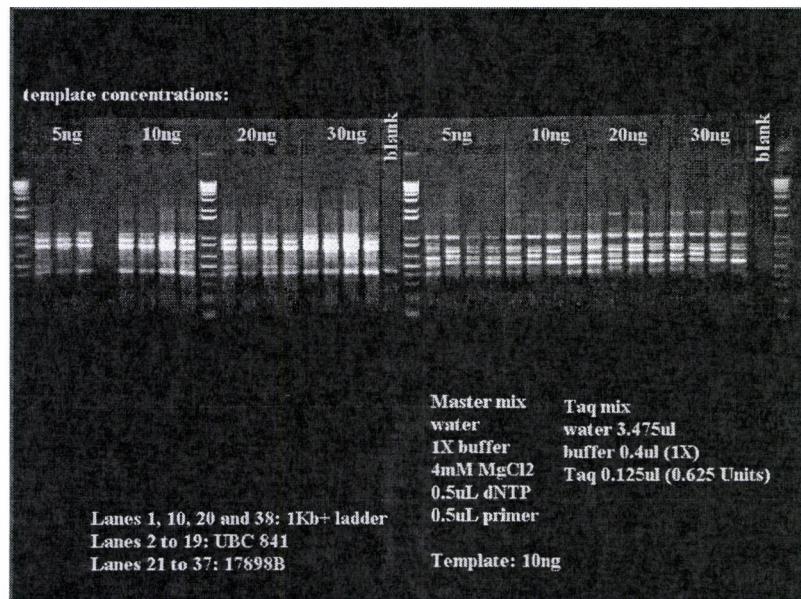


Fig.16 : Polymorphismes produits par différentes concentration d'ADN testées pour l'amplification

De 4 concentrations testées (5 ng/μl; 10 ng/μl; 20 ng/μl et 30 ng/μl) sur un échantillon de 4 accessions en présence de deux amorces, ISSR (17898B) et RAPD (UBC 841), il s'est avéré qu'à partir de 10 ng/μl, des amplifications polymorphiques ont été enregistrées. La concentration de 10 ng/μl a été choisie pour les réactions ultérieures.

3.3 Optimisation des amorces

Les résultats des amplifications obtenues avec les amorces ISSR et RAPD sur les embryons sont présentés dans le tableau les figures 17 et 18.

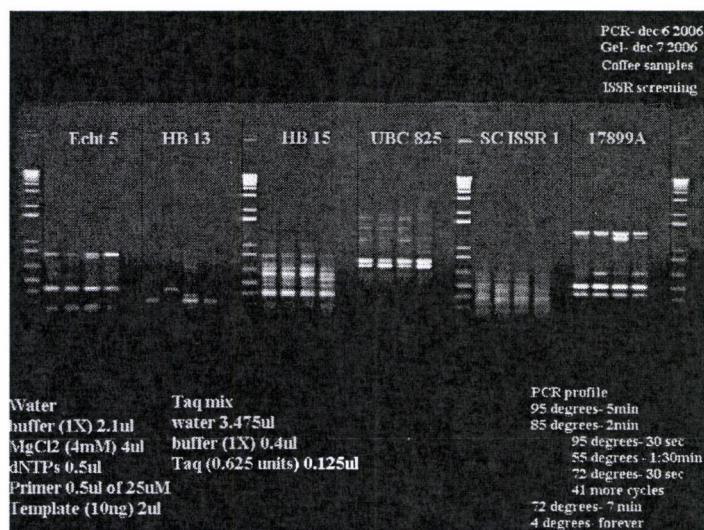


Fig. 17 : Profils d'amplification polymorphique générée par 6 différentes amorces ISSR

L'analyse des profils générés par 6 amorces ISSR révèle ces dernières polymorphisme ont produit des bandes bien démarquées et d'intensité variable. Les amorces UBC 825 et 17899A ont été choisies pour la recherche de diversité des accessions des cafiers étudiées.

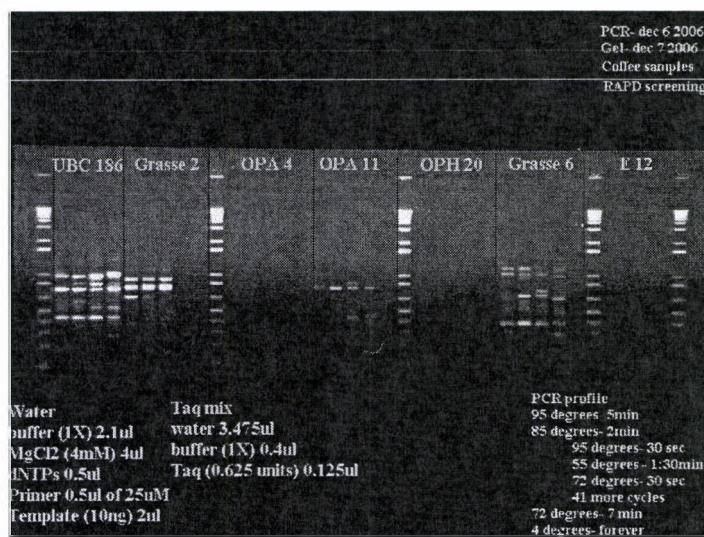


Fig. 18 : Profils d'amplification polymorphique générée par 6 différentes amorces ISSR

Il apparaît à l'examen de la figure 18, présentant les polymorphismes obtenus avec les amorces RAPD testées, que l'amorce Grasse 6 et OPA11 donnent des bandes plus polymorphiques que les autres. Elles sont retenues pour l'analyse de diversité au sein d'accessions en études.

Le tableau 7 présente l'analyse des données sur amorces ISSR et RAPD révélées par les profils moléculaires et les produits d'amplification qu'elles ont générés.

Table 7: Séquences des nucléotides des amorces utilisées à la détection des marqueurs RAPD et ISSR

Amorces	Séquences des amorces (5' – 3')	Nombre de fragments amplifiés	Taille des fragments amplifiés	Nombre de fragments polymorphiques
Amorces RAPD				
UBC 186	GTGCGTCGCT	27	200-2000	27
Grasse 2	GTGGTCCGCA	8	150-1200	8
OPA 4	AATCGGGCTG	0	0	0
OPA11	CAATGCCGT	13	300-2000	13
OPH 20	GGGAGACATC	0	0	0
Grasse 6	CGTCGCCCAT	9	300-1200	6
E 12	TTATGCCCCC	0	0	0
Amorces ISSR				
Echt 5	AGACAGACGC	5	250-1000	2
HB 13	GAGGAGGAGGC	8	250-800	7
HB 15	GTGGTGGTGGC	8	300-1000	2
UBC 825	ACACACACACACACT	7	450-1100	7
SC ISSR 1	(AG) 8RG	7	250-850	0
17899A	CACACACACACAAG	9	250-1400	5

Les six amorces ISSR examinées ont produit globalement 44 bandes dont 23 (52 %) étaient polymorphes (tableau 7). Les amorces UBC 825 et 17899A ont produit des bandes intenses qui étaient fortement polymorphes. En effet l'amorce UBC 825 a détecté 7 bandes qui étaient toutes (100 %) polymorphes tandis que l'amorce 17899A produisait 9 bandes dont 5 (56 %) étaient polymorphes. Ces amorces ont été choisies pour les analyses ultérieures.

