INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2001-10008

FIRST ANNUAL REPORT

covering period from 01/11/01to 31/10/02

Title: CBDRESIST

Project homepage: http://www

Keywords: (5 maximum) Coffea, Colletotrichum kahawae, durable resistance

Annexe 1 **COVER PAGE**

Contract number:
ICA4-CT-2001-10008

TITLE: CBDRESIST

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(SUMMARY)
FIRST ANNUAL REPORT
November 2001 - October 2002

WP1 Breeding for durable resistance

Some *Coffea eugenioides* genotypes were collected by Coffee Research Foundation in the forests of Mt. Elgon (Kenya); no CBD could be observed *in situ*; these genotypes still have to be tested. Some resistance was found in Ethiopian germplasm from Cameroon, it has to be confirmed.
In order to confirm the results of earlier hypocotyl tests conducted at Cirad, seeds of 25 Colombian Catimor and Sarchimor lines were send to Kenya and Cameroon for field establishment in CBD infested zones.

Some Resistant x Susceptible F2 progenies derived from well identified resistant individual trees have been prepared and established in the field during the last joint Research Project funded by the EC. They will be used to study the inheritance of resistance, especially in hybrids derived from Ethiopian entries. No material was available for tests in Year 1. Another study, on inheritance of the resistance found in Timor Hybrids, has been postponed due to bad germination of seeds.

Search for markers of resistance is still pending availability of F2 seeds.

WP2 Host x Pathogen interaction

The tree-to-tree variation within entries often seen as homogeneous was highlighted by the few tests conducted. However enough replications are necessary to confirm the presence of interaction, moreover to identify the genotypes (Coffee and Fungus) responsible for that interaction.
The achievement of proper ring tests is thus conditioned by the availability of seeds.
It is proposed, for a better co-ordination of results, that all partners simplify their tests results into 5 classes of susceptibility (0 to 4) and calculate a mean grade by dividing the weighted total by the total number of seedlings.

WP3 The pathogen

29 Isolates were collected by CRF in various regions of Kenya, and from resistant varieties. Tests were initiated both at CRF and at CIRAD to check their aggressiveness. They will have to be confirmed by further replications. A collection of isolates was also made in Rwanda in a germplasm collection.
A complementary study of the diversity (morphology, aggressiveness, molecular biology) of the fungus is pending the availability of isolates collected in the wild on *Coffea eugenioides*. Indeed this species may be the initial host of the disease before it spread to *C. arabica*.

WP4 Epidemiology

A thorough study of the evolution of the disease and of crop losses was made in Cameroon. Three sites were chosen, with two varieties on one of the sites. Half the trees are under shade, the others in full sunshine, with some variation due to the presence of banana trees. Three branches per tree were monitored. The analyses are not completed yet. The same trees will be monitored for 4 years. In Kenya the sites for a similar study were earmarked but the study will start in Year 2.

Search for antagonistic micro flora started in Kenya. Antagonistic effects were detected in the lab. The multiplication of these microorganisms will start next year. Meanwhile at CIFC it was evaluated the antagonistic effect of the extract Gal-02 from the fungus *Ganoderma* sp. on CBD isolates. The conidia germination of some isolates was significantly inhibited *in vitro* by the extract Gal-02, while for others the results were not conclusive. However, the conidia germination of all the *C. kahawae* isolates tested was inhibited *in vivo*. The differentiation of melanized appressoria of the different CBD isolates was also inhibited by Gal-02, both *in vitro* and *in vivo*. In what concerns the percentage of hyaline appressoria, the results of the *in vitro* and *in vivo* studies were not coincident. Even in the *in vivo* studies, contradictory results were obtained for the some isolates, in the different experiments. So, apparently it seems that Gal-02 had no effect in the appressoria melanization.

FIRST SCIENTIFIC ANNUAL REPORT
November 2001 - October 2002

WP1 Breeding for durable resistance
Objectives
Identify and confirm sources of resistance, better understand the transmission of resistance: Search for molecular markers of resistance using segregating F2 progenies

WP1 T1: Sources of resistance

Look for resistance in *Coffea eugenioides*, Confirm field resistance of genotypes earmarked, through hypocotyl test.

Various attempts were made by the Kenyan partner to survey the zones where *C. eugenioides* grows wild (near Mt Elgon in Western Kenya). To date, some cuttings of *C. eugenioides* have been made and established on the Research Station.

Other sources of resistance
Catimor: derived from Caturra x Hybrid of Timor (HDT) cross. (Table 1)
Some resistance was found using hypocotyl tests at Cirad, especially in lines derived from HDT1343 ("Colombia"), and also in lines derived from CIFC832/2 (Sarchimor).

Table 1: Results of CBD resistance tests at Cirad on Catimor lines
Sorted by decreasing resistance to isolate CM 732

CATIE No.	Int.	Tree	IDI	class 0	class 1	class 2	class 3	class 4	R (*)	S (*)	Resistance
T18126	3	5	7	71	29	0	0	0	100	0	R
T18126	3	10	27	27	36	36	0	0	100	0	R
T18123	3	5	30	17	44	39	0	0	100	0	R
T18126	3	6	36	17	28	50	6	0	94	6	R
T18123	3	4	36	10	40	46	2	2	96	4	R
T18138	2	3	38	15	25	57	2	2	96	4	R
T17935	3	9	46	0	35	55	0	10	90	10	R
T17933	3	7	46	0	15	85	0	0	100	0	R
T18123	3	3	53	3	25	48	5	19	76	24	R
T17931	3	11	62	0	4	62	17	17	66	34	MS
T17933	3	8	62	0	4	65	10	21	69	31	MS
T17933	3	6	62	0	6	65	4	25	71	29	MS
T18138	2	6	62	3	24	29	8	36	56	44	MS
T17933	3	2	62	1	1	61	22	15	63	37	MS
T18141	2	3	64	6	2	56	2	34	64	36	MS
T17935	3	3	65	4	1	56	8	31	61	39	MS
T18138	2	4	65	4	5	47	10	33	57	43	MS
T17931	3	4	69	0	4	48	16	32	52	48	MS
T18141	2	10	75	0	0	44	12	44	44	56	S
Caturra	-	-	79	0	0	32	19	49	32	68	S
T17931	3	3	82	0	0	32	9	59	32	68	S
Caturra	-	-	86	0	0	24	5	70	25	75	S
Caturra	-	-	90	0	0	18	3	80	18	82	S
T17931	3	2	97	0	0	6	2	92	6	94	S