Les 7 amorces RAPD utilisées ont amplifié 57 bandes dont 54 (95%) ont été polymorphiques (tableau 8). Les UBC 186 et OPA 11 ont produit 27 et 13 bandes RAPD qui ont été toutes polymorphiques. Ces amorces ont ainsi été sélectionnées pour les analyses ultérieures.

3.4 Amplification et analyse des marqueurs

Après différentes optimisations des paramètres nécessaires pour l'amplification des marqueurs ISSR et RAPD les profils moléculaires de ces marqueurs sont présentés dans les figures 19 et 20 pour l'amplification avec les amorces ISSR et par les figures 21 et 22 pour les amorces RAPD.

Analyse avec ISSR

Les 2 amores ISSR (UBC 825 et 17899A) ont révélé après amplification au PCR des profils polymorphiques représentés par les figures 19 et 20.

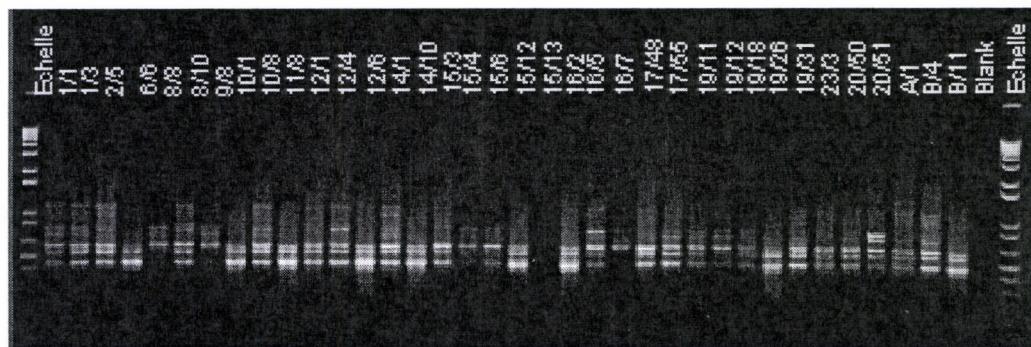


Fig. 19: Profils électrophorétiques de l'ADN génomique de différentes accessions du *Coffea canephora* (var Robusta) générés par ISSR après amplification à l'aide de l'amorce UBC 825 en gel d'agarose 2%. Les rangées 1 et 39 contiennent l'échelle 1-Kb + DNA ladder. Les rangées 2 à 37 contiennent les produits d'amplification de différentes accessions du *Coffea canephora* (var. Robusta).

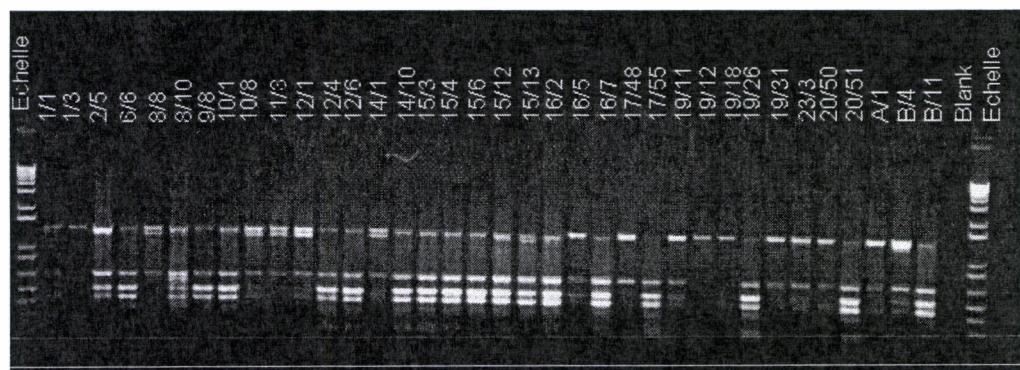


Fig.20: Profils électrophorétiques de l'ADN génomique de différentes accessions du *Coffea canephora* (var Robusta) générés par ISSR après amplification à l'aide de l'amorce ISSR 17899 en gel d'agarose 2%. Les rangées 1 et 39 contiennent l'échelle 1-Kb + DNA ladder. Les rangées 2 à 37 contiennent les produits d'amplification de différentes accessions du *Coffea canephora* (var. Robusta).

L'analyse de ces profils a mis en évidence des distances génétiques parmi les accessions qui ont varié de 0 à 0,67 représenté par le tableau 8. Environ 40% de distances génétiques enregistrées ont été de 0,40. Les accessions KR19/12 et KR19/18 ont présenté des profiles d'amplification identiques avec la distance génétique de 0. De même les accessions KR10/1 et KR12/6 aussi bien que les accessions KR19/31 et KR20/50 ont également révélé des profils identiques. Par contre les accessions KR15/12 et KR8/8 ont été les plus génétiquement distantes.

Un dendrogramme (figure 21) a été construit à partir des coefficients de similarité en utilisant le programme RAPDistance program v.1.04 (Armstrong *et al.* 1994). Quatre groupes principaux ont été identifiés. L'accession KR14/1 a semblé être un hors groupe.

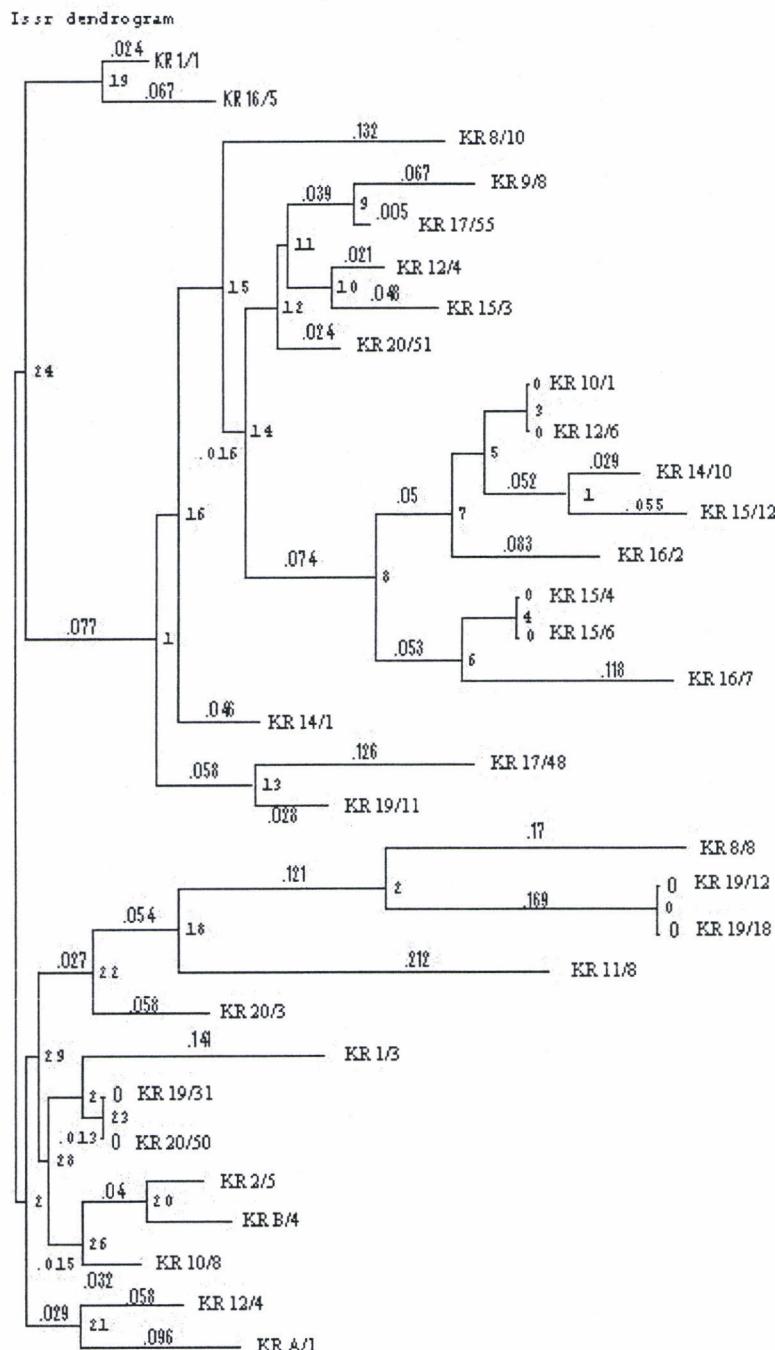


Fig 21 : Dendrogramme de relations génétiques parmi 32 accessions du *Coffea canephora* (var. *Robusta*) révélées par la matrice de similarité de Jaccard en utilisant les données de ISSR. Les valeurs au-dessus de branches indiquent les distances basées sur l'analyse des neighbour-joining (NJ).

Analyse avec RAPD

Comme pour les données ISSR, l'analyse des polymorphismes révélés par les 2 amores RAPD, UBC 186 (fig. 22) et OPA 11 (fig. 23) a été complétée par l'étude de degré de parenté entre les accessions.

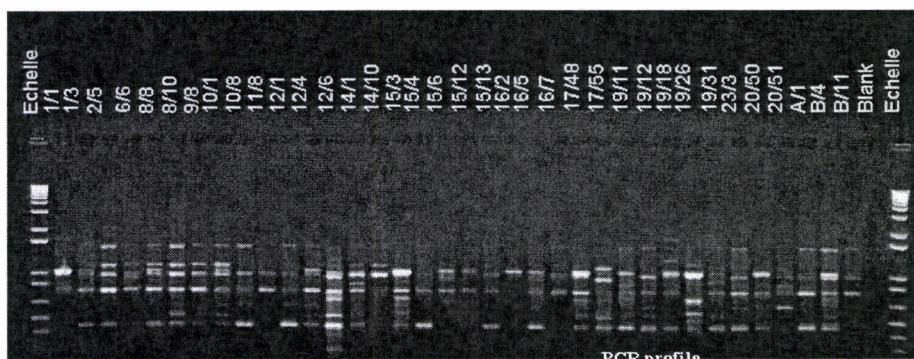


Fig. 22: Profils électrophorétiques de l'ADN génomique de différentes accessions du *Coffea canephora* (var. Robusta) générés par RAPD après amplification à l'aide de l'amorce UBC 186 en gel d'agarose 2%. Les rangées 1 et 39 contiennent l'échelle 1-Kb + DNA ladder. Les rangées 2 à 37 contiennent les produits d'amplification de différentes accessions du *Coffea canephora* (var. Robusta).

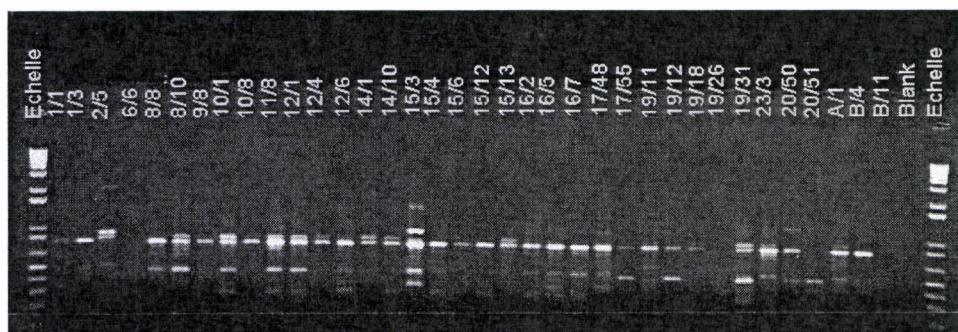


Fig. 23 : Profils électrophorétiques de l'ADN génomique de différentes accessions du *Coffea canephora* (var. Robusta) générés par RAPD après amplification à l'aide de l'amorce OPA 11 en gel d'agarose 2%. Les rangées 1 et 39 contiennent l'échelle 1-Kb + DNA ladder. Les rangées 2 à 37 contiennent les produits d'amplification de différentes accessions du *Coffea canephora* (var. Robusta).

Les données des distances génétiques (tableau 9) ont montré que la plupart des accessions sont éloignées les unes des autres. Les valeurs varient en effet dans les limites 0,17 à 0,89. Les descendants de KR8/8 et de KR8/10 sont les plus rapprochés, suivies de celles de KR 15/4 et de KR 12/1. Les plus éloignées sont celles issues des accessions KR15/3 et KR1/1.

Un dendrogramme (fig. 24) a été construit à partir des coefficients de similarité utilisant le RAPDistance program v.1.04 (Armstrong *et al.* 1994). Il n'y a pas eu des principaux groupes discriminatoires identifiés. Il y a eu des accessions provenant de même famille comme KR 8/8 et KR 8/10 qui forment un regroupement tout comme aussi beaucoup d'autres familles différentes qui se retrouvent dans un même regroupement.