(*) R = class 0 + class 1 + class 2 S = class 3 + class 4 (percentages)
IDI: Index of Disease Intensity (%) = weighted cumulated percentages of seedlings in each class
Int. : source of resistance introgression: 1 = CIFC832/1, 2 = CIFC832/2, 3 = CIFC1343

Wild coffee from Ethiopia and other germplasm from Cameroon
Due to a bad germination, no tests were conducted at Cirad on these materials from the Cameroon collections.

The tests conducted at CIFC (Figure 1) indicate a wide range of resistance within this germplasm, although the Caturra control did not exhibit its high susceptibility.

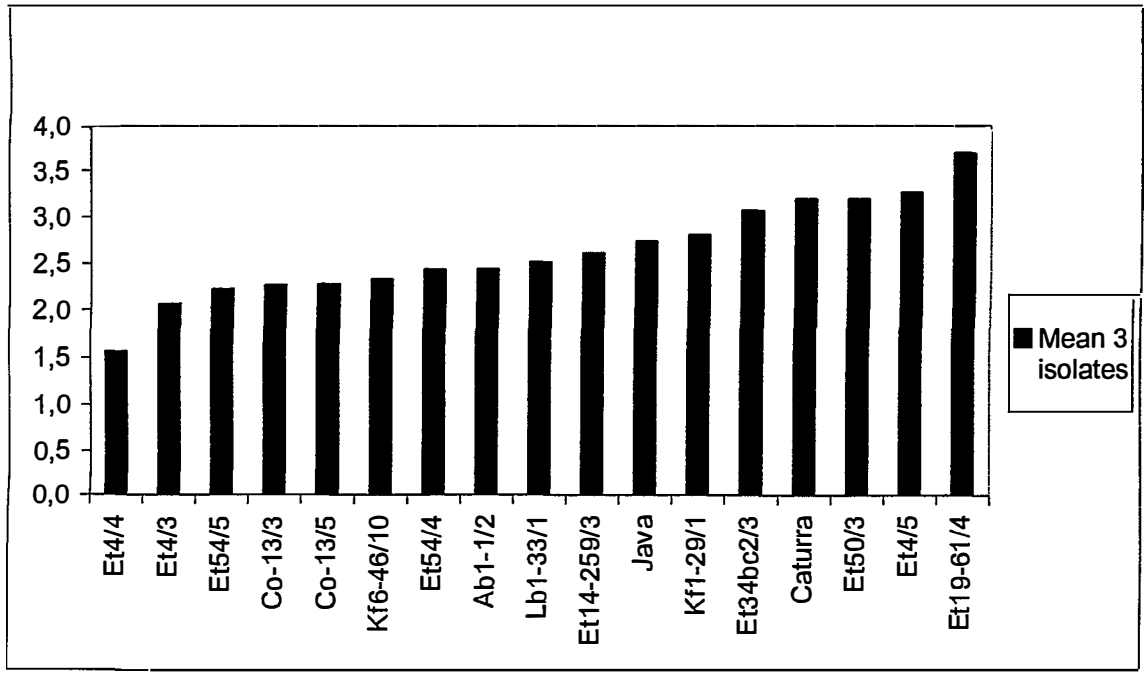

Figure 1: Results of resistance tests conducted at CIFC
(mean grade)

WP1 T2 Inheritance of CBD resistance
Analyse the inheritance in F2 progenies from a Resistant x Susceptible cross, using hypocotyl tests.

Some Resistant x Susceptible F2 progenies derived from well identified resistant individual trees have been prepared and established in the field during the last joint Research Project funded by the EC. However no seeds could be available in Year 1 from Kenya as the trees were still too young.

Based on the results of the tests of Catimor lines a scheme was proposed to study the inheritance of resistance in this material. A scheme was proposed based on crosses between moderately resistant, moderately susceptible, and susceptible lines (Table 2). However the seeds received in January 2002 failed to germinate, and this study is postponed to Year 2 of the Project.

Table 2: Proposed scheme for the study of inheritance of resistance, Progenitor T17933

Hybrids	Mother ("T")	Trees tested	mean IIM % Caturra (**)	SD	Father ("T")	Trees tested	mean IIM % Caturra (**)	SD	Received (50 to 100 g parchment)
C7	17933	2,6,7,8	53	17	18130		susceptible		R4 T39,40,41,42
X14	18130		susceptible		17933	2,6,7,8	53	17	R4 T35,36,37,38
D9	17933	2,6,7,8	53	17	18138	3,4,6	77	23	R4 T31,32,33,34
X11	18138	3,4,6	77	23	17933	2,6,7,8	53	17	R4 T7,8,9,10
E6	17933	2,6,7,8	53	17	18140 *				R3 T15,16,17,18
F13	18130		susceptible		18141	3,10	85	8	R3 T19,20,21
X10	18140				17933	2,6,7,8	53	17	R3 T3,4,5,6
D23	18141	3,10	85	8	18138	3,4,6	77	23	R7 T27,28,29,30
Progenitors									
T17933	17933	2,6,7,8	53	17					R2 T39,40,41,42
T18130	18130		susceptible						R7 T15,16,17,18
T18138	18138	3,4,6	77	23					R4 T27,28,29,30
T18140	18140 *								R4 T27,28,29,30
T18141	18141	3,10	85	8					R3 T23,24,25,26

introgression: CIFC 832/2	Possibly different sources of resistance?
introgression: CIFC1343	
Introgression:832/1	No resistance

MR (yellow): moderately resistant, MS (green): moderately susceptible, S (white): susceptible

* not included in the tests, probably MS?