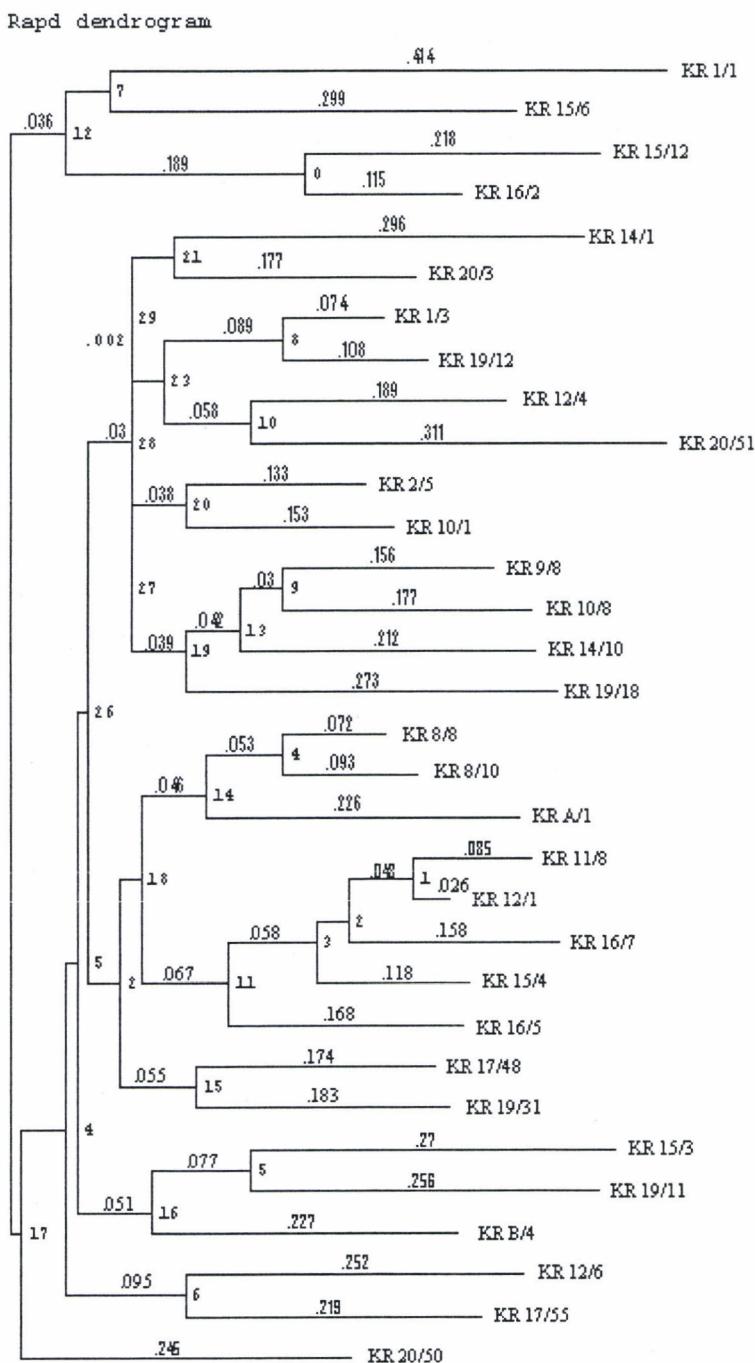


Fig. 24 : Dendrogramme de relations génétiques parmi 32 accessions du *Coffea canephora* (var. *Robusta*) révélées par la matrice de similarité de Jaccard en utilisant les données de RAPD. Les valeurs au-dessus de branches indiquent les distances basées sur l'analyse des neighbour-joining (NJ).

Combinaison de marqueurs RAPD et ISSR

Comme les amorces RAPD et ISSR ciblent de différentes régions du génome, les deux systèmes ont été analysés ensemble. Il s'est avéré que les distances génétiques ont été aussi élevées que celles révélées par le RAPD et vont de 0,24 à 0,79. Plus de 70% des valeurs enregistrées ont été égales ou supérieures à 0,5. Les descendants des accessions KR16/2 et KR15/12 sont les plus rapprochées génétiquement tout aussi bien que celles de KR12/1 et KR11/8. Par contre les descendants des accessions KR12/6 et KR1/1, KR15/3 et KR11/8, ainsi que celles des KR15/12 et KR8/8 ont été les plus génétiquement éloignées.

Le dendrogramme (fig. 25) construit à partir des valeurs des distances génétiques a révélé deux principaux groupes incluant les descendants de différentes familles sans mettre en évidence une tendance particulière. Aucune corrélation ($r^2 = 0,0036$) n'a été trouvée entre les données des matrices de distances génétiques obtenues avec les amorces RAPD et ISSR.

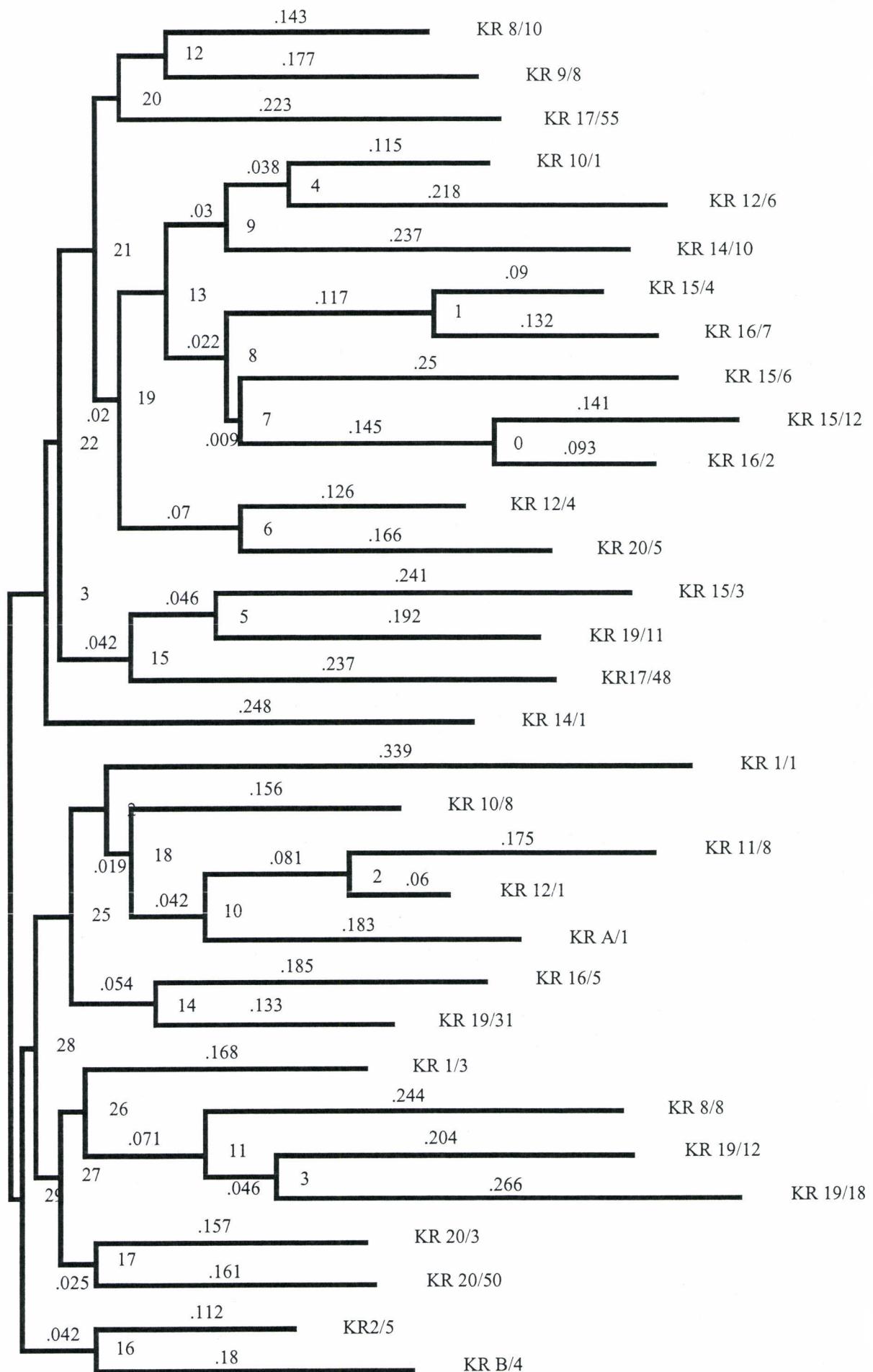


Fig. 25 : Dendrogramme de relations génétiques parmi 32 accessions du *Coffea canephora* (var. *Robusta*) révélées par la matrice de similarité de Jaccard en utilisant les données combinées de ISSR et RAPD. Les valeurs au-dessus de branches indiquent les distances basées sur l'analyse des neighbour-joining (NJ).

4 DISCUSSION

Plusieurs milliers d'accessions de café sont conservées ex-situ et in-situ dans des collections de banques de semence ou dans des stations expérimentales à travers le monde. La majorité d'entre elles ne sont pas adéquatement décrites pour leur utilisation dans des programmes d'amélioration génétique. La plupart des données sur la diversité génétique ont été estimées à partir des caractéristiques agronomiques ou morphologiques. Ces caractéristiques sont souvent influencées par l'environnement. L'évaluation de la diversité basée sur ces paramètres est souvent entravée par des confusions dues aux grandes variations pour un trait phénotypique spécifique dans une population donnée. Cet aspect est particulièrement important pour une espèce à pollinisation croisée comme le cafier robusta. Contrairement aux caractères morphologiques, des marqueurs moléculaires ne sont pas influencés par l'environnement (Kuleung *et al.*, 2006). Des études sur la variabilité et de la diversité génétique chez le cafier robusta utilisant des marqueurs moléculaires sont relativement limitées.

Dans la présente étude nous avons évalué la variation génétique des accessions de cafier robusta disponibles en DR - Congo en utilisant des marqueurs d'ISSR et de RAPD. Les amorces de RAPD ont détecté un niveau de polymorphisme plus élevé (95%) que les marqueurs d'ISSR (52%). Chaque accession a été identifiée individuellement en se basant sur les empreintes génétiques générées par les amorces de RAPD. Les deux types de marqueurs étaient efficaces dans la détection de la variabilité génétique des accessions analysées. Cette grande variabilité génétique serait en partie due à l'allogamie de *C. robusta*, mais elle serait aussi attribuable à une grande différence génétique des clones qui ont constitué le patrimoine héréditaire initial. En fait les progénitures examinées sont les produits des générations de quelques intercroisements de 15 différentes familles issues de lignées ayant des liens maternels. Malgré cette observation, les résultats enregistrés n'ont montré aucune tendance à la dérive génétique dans ces populations.

L'intérêt de tels résultats résiderait dans la sélection et amélioration génétique pour des caractères comme la résistance à la trachéomycose. En effet, 14 accessions de cette collection ont été testées pour la résistance à la trachéomycose. Elles ont montré à des degrés divers une résistance à cette maladie. Ceci indique que ces accessions peuvent être utiles dans des programmes d'amélioration, non seulement pour l'accroissement de rendement pour lequel ils ont été initialement sélectionnés, mais également pour la résistance à la trachéomycose en RD Congo.

Prakash *et al.*, (2005) avaient également analysée la diversité génétique du patrimoine héréditaire du café robusta en Inde en comparaison avec des accessions provenant du centre

de la diversité génétique du café en Afrique. Ils ont également trouvé un niveau élevé de variation génétique des accessions analysés en utilisant des marqueurs de SSR et d'AFLP. Aga *et al.*, (2005) ont rapporté quant à eux des niveaux de polymorphisme assez bas (25%) dans le matériel génétique de *Coffea arabica* d'Ethiopie à l'aide des amorces d'ISSR comparé à 52 % de polymorphisme observé dans la présente étude. Le niveau de la variabilité génétique dans les accessions de *C. arabica* d'Ethiopie examinées par Chaparro *et al.* (2004) à l'aide également des amorces de RAPD était aussi plus beaucoup bas (34,2%) que le 95% observé avec les échantillons de *C. robusta* de DR Congo. Ce niveau bas de la variabilité génétique chez *C. arabica* rapporté dans plusieurs études pourrait être attribué en partie à sa nature autogame (Chaparro *et al.*, 2004).

D'autres études antérieures ont montré de niveaux différents de polymorphisme dans différentes espèces quand RAPD et ISSR ont été comparés. Par exemple, Fang et Roose (1997) ont rapporté un niveau élevé de la variation génétique en utilisant des marqueurs de RAPD qu'avec ISSR chez des espèces du genre *Citrus*. D'autre part, plusieurs auteurs ont détecté un niveau de polymorphisme plus élevé avec le système d'ISSR qu'avec RAPD chez d'autres espèces de plante (Nkongolo *et al.*, 2005 ; Nagoaka et Ogihara 1997 ; Raina *et al.*, 2001 ; Rus-Kortekaas *et al.*, 1994 ; Qian *et al.*, 2001).