** % of Disease Intensity Index for Caturra (generally close to 100%)

SD: standard deviation for all replications

WP1 T3: Search for markers of resistance

This study is conditioned by the availability of F2 seeds, and also by the possibility to get enough DNA from susceptible F2 seedlings in the nursery. A technique will be tested to "rescue" these susceptible plants after infection has been seen.

WP2 (Host x Pathogen interaction):

Validate the screening tests (ring test). The objective is to come up with a standard testing methods and/or to be able to compare results obtained in various conditions. Nor enough seeds with good germination rate were available in Year 1. This will be continued in Year 2.

Host x pathogen interaction

The results of tests conducted at CIFC (Figure 2) may indicate some interaction between some of the genotypes and the 3 isolates used. Also, the possible variation within one variety is highlighted by variety ET4. Indeed, trees 3 and 4 are the most resistant whereas tree 5 is almost the most susceptible. However, in the absence of replication, and given the low susceptibility shown by Caturra, no conclusion can be made at this stage.

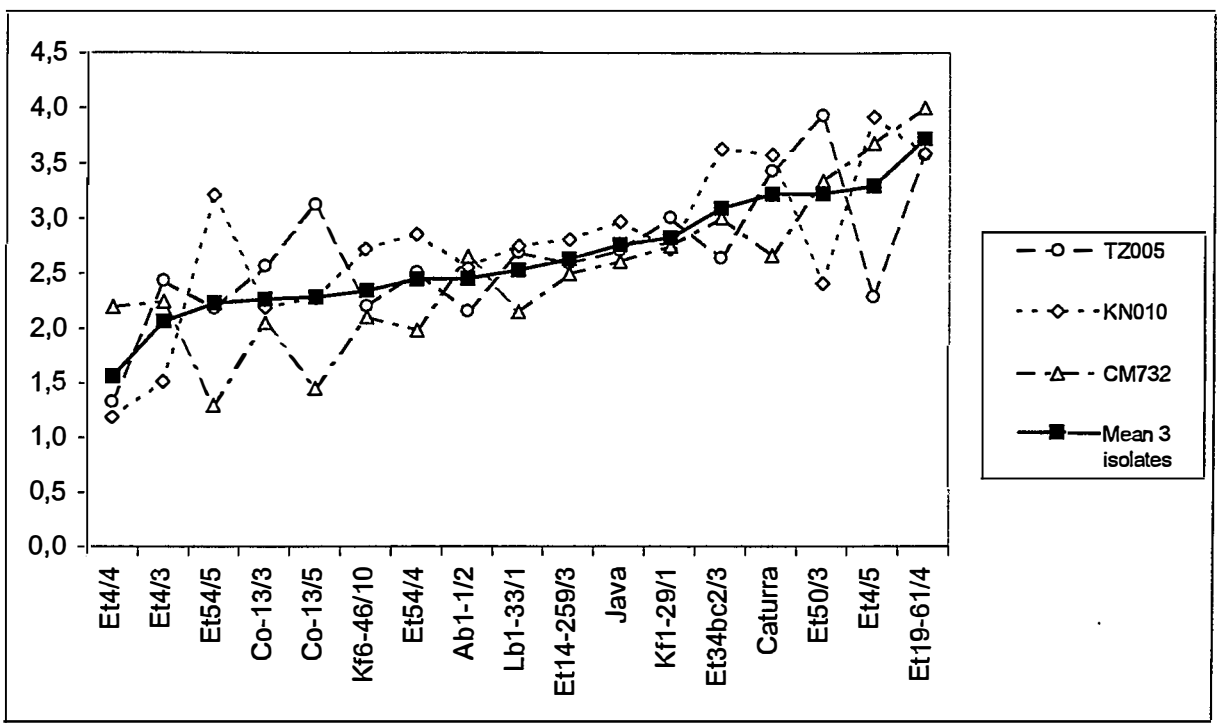

Figure 2: Results of resistance tests conducted at CIFC with 3 reference isolates
(Mean grade observed)

In order to draw reliable conclusions on this interaction, it will be necessary to ensure the availability of enough plant material for various partners, and that replicates are made systematically for all tests.

The synthetic indices used to evaluate the susceptibility are not the same for the various partners. Although they are highly correlated it is suggested to use the same index, for instance the mean grade:

Mean grade = weighed sum of seedlings in each class, divided by total number of seedlings (seedlings without symptoms are allocated to a class "zero")

The 12 classes used in Kenya should then, for calculation purpose, be translated to 0 to 4 instead of 1 to 12, as shown in Table 3.

Table 3: Comparison of scoring methods and calculations of mean grade

Mean grade = Weighted total of seedlings per class / total number of seedlings

12 classes														4 classes						
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**T**	**Mean grade**	**1**	**2**	**3**	**4**	**5**	**T**	**Mean grade**
1	2	3	1	0	2	3	2	3	3	4	2	26	7,38	1	5	3	8	9	26	3,73
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**T**	**Mean grade**	**0**	**1**	**2**	**3**	**4**	**T**	**Mean grade**
1	2	3	1	0	2	3	2	3	3	4	2	26	6,38	1	5	3	8	9	26	2,73

WP3 The pathogen

Collection of strains (Kenya)

On *Coffea eugenioides*

Sampling of isolates included *C. eugenioides* in Mt Elgon forest. The disease is believed to have originated from the forest. No CBD infection was evident, so that only clean berries and twigs were collected. No *Colletotrichum* isolates were obtained from *C. eugenioides* tissues.

In farmers' fields
The objective is to check if the fungus evoluates, naturally and/or in contact with resistant varieties.
C. kahawae was isolated from the berries in farmers' fields and about 80 monoconidial isolates have been obtained. Isolates of *Colletotrichum kahawae* were obtained from the infected resistant and susceptible varieties and from as many geographically diverse locations as possible. 29 of these isolates are listed below. Their origins still has to be precised.

KC01, KC04, KC05, KC07, KC10
KE09, KE15, KE30
KM11, KM13, KM14, KM19, KM25, KM29, KM30, KM45
KP01,
KW03, KW06, KW09, KW14, KW15, KW17, KW29, KW31, KW33, KW39, KW41, KW42

The isolates will be used in future studies on pathogen diversity and nature of resistance. More collection will be made in areas where success rate was low and on wild coffee populations.

Some of these isolates were dispatched to Cirad in April 2002 (Table 4) to be evaluated in Cirad conditions, and in comparison with isolates from other countries. Their origins are documented.

Table 4: List of CBD isolates collected in Kenya and dispatched to Cirad in April 2002.

Isolate	Location of origin	Isolated from (host cultivar)	Remarks
KW3	Kakamega, Western Province	Resistant	positive reaction with Rume Sudan and "Cat"(imor?)
KW33	Kitale, Rift Valley Province	Resistant	positive reaction with "Cat"(imor?)
KC4	CRF Ruiru	Susceptible	positive reaction with "Cat"(imor?)
KW41	West Pokot Rift Valley Province	Susceptible	positive reaction with "Cat"(imor?)
KE9	Lyego, Muranga	Susceptible	positive reaction with "Cat"(imor?)
KM29	CRF Ruiru	HdT x Geisha9	Std negative reaction with Rume Sudan and "Cat"(imor?)

WP4 Epidemiology

Evolution of the disease, crop losses

Work was only initiated in **Kenya** by the selection of sites.

In **Cameroon** a complete season was followed.
Trees were earmarked in 3 sites, in one of them on 2 varieties. The shade was taken into account in the choice of trees, so that half the trees are under permanent shade, the other half without shade. Also, the presence of banana trees near the trees was noted. Each tree is precisely located in the field, and will be followed over 4 years, so as to have a better knowledge of the evolution of the disease over the time and space. The data could not be analysed yet. They will be checked before starting the next season.

Evaluation of the role of antagonistic micro flora

In **Kenya** Coffee berries with CBD lesions were washed and the washing plated on malt extract agar with an antibiotic (chloramphenicol). The cultures that grew were observed for a growth pattern that indicated antagonism. These were isolated and grown as dual cultures with *C. kahawae* to confirm the antagonism. Means of multiplying the antagonists in mass using low technology will be evaluated within next project year. Field application will be by a manual Knapsack sprayer. Coffee berries and leaves were sent to CIFC (Portugal) for isolation of antagonists.

At **CIFC** it was evaluated the antagonistic effect of the extract Gal-02 from the fungus *Ganoderma* sp. (collected in rosemary and supplied by Dr. Wagner Bettiol - Embrapa Meio Ambiente, Brazil) on CBD isolates.

Material and methods

Plants and fungus isolates
Susceptible young leaves of Catimor were inoculated with isolates of *C. kahawae* (CIFC Q2) from Kenya, (CIFC Z1) from Zimbabwe, (CIFC Ca1) from Cameroon, (CIFC A6) from Angola, (CIFC M2) from Malawi, (CIFC R1) from Rwanda.

Treatment of coffee leaves with Gal-02
The lower epidermis of detached young leaves was sprayed with a Gal-02 solution diluted 1:10 in 10% ethanol and kept at room temperature. Two hours later the leaves were inoculated with different CBD isolates. Leaves sprayed with 10% ethanol were used as controls.

Inoculation and incubation
The inoculation was done in young leaves according to the technique described by van der Vossen *et al.* (1976) with slight modifications. The young leaves were placed on aluminium trays lying down on a nylon sponge and then inoculated with conidia suspension ($2x10^6$/ml) by means of an atomizer connected to a pressure pump. The trays were covered with a glass plate and put in a phytotron at 22ºC. The incubation period lasted 24h in which the inoculated material was kept in dark conditions. The disease assessment was made according to van der Graaf´s scale (1981).

Light microscopic observations

Conidia germination and appressoria formation in vitro - Glass slides were sprayed with a Gal-02 solution diluted 1:10 in l0% ethanol and kept at room temperature during 2h. Glass slides sprayed with 10% ethanol were used as controls. Germination and appressoria formation "*in vitro*" were evaluated by placing aliquots of 40 µl of the conidia suspensions in the glass slides (previously treated with Gal-02 and with 10% ethanol), which were kept in moist chamber during 17h at 22ºC. After this time the germination was stopped with an aqueous solution of 3% formaldehyde and the fungal structures were stained with a drop of blue lactophenol. The percentage of germinated conidia and appressoria (melanized and hyaline) formed were made on a minimum of 6 microscope fields of 100 conidia each/experiment (Silva *et al.*, 1985)

Conidia germination and appressoria formation in vivo - Germination "*in vivo*" and appressoria formation were evaluated on leaf pieces (±5cm^2), 17h after the inoculation, following the technique described by Silva *et al.* (1985). The leaf pieces were painted with transparent nail polish on the lower surface. About 24h later, the nail polish (leaf replica) was removed with the help of tweezers and stained and mounted with blue lactophenol. Countings of the germinated conidia and appressoria (melanized and hyaline) formed were made on a minimum of 6 microscope fields of 100 conidia each/experiment.