Comparaison des marqueurs de RAPD et d'ISSR

Dans la présente étude, les marqueurs de RAPD ont détecté de plus haut niveau de polymorphisme dans les accessions de café robusta comparé aux marqueurs d'ISSR. Les analyses de RAPD ont montré également de plus grandes distances génétiques parmi les accessions étudiées que les données d'ISSR. Les différences entre les variations détectées par les amorces de RAPD et celles d'ISSR résultent de différentes régions génomiques visées. Les marqueurs de RAPD indiquent le polymorphisme dans les régions codantes et non codantes, y compris des séquences répétées ou des séquences uniques qui couvrent le génome entier (Williams *et al.*, 1990). Des sites de microsatellite sont dispersés à travers tout le génome et sont hypervariables (Echt *et al.*, 1996). Le plus souvent les amorces d'ISSR détectent plus de polymorphisme que des amorces de RAPD en raison du niveau élevé de la variabilité dans des sites de microsatellite qui subissent un processus évolutionnaire différent dû aux forces de sélection (Qian *et al.*, 2001).

Bien que dans la présente étude, les amplifications de RAPD aient été reproduites plusieurs fois dans les mêmes conditions, beaucoup d'études ont rapporté la faible reproductibilité des profils de bande de RAPD comparés aux résultats d'ISSR. Ceci peut être le résultat d'un certain nombre de facteurs. D'abord, des amorces de RAPD sont composées

de 10 oligonucléotides arbitraires qui sont utilisés dans des conditions d'amplification non spécifiques. Ceci résulte à des emplacements multiples d'attachement des amores avec des degrés variables de similitude. En second lieu, il est plus difficile d'obtenir des bandes distinctes dans les analyses des variabilités intraspecificques ou interspecificques parce qu'il y a beaucoup de bandes de mobilité semblable qui proviennent des régions non-homologues (Sanchez de la Hoz *et al.*, 1996).

En conclusion, les marqueurs de RAPD ont semblé être plus informatifs que des marqueurs d'ISSR pour la caractérisation, l'analyse et l'utilisation des ressources génétiques de café robusta. Les deux systèmes sont basés sur l'application de PCR et ils sont faciles à appliquer a une analyse a grande échelle de matériels dans un programme d'amélioration génétique. Pour une évaluation efficace de la variabilité génétique de ressources génétiques, ces deux types de marqueurs moléculaires devraient être utilisés conjointement pour couvrir une plus grande partie du génome. Dans la présente étude, le niveau de polymorphisme détecté était très élevé bien que les accessions aient été dérivées de familles ayant des liens maternels assez étroits. Eu égard a cette variabilité génétique, au niveau appréciable de la résistance à la trachéomycose ainsi qu' a la productivité individuelle, ces accessions ont un grand potentiel dans l'amélioration de Cafier robusta pour la résistance aux maladies et pour un rendement élevée. Leur distribution au niveau des planteurs serait recommandée pour la régénération de plantations de cafier robusta qui sont dégénérées par l'introduction des gènes venant d'autres accessions locales à faible rendement et faible résistance aux maladies.

WP 4 : Epidémiology

Essai : Incidence des tissus fusariés sur la conservation du pouvoir infectieux et la transmission de la maladie

1. Objectif

La présence d'un inoculum primaire dans un site figure parmi les facteurs les plus importants dans la propagation d'une maladie épidémique. Dans le cas de la trachéomycose du cafier une hypothèse a été émise sur la possibilité de contamination de différents débris. Cet essai est entrepris en vue de déterminer la capacité survie et de contamination de l'agent pathogène à des niveaux différents dans le bois parasité.

2. Matériel et Méthodes

2.1 Site expérimental

L'essai a été conduit dans le Jardin Expérimental de la Faculté des Sciences Agronomiques de l'Université de Kinshasa dont les conditions climatiques ont été précédemment décrites (tableau 1).

2.2 Matériel végétal

Au cours de notre expérimentation, nous avons utilisé les plantules de l'espèce *Coffea canephora* var *Robusta* (le génotype KR 12/5). Elles sont obtenues à partir des semences récoltées dans les blocs cafiers de la station de recherche de l'Institut National d'Etudes et de Recherches Agronomiques (INERA) de Kiyaka. Les graines ont été semées le 02 avril 2003 puis transplantées après germination dans des sachets en polyéthylène de dimension 15 x 35 x 0,5cm remplis de terreau forestier.

2.3 Types d'inoculum et inoculation

Un échantillon de tige de cafier, âgé de 12 mois, parasité par le *G. xylosporus* et montrant les périthèces a constitué la source d'inoculum, représenté par 4 sortes en suivant un transect allant de la surface vers la profondeur. A chaque niveau des morceaux de tissus de dimension approximative de 3 mm x 4 mm x 1 mm sont prélevés et taillés en vue de constituer l'inoculum.

- Niveau superficiel l'écorce : il s'agit des endroits de l'écorce (fig.26) portant des périthèces



Fig. 26 : Endroits de prélèvement des périthèces localisés dans les crevasses sur l'écorce d'un caféier atteint de la trachéomycose.

- Niveau sous-cortical nécrosé : les tissus nécrosés de bois immédiatement en dessous de l'écorce portant périthèces sont prélevés comme 2^{ème} type d'inoculum.
- Niveau profond en dessous du précédent : les tissus nécrosés de bois sous-jacents au niveau précédent ont été le 3^{ème} type d'inoculum.
- Fragment de culture gélosé du parasite : dans une culture du pathogène en boîte de Pétri sur milieu SNA, il a été prélevé un morceau de celui-ci à l'endroit où une forte concentration de conidies a été observée.

Avant l'inoculation les morceaux de tissus représentant les différents types d'inoculum ont été désinfectés à l'alcool éthylique 70 % dont l'excès a été immédiatement éliminé par un passage rapide dans une flamme de bec Bunsen. L'inoculation a été réalisée en insérant ces inoculums dans une entaille faite dans la tige de caféier à 1 cm en dessous des feuilles cotylédonaires. Cet endroit a été au préalable désinfecté à l'alcool éthylique qui a été laissé s'évaporer pendant 10 minutes. Après insertion des inoculums, le tout a été maintenu en place par une ligature faite avec du parafilm.

2.4 Variables observées

L'appréciation du pouvoir a porté sur les paramètres suivants :

2.4.1 Durée d'incubation

Les plantes sont observées tous les 7 jours et l'on note l'apparition des premiers symptômes qui apparaissent.

2.4.2 Taux de mortalité

Il est noté en relevant le nombre de plantules mortes par rapport au nombre total des plantules du traitement. Il est exprimé en pourcentage.

3. Résultats et discussion

Les données du présent essai sont en cours d'enregistrement. Elles concernent le délai d'apparition de différents symptômes de la trachéomycose et leur taux de sévérité.