Statistical analysis

Arcsine transformed percentages and the Student test were used for statistical analysis.

Results

Antagonistic studies of Gal-02

As shown in the Tables 5 – 16, the conidia germination of the isolates Z1, M2 and R1 was significantly inhibited *in vitro* by the extract Gal-02, while for the isolates Ca1, Q2 and A6 the results were not conclusive. However, the conidia germination of all the *C. kahawae* isolates tested was inhibited *in vivo*. The differentiation of melanized appressoria of the different CBD isolates was also inhibited by Gal-02, both *in vitro* and *in vivo* (Figs 1 and 2). In what concerns the percentage of hyaline appressoria, the results of the *in vitro* and *in vivo* studies were not coincident. Even in the *in vivo* studies, contradictory results were obtained for the isolates A6, M2 and R1, in the different experiments. So, apparently it seems that Gal-02 had no effect in the appressoria melanization.

Table 5. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **Z1** (from Zimbabwe) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	74,3	31,3	4,1***	91,8	2,7	18,1***	3,7	27,2	2,8***
II	60,5	1,7	5,9***	74	0	-	16,1	0,5	2,5***

*C= control
***(P 0,01)

Table 6. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **Z1**(from Zimbabwe) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	70,2	46	1,5***	94,2	1	26,4***	2,5	8	3,3***
II	65	50,2	0,9***	88,5	4,2	17,8***	5,5	6	0,1***

*C= control
***(P 0,01)

Table 7. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **Ca1** (from Cameroon) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	37,3	79,3	4,8***	86,7	2,7	26***	4,2	0	-
II	82,2	1	10,6***	64,3	0	-	16,8	1	4***

*C= control
***(P 0,01)

Table 8. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **Ca1** (from Cameroon) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	80,2	57,3	2,9***	96,8	0	-	0,5	3,5	4,4***
II	94,8	97,3	1,1***	94,2	3	23,2***	3,5	8	2,6***

*C= control
***(P 0,01)

Table 9. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **Q2** (from Kenya) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	17,5	48,3	5,3***	62,3	7,7	7,1***	8,2	12,3	2,1***
II	33,8	62,3	2,4***	93,7	1,3	23***	1	6,5	3,5***

*C= control
***(P 0,01)

Table 10. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **Q2** (from Kenya) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	68,8	60,5	1,7***	75,7	2,5	14,0***	11,5	7	1,6***
II	78,3	70,3	1***	89,2	7,8	11,7***	6,3	3,7	1,1***

*C= control

***(P 0,01)

Table 11. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **A6** (from Angola) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	25,3	3,3	5,8***	88,3	0	-	3,5	0,17	7,3***
II	53,2	54,2	0,2***	94,3	31,3	3,1***	3,5	2,3	1,4***

*C= control ***(P 0,01)

Table 12. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **A6** (from Angola) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	68,8	8,5	4,9***	75,7	0,5	8,1***	11,8	0	-
II	45,8	62	0,6***	94,3	21,5	15,2***	1,8	3,2	0,8***

*C= control
***(P 0,01)

Table 13. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **M2** (from Malawi) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	64,2	21,8	2,9***	78,7	0,3	8,7***	10,7	0,3	2,2***
II	37,5	5,5	9,6***	66,8	0,3	38,1***	10	1	10,5***

*C= control
***(P 0,01)

Table 14. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **M2** (from Malawi) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	68,8	37,3	2,4***	84,8	13,7	8,4***	3	4,7	0,8***
II	40	30,3	1,3***	89,2	0	-	2,7	1,3	1,7***

*C= control
***(P 0,01)

Table 15. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **R1** (from Rwanda) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	50,2	1,7	9,4***	75	0	-	14,8	0,7	4,7***
II	30,8	1,3	14,5***	70,3	0	-	5,7	0	-

*C= control
***(P 0,01)

Table 16. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **R1** (from Rwanda) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	59	44,8	4,4***	85,2	9,2	15,5** *	1,8	10,3	5***
II	61,7	36	1,7***	96	41,5	10***	8	6,2	3,3***

*C= control
***(P 0,01)

The reduced percentages of conidia germination and appressoria (melanized) formation in the coffee leaves treated with Gal-02, comparatively to the controls, gave rise to a small number of lesions although the symptoms appeared at the same time in treated and non-treated leaves.
At CIFC it was initiated the study of the modifications induced by Gal-02 in the plant responses (local protection/systemic acquired resistance). The same kind of studies was initiated with the antagonistic bacteria *Bacillus subtilis* (from the CIFC collection).

Isolation, selection and identification of antagonistic micro organisms
At the end of September we received coffee leaves and berries from Dr. Gichuru (Coffee Research Station, Kenya) for bio control studies. The washing technique was used for microflora isolation. It was identified the fungus *Epicoccum* sp..8 The antagonistic effect of this fungus on CBD will be investigated.

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Management annual report

Organisation of the collaboration

Overview of how cooperation between partners proceeded during the period.

Co-operation between partners has been greatly facilitated by the possibility to have email contacts, thus relatively fast interaction. However responses were sometimes delayed due to the unavailability of some correspondents. It is therefore suggested that at least two persons per partner are designated as correspondents, and share the knowledge of all aspects of the Project.

Meetings

Summary of project meetings; date, place; purpose, participants, results.

The first General Meeting took place in February 2002 at Ruiru, Kenya. The purpose was to define more precisely the Work Packages and tasks to be achieved by each partner.
The co-ordinator failed to structure properly the first general meeting. He did not want to have mere presentations made; indeed the team had been working in partnership for several years before. Instead, he tried to stress on exchange and co-ordination of methods; this led to informal discussions.

Outline of meetings planned for the following year.
The second General Meeting is scheduled for November/December 2003 (Year 3) in Portugal. Each partner will present achievements and the plans for the two last years will be discussed.

Exchanges

Draw attention to any exchanges of personnel (training periods/stays of more than three months in other laboratories). Include details of any exchanges already foreseen for the following year.
Exchanges could not be organised in Year 1 because work was not advanced enough. Exchanges of 1 to 3 months are planned in Year 2: Kenyan breeder to visit CIRAD for joint analysis of host x pathogen interactions, Cameroonian pathologist to visit CIRAD to analyse results of the first season of epidemiology, Kenyan pathologist to visit CIFC to study antagonists. It is also planned to have a Kenyan student working on a PhD thesis on identification of markers of resistance, taking into account the new opportunity that CRF is installing a molecular biology lab. Slight modifications to the Work plan/Tasks will have to be made accordingly.
Our feeling is that not enough exchanges have been planned, due to the donor's instructions (limitations in travels). This is responsible for numerous misunderstandings, and for difficulties in achieving truly co-ordinated work.

Problems

Give details of any problems encountered or foreseen with management, administrative and financial aspects of the contract.

Management

Communication has not been satisfactory. There is a feeling by some AC partners - also found in other Inco projects- that only information regarding activities strictly and specifically funded by the Inco Project should be circulated. Even so, data directly connected to the Project are not circulated early enough to be reported and to provide guidelines for the following years.

Administration

The large size of the Administrations did not facilitate the administration of the project. However the co-ordinator himself made all possible efforts to ensure a smooth communication between the administrations of the various partners. There is no Administration-to-Administration relation; they communicate through the co-ordinators, so that the messages have to be translated at both ends.

Finance

The delays in Bank Transfers to Africa did not help to start the work in time. The African partners are usually totally dependant on such funds to initiate activities.
The budget had been prepared assuming equipment would be purchase free of tax in Africa. This applied for one partner only. Numerous exchanges and procedures were necessary to try to solve these questions.

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WP1 Breeding for durable resistance

Sources of resistance

Catimor and Sarchimor

"Colombia Catimors" are derived from Hibrido de Timor CIFC 1343. They proved to be CBD resistant in Kenya due to one gene called "T". In earlier investigations we also found some resistance in "Sarchimors", a Catimor like cross between Vila Sarchi (a dwarf mutant equivalent to Caturra) and Hibrido de Timor CIFC 832/2. The gene(s) inherited from CIFC 832/2 are not known, but the level of resistance looks lower than for CIFC 1343. No resistance was found in Catimors derived from CIFC 832/1.

Thanks to co-operation with Costa Rica we have been able to further investigate these sources of resistance, by testing a large number of lines using hypocotyl tests. Some results are given in Table 1.

Some variation is found within the lines. It has to be confirmed by replicating the tests for the lines that look heterogeneous for resistance (e.g. T17931). However the range of susceptibilities fits roughly with the origins of these Catimor lines. One gene of resistance has been identified in Hibrido de Timor CIFC1343. The resistance found in Catimors derived from CIFC1343, except for T17931, especially tree No.2, looks stronger than the resistance of "Sarchimors" (derived from CIFC832/2 and Vila Sarchi). From previous results CIFC832/1 seems to carry no resistance gene.

Table 1: Results of CBD resistance tests at Cirad on Catimor lines
Sorted by decreasing resistance to isolate CM 732

CATIE No.	Int.	Tree	IDI	class 0	class 1	class 2	class 3	class 4	R (*)	S (*)	Resistance
T18126	3	5	7	71	29	0	0	0	100	0	R
T18126	3	10	27	27	36	36	0	0	100	0	R
T18123	3	5	30	17	44	39	0	0	100	0	R
T18126	3	6	36	17	28	50	6	0	94	6	R
T18123	3	4	36	10	40	46	2	2	96	4	R
T18138	2	3	38	15	25	57	2	2	96	4	R
T17935	3	9	46	0	35	55	0	10	90	10	R
T17933	3	7	46	0	15	85	0	0	100	0	R
T18123	3	3	53	3	25	48	5	19	76	24	R
T17931	3	11	62	0	4	62	17	17	66	34	MS
T17933	3	8	62	0	4	65	10	21	69	31	MS
T17933	3	6	62	0	6	65	4	25	71	29	MS
T18138	2	6	62	3	24	29	8	36	56	44	MS
T17933	3	2	62	1	1	61	22	15	63	37	MS
T18141	2	3	64	6	2	56	2	34	64	36	MS
T17935	3	3	65	4	1	56	8	31	61	39	MS
T18138	2	4	65	4	5	47	10	33	57	43	MS
T17931	3	4	69	0	4	48	16	32	52	48	MS
T18141	2	10	75	0	0	44	12	44	44	56	S
Caturra	-	-	79	0	0	32	19	49	32	68	S
T17931	3	3	82	0	0	32	9	59	32	68	S
Caturra	-	-	86	0	0	24	5	70	25	75	S
Caturra	-	-	90	0	0	18	3	80	18	82	S
T17931	3	2	97	0	0	6	2	92	6	94	S

(*) R = class 0 + class 1 + class 2 S = class 3 + class 4 (percentages)
IDI: Index of Disease Intensity (%) = weighted cumulated percentages of seedlings in each class
Int. : source of resistance introgression: 1 = CIFC832/1, 2 = CIFC832/2, 3 = CIFC1343

Confirmation of these results is sought by testing these lines in the fields, in areas infested by the disease. For this purpose we organised a transfer of seeds through quarantine in Montpellier from Costa Rica to Kenya and to Cameroon. The list of lines is given in Table 2. They have been germinated in 2002.