Les données du présent essai concernant le délai d'apparition de différents symptômes de la trachéomycose et leur taux de sévérité ont été enregistrées et sont présentées dans le tableau 11.

Tableau 11: Délai (jours) d'apparition et taux (%) de différents symptômes et mortalité enregistrés après inoculation

Inoculum	Flétrissement		Brunissement		Chute des feuilles		Dessèchement des feuilles		Mortalité	
	Jour	%	Jour	%	Jour	%	Jour	%	Jour	%
Ecorce	17	11,1	22	11,1	50	24,05	N.O.	0	N.O.	0
Sous écorce	51	11,1	56	11,1	58	22,1	61	11,1	66	11,1
Tissu nécrosé	60	33,3	65	33,3	66	31,7	N.O.	0	N.O.	0
Fragment gélosé	93	22,2	98	22,2	100	39,4	103	22,2	105	22,2
Non traité	N.O.	0	N.O.	0	45	38,8	N.O.	0	N.O.	0
PPDS	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Légende :

N.O. : Non observé

N.S. : Non significatif.

L'analyse des données montre que l'induction des symptômes de la maladie a été observée avec tous les types d'inoculum. Par contre la mortalité a été enregistrée uniquement sur les plants inoculés à l'aide des tissus immédiatement sous écorce et le fragment de culture gélosée du parasite. L'absence de cette manifestation chez les sujets inoculés avec les écorces portant périthèces serait être due à l'identité de ces périthèces qui seraient autres que ceux de *G. xylosporus*, capables pourtant de provoquer des symptômes courants sauf la mortalité. Une telle observation a été enregistrée lors des inoculations faites avec le *F. solani*, *F. stilboides* et *F. falciforme* (Tshilenge *et al.*, 2004). Les tissus profonds qui n'ont pas aussi provoqué la mortalité pourraient être hors d'atteinte du parasite et, par conséquent, rester non contaminants.

CABI

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

Fifth Annual Report (November 2005 to October 2006)

INCO-DEV COFFEE WILT PROJECT

(Contract no. ICA4-CT-2001-10006)

Work Package 1

CAB International Annual Progress Report

Nov. 2005–Oct. 2006

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1. Project partners

CAB International (CABI), United Kingdom (leading Work Package 1)

Centre de Cooperation Internationale en Recherche Agronomique pour Le Developpement (CIRAD), France

Coffee Research Institute (CORI), Uganda

Université Catholique de Louvain (UCL), Belgium

University of Kinshasa (UoK), Democratic Republic of Congo

2. Overall objective

To improve knowledge of the coffee wilt pathogen, *Gibberella xylospora*, with respect to genetic diversity and variation in aggressiveness.

3. Specific activities

Specific activities for which CABI E-UK) is responsible for, or involved in:

3.1 Maintain a collection of the anamorph (*F. xylospora*) and teleomorph (*Gibberella xylospora*) forms of the CWD pathogen obtained from wilt-affected coffee trees in Uganda, DRC and other regions of Africa as appropriate. Deposit representative strains in a designated facility to facilitate secure, long-term storage.

3.2 Investigate the extent of diversity among strains, across geographical locations and over time, principally using a range of genetic approaches.

3.3 Maintain baseline data relating to those strains acquired and held by CABI and its partners on an electronic database.

3.4 Synthesise results of research activities undertaken by project partners contributing to WP1.

4. Research progress

Note: It was expected that, under the original contractual arrangements, CABI's inputs to the project would cease at the end of October 2004. However, as a result of a successful application to the EU for an extension to the project CABI was able to continue its research since that time. However, given that the extension was not funded this research has relied on funds remaining from the original phase of the project and, as such, was very limited.

Complementary reports: Full details of the research undertaken by CABI throughout the life of the project, and of its findings and implications, are available in a separate report entitled 'Individual partner final report: CABI E-UK' submitted to CIRAD in 2007. A further report entitled 'Synthesis of activities and findings of research activities undertaken by partners on pathogen variability and contributing to Work Package 1', which provides an overview of the research undertaken by all project partners contributing to Work Package 1 'Pathogen variability' and its implications, is also available.

4.1 Establish a collection of anamorph and teleomorph forms of the CWD pathogen and deposit representative strains for secure, long-term storage

An extensive collection of nearly 300 strains of various *Fusarium* species is now held at the CABI E-UK Centre. Most of these are the anamorphic form, *F. xylarioides*, were isolated from coffee plants affected by CWD, with identifications confirmed by CABI mycologists. The collection also includes other recognised pathogens of coffee such as *F. stilboides*, *F. lateritium*, *F. solani* and *F. oxysporum*, which were included in a number of the characterisation studies completed at CABI and elsewhere. Although no new strains were acquired during this reporting period, work to confirm the identity of existing strains on the basis of morphological characters continued. A number of strains were also deposited in the CABI Genetic Resources Collection (GRC) for long-term storage under liquid nitrogen and also in a freeze-dried state, bringing the total number to 69. These strains are considered representative of the range of *Fusarium* species, geographic origins, host plant species/clones and time of recovery from CWD affected trees in relation to the larger collection available.

4.2 Investigation of the extent of molecular variability diversity among strains

Ongoing studies of genetic variability within a wide range of fusaria associated with CWD was completed in early 2006. This study originally focused on 62 representative strains of *F. xylarioides*, *F. solani*, *F. stilboides*, *F. lateritium* and *F. oxysporum* but was expanded to include several other groups of additional strains. These additional groups included seven strains from *C. arabica* in Ethiopia, 28 strains from *C. canephora* in Tanzania and 32 strains obtained from the on-farm CWD mapping site at Mayuge in central Uganda. These three groups were compared to each other and to selected individuals from the representative set using ISSR and IGS analysis. The Tanzanian group was also subjected to mtDNA RFLP analysis. New dendograms based on the IGS and mtDNA RFLP analyses support the genetic population structures reported previously. The production of dendograms for ISSR data has been problematic and a clear conclusion has yet to be reached. A manuscript detailing this work is currently being prepared for submission to a recognised journal.

4.3 Establishment and maintenance of baseline data relating to those strains acquired and held by CABI and its partners on an electronic database.

The input of data into the electronic database, including that for newly acquired strains continued during the reporting period. The database now holds comprehensive information relating to nearly 300 strains held by CABI, UCL, CIRAD and their partners in Africa. However, information reflecting the findings of the investigations of genetic variability, pathogenic variability, mating type and other studies remains limited. It is hoped that further information specifically to reflect the findings of the research may be provided by the partners and input to the database in the future.