Table 2: List and seed quantities of Catimor and Sarchimor lines received for dispatch in 2002 and for field establishment in 2003

Variety	Line	Dispatch to IRAD (g)	Plants obtained Nkolbisson nursery, October 2002	Dispatch to CRF (g) (*)
Colombia	R1	71	90	87
	R2	92	79	89
	R3	80	200	109
	R4	81	59	90
	R5	79	8	81
	R6	81	61	89
	R7	84	78	87
	R8	85	111	77
	R9	75	79	90
	R10	81	5	87
	R11	78	72	79
	R12	83	24	88
	R13	83	64	82
	R14	86	40	83
	R15	85	19	88
	R16	83	59	97
	R17	84	52	95
	R18	94	41	94
	R19	79	51	87
	R20	86,6	53	89
Sarchimor	R1	95	97	90
	R2	86	10	88
	R3	82	10	81
	R4	79	30	88
	R5	89	70	88
	R6	90	6	80
	R7	86	69	97
	R8	78	0	81
	R9	79	56	90
	R10	95	100	89
	R11	92	103	85
	R12	71	76	102
	R13	83	50	82
	R14	83	60	92
	R15	77	80	92
Total		2 916	2 062	3 093

(*) "very bad germination", no detail received

Ethiopan accessions and other germplasm

IRAD collections were sampled, especially trees suspected to be CBD resistant. The seeds were received at Cirad in December 2001 and February 2002. The germination of the seeds kept at Cirad was too low for inoculation. Part of the seeds were dispatched to CIFC (Portugal) as shown in Table 3. Results obtained at CIFC are shown in this partner's report.

Table 3: List of seed samples received from Cameroon, 2001-2002

Foumbot Station (December 2001)				Santa Station (March 2002)			
Field	**Variety**	**Tree**	**Weight (g)**	**Field**	**Variety**	**Tree**	**Qty (*)**
Coll	Et02	C4	39.95	Coll.	Ab1	C2	++
Coll	Et02	C6	94.92	Coll.	Ab1	C3	-
Coll	Et03	C2	48.30	*x*	*Caturra*	*x*	++
Coll	Et03	C3	15.57	Coll.	Co	C3	++
Coll	Et04	C1	33.74	Coll.	Co	C5	++
Coll	Et04	C2	52.38	Coll.	Et14	C3	++
Coll	Et04	C3	143.65	Coll.	Et19	C1	++
Coll	Et04	C4	162.37	Coll.	Et19	C2	+
Coll	Et04	C5	273.30	Coll.	Et19	C3	++
Coll	Et06	C2	34.25	Coll.	Et19	C4	++
Coll	Et06	C3	32.50	Coll.	Et19	C5	?
Coll	Et11c	C5	64.87	Coll.	Et2	C2	+++
Coll	Et17	C5	52.30	Coll.	Et4	C2	-
Coll	Et17	C6	27.89	Coll.	Et50	C2	+
Coll	Et19	C1	62.38	Coll.	Il3	C6	+
Coll	Et19	C1	36.26	Coll.	Jm1	C6	-
Coll	Et19	C3	63.74	Coll.	Kf1	C1	+++
Coll	Et33	C4	92.55	Coll.	Kf6	C2	-
Coll	Et34	C4	105.04	EV73	Kf6	C10	+++
Coll	Et34 bc1	C1	25.56	Coll.	Kf9	C3	+
Coll	Et34bc2	C3	128.98	Coll.	LB	C1	++
Coll	ET34bc5	C1	46.72	Coll.	Mot	C1	+++
Coll	Et34bc5	C2	32.93	Coll.	MoT	C3	++
Coll	Et40	C2	67.06				
Coll	Et50	C3	160.92				
Coll	Et54	C2	87.79				
Coll	Et54	C4	128.17				
Coll	Et54	C5	213.43				
Coll	Et55	C2	74.07				
Coll	Et55	C5	70.38				
Coll	Et59	C3	16.80				
SG	Java	X	7500				

(*) rough estimate, + = 50 g

Table 4: Seeds from Cameroon dispatched to Portugal in April 2002

CP No.	Station	Field	Variety	Tree	Weight (g)
1238	Foumbot	Collection	ET 4	3	51
1239	Foumbot	Collection	ET 4	4	52
1240	Foumbot	Collection	ET 4	5	73
1242	Foumbot	Collection	ET 34	bc2.3	45
1244	Foumbot	Collection	ET 50	3	52
1246	Foumbot	Collection	ET 54	4	46
1247	Foumbot	Collection	ET 54	5	73
1249	Foumbot	CS	JAVA	mixed	500
1303	Santa	Collection	Ab 1	L1C2	243
1305	Santa	Collection	Bb 1	L175C1	518
1307	Santa	Collection	Bb 1	L175C3	231
1308	Santa	Collection	CO	L13C3	210
1309	Santa	Collection	CO	L13C5	150
1310	Santa	Collection	ET 2	L175C2	445
1312	Santa	Collection	ET 14	L259C3	145
1313	Santa	Collection	ET 19	L61C1	413
1316	Santa	Collection	ET 19	L61C4	151
1322	Santa	Collection	KF 1	L29C1	418
1324	Santa	Collection	KF 6	L46C10	511
1326	Santa	Collection	Lb 1	L33C1	303
1328	Santa	EV73	Mot	L62C1	146
1329	Santa	EV73	Mot	L62C3	226
1330	Foumbot	CS	Java	mixed	400

1328 to 1249: Seeds received December 2001
1303 to 1330: Seeds received March 2002

From Kenya (CRF) we expected to receive at least seeds from the progenitors of the F1 and F2 (R x S) hybrids. However no seeds were received from Kenya until the end of Year 1.

Inheritance of CBD resistance

Based on the results of the hypocotyl tests for the progenitors (see WP2) the assessment of resistance of F2 seeds of MR x MS, MR x S, MS x S hybrids has been planned as follows (Table 5), in order to study the F2 segregation of these resistance factors.