4.4 Synthesis of research activities undertaken by CABI E-UK and partners contributing to WP1.

As highlighted in the 2004-5 annual report, comprehensive fungal collections have been established by CABI and the other project partners, at CIRAD, UCL, CORI and UoK. Each organisation holds its own unique collection of strains although, a number of the more important strains have been identified and are now held in at least two collections. This replication will help to ensure the longer term security of strains.

In Uganda, monitoring of CWD incidence on coffee trees continued in the Kawanda Coffee Collection and the Botanical Gardens at Entebbe. No new incidences of disease were reported in this reporting period, therefore no new isolations of *G. xylosporus* were made. During this period it has also been noted that there has been no incidence of CWD affecting *C. arabica* anywhere in Uganda, supporting previous field observations and also our current understanding of host specificity, geographic delimitation and other attributes of the pathogen. The monitoring of CWD on selected farms did not continue as funding came to an end. Monitoring did, however, continue on field sites at CORI where it was observed that there was variability in the rate of CWD symptom development on plants of different *C. canephora* clones. This suggests that some field resistance exists and also that the progress of the disease was more rapid in the wet season and in susceptible clones.

At UCL, previous work involved the use of *MAT-PCR* to characterise 20 *C. canephora* associated strains of *F. xylosporus* from Equator Province (DRC) as mating type *MAT-1*. Crossing results have shown preliminary *MAT-PCR* results to be correct, with all 20 strains from Equator Province found to be mating type *MAT-1*. Also described in previous reports was the method for *in vitro* production of perithecia between compatible *C. arabica*-associated strains (Biological Species 1, BS1) and *C. canephora*-associated strains (BS2). BS1 and BS2 by definition are reproductively isolated from one another and other populations. Recently a third category has been identified, BS3, in a cross between BBA 62457 (*C. excelsa* *MAT-2*) and ATCC 15664 (*Coffea* sp. *MAT-1*). The perfect state between these two strains was achieved and 10 ascospores were isolated. Of these 10 progeny two appear to be highly fertile, similar to the female parent BBA 62457 and the other eight resembled the male parent (the 10 progeny were backcrossed with both parents for *MAT* identification but due to time restraints and lack of funding the study could not be completed). A fourth group became apparent comprising four strains originating from Central and West Africa. All of these strains appeared to be incompatible with each other and members of the BS1-BS3. These four strains have been assigned to a residual sterility group (SG4). In conclusion it would appear that what is referred to as a single species is in fact at least four distinct reproductively isolated populations/biological species and should perhaps be referred to as the *G. xylosporus* complex. *MAT* sequencing results also confirmed the presence of four distinct clades corresponding to the BS/SG groups. Analysis of the left idiomorph flanking region revealed the presence of a 60bp region containing mating

type-specific sequences common to both *G. xylarioides* and the *G. indica* complexes. Surprisingly, *C. arabica*-associated *G. xylarioides* *sensu lato* *MAT-1* strains IMI 375907 and BBA 62458 possess the sequence type for *MAT-2* strains in this region. It may be useful to note that the *G. xylarioides* *MAT* idiomorph primers are able to successfully amplify corresponding regions not only in strains belonging to the complex but also others in the *Gibberella fujikurio* complex and may be used as a tool to identify undescribed cryptic species of *G. xylarioides* and closely related species.

At CIRAD, genetic variability was assessed among 182 single-spore derived strains collected from all the geographical zones affected by Coffee Wilt (DRC, Uganda, Tanzania, Ethiopia) and several different coffee species (*C. canephora*, *C. arabica*, *C. sp*). Included in these strains were several ‘historical’ strains collected during the first emergence in the 1950-1960s. Strains of *F. stilboides* and *F. decemcellulare* were also included in this study (materials and methods developed for DNA extraction, the 11 microsatellite markers and PCR amplification have all been described in previous reports). Analysis of the dendrogram revealed four distinct groups belonging to the species *F. xylarioides* and a 5th group made up of other species of *Fusarium* strains comprising strains as follows:

The Canephora strains group: Strains isolated from coffee trees belonging to the species *C. canephora*, in DRC, Uganda and Tanzania during the contemporary period between 1997 and 2002 (except RDC001, collected by Meyer in 1962 in DRC). The totality of that group is 100% uniform for the 11 microsatellite markers.

West African group: Two historical strains collected in Ivory Coast and Guinea, which are identical.

Central African group: Two strains collected during the 1st emergence of the disease. DSMZ 62457 from CAR was collected from *C. excelsa*. The origin of ATCC15664 is unknown.

The Arabica strains group: Strains collected between 1971 and 2002 from *C. arabica* in Ethiopia. These strains are identical.

Fusarium spp. group: Reference strains of different species of *Fusarium* and strains isolated from diseased coffee trees, present as saprophytes or secondary pathogens not responsible for Coffee Wilt. It was noted that strain ATCC 36325, mistakenly identified as being *F. xylarioides*, does not belong to this species.

The origin of the ‘historical’ strains still is unclear, as CWD does not appear to have been endemic in Guinea or Ivory Coast. Both regions were surveyed at the end of the 19th century and beginning of the 20th century for the collection of wild coffee trees when there were numerous transfers of wild material from forests to plantations. Coffee growing was the subject of intense activity and surveillance over that period. Therefore, if the disease was present at that time and causing problems it probably would have been reported. CWD was first reported in Ivory Coast in 1950 by Delassus and in Guinea by Conakry in 1958. A likely hypothesis is of an accidental introduction due to exchanges of planting material in the French-speaking zone from CAR. These two historical strains may be considered to be individuals representative of the original diversity in CAR. Strain MUCL 14186, collected in 1960, is a historical sample from the first emergence of the disease in DRC. The presence of the disease in RDC was considered to be an accidental introduction linked to planting material exchanges. When considering the historical strains, three groups stand out – one representing the Ivory Coast and Guinea populations, a second representing CAR and a third representing the disease in DRC (strain RDC001). If it is considered that the centre of origin of the disease is CAR and that the disease was due to accidental introductions during the first emergence between 1940-1960s in the other countries, then these historical strains can be considered to be strains representative of the original diversity of the *G. xylarioides* population. The strains

collected from *C. canephora* in DRC, Uganda and Tanzania were identical as were the *C. arabica* group collected in Ethiopia. The absence of diversity shown using the microsatellite markers studied raises the question of the role of the sexual cycle of this fungus. Abundant perithecia occur under natural conditions and this fungus is also heterothallic having two mating types MAT1 and MAT2. As those mechanisms are sources of recombination, the reason for the very low genetic diversity observed raises further questions.

5. Promotion/dissemination outputs

Full details of promotion and dissemination outputs produced as a result of, or relating to, the work undertaken under Work Package 1 are available in the ‘Individual Partner Final Report’ produced by CABI and associated Appendix 1. These outputs include journal publications, oral presentations, poster presentations, CABI reports and other related publications and articles.

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