Table 5: Seeds effectively available in January 2002, Progenitor T17933

Hybrids	Mother ("T")	Trees tested	mean IIM % Caturra (**)	SD	Father ("T")	Trees tested	mean IIM % Caturra (**)	SD	Received (50 to 100 g parchment)
C7	17933	2,6,7,8	53	17	18130		susceptible		R4 T39,40,41,42
X14	18130		susceptible		17933	2,6,7,8	53	17	R4 T35,36,37,38
D9	17933	2,6,7,8	53	17	18138	3,4,6	77	23	R4 T31,32,33,34
X11	18138	3,4,6	77	23	17933	2,6,7,8	53	17	R4 T7,8,9,10
E6	17933	2,6,7,8	53	17	18140 *				R3 T15,16,17,18
F13	18130		susceptible		18141	3,10	85	8	R3 T19,20,21
X10	18140				17933	2,6,7,8	53	17	R3 T3,4,5,6
D23	18141	3,10	85	8	18138	3,4,6	77	23	R7 T27,28,29,30
Progenitors									
T17933	17933	2,6,7,8	53	17					R2 T39,40,41,42
T18130	18130		susceptible						R7 T15,16,17,18
T18138	18138	3,4,6	77	23					R4 T27,28,29,30
T18140	18140 *								R4 T27,28,29,30
T18141	18141	3,10	85	8					R3 T23,24,25,26

introgression: CIFC 832/2	Possibly different sources of resistance?
introgression: CIFC1343	
Introgression:832/1	No resistance

MR (yellow): moderately resistant, MS (green): moderately susceptible, S (white): susceptible

* not included in the tests, probably MS?

** % of Disease Intensity Index for Caturra (generally close to 100%)

SD: standard deviation for all replications

The seeds have been received in January 2002 and were germinated in April 2002. The germination rate was close to zero, thus no inoculation was possible. This study will be undertaken again in Year 2.

WP2 Host x Pathogen interaction

Ring test

Part of the seeds received from Cameroon were supposed to be tested in Cameroon, France, and in Portugal using the same isolates. However IRAD was not ready to conduct the tests, and the germination rate at Cirad was too low to make inoculations, so that results were obtained only in Portugal (see CIFC report).

Host Pathogen interaction

Various Catimor entries were tested using at least three reference isolates, namely CM732, KN009, and TZ005, known as presenting various levels of aggressiveness.
The results will be presented in the next report, as the meaning of a significant although very low genotype x isolate interaction has not been fully investigated yet. It is assumed that some of the genotypes, or some of the isolates, contribute to the interaction more than others.

WP3 Pathogen diversity

The molecular biology work is planned to start in Year 2, provided enough new isolates can be collected, especially from *Coffea eugenioides*, one putative parent of *C. arabica*. In the meantime isolates have been collected from resistant varieties in Kenya (Table 7).

Some isolates were collected in Rwanda in a germplasm collection. They are listed in Table 6.

Table 6: Isolates collected from Rwanda in March 2002

No.	Location	Variety	Comments
1	**Sowerakori**	BM139	*susceptible,*
2	*Rubona*	*pupurascens*	*susceptible*
3	Rubona	1104 Blue Mountain Jamaica	Partial resistance: gene k?
4	Rubona	6674 Java	Same resistance as Java Cameroon?
5	*Rubona*	*15706 CIFC*	*Probably T15706?*
6	Rubona	K7 6711	Partial resistance: gene k?
7	*Rubona*	*8224 CIFC*	*Catuai vermelho susceptible?*
8	Rubona	20274 Blue Mountain Guatemala Arigola	Partial resistance: gene k?
9	Rubona	5716 Harrar (R3) Dugoda Lemita Arussi Ethiopia	
10	*Rubona*	*POP3*	*Catimor Portugal: susceptible*
11	**Mwito**	Catimor	*Susceptible*
12	Rubona	5523 Blue Mountain 13 Jamaïque	Partial resistance: gene k?
13	Rubona	Sarchimor	Partial resistance: gene T?
14	*Rubona*	*Pop2*	*Catimor Portugal susceptible*
15	Rubona	Cat AX CIFC	Catimor from T1343
16	Rubona	5518 Blue Mountain Kenya	Partial resistance: gene k?
17	Rubona	5714 Harrar (R1) Dugoda Lemita Arussi Ethiopia	
18	Rubona	Ruiru II F2	F2 progeny from Ruiru II
19	*Rubona*	*Pop 4*	*Catimor Portugal susceptible*
20	Rubona	5491 blue Mountain Jamaïque 13-1066	Partial resistance: gene k?
21	Rubona	521 blue Mountain	Partial resistance: gene k?
22	Rubona	5712 Sidamo Ethiopia	Partial resistance?
23	*Rubona*	*Coleção*	*Catimor from collection Brazil*
24	Rubona	Icatu R.P.C.	Arabica x canephora hybrid
25	*Rubona*	*Pop1 Rubona Rwanda*	*Catimor Portugal susceptible*
26	Rubona	Icatu L.C.H	Arabica x canephora hybrid

Shaded grey: isolation achieved

Table 7: List of CBD isolates collected in Kenya and dispatched to Cirad in Arpil 2002.

Isolate	Location of origin	Isolated from (host cultivar)	Remarks
KW3	Kakamega, Western Province	Resistant	positive reaction with Rume Sudan and "Cat"(imor?)
KW33	Kitale, Rift Valley Province	Resistant	positive reaction with "Cat"(imor?)
KC4	CRF Ruiru	Susceptible	positive reaction with "Cat"(imor?)
KW41	West Pokot Rift Valley Province	Susceptible	positive reaction with "Cat"(imor?)
KE9	Lyego, Muranga	Susceptible	positive reaction with "Cat"(imor?)
KM29	CRF Ruiru	HdT x Geisha9	Std negative reaction with Rume Sudan and "Cat"(imor?)

WP4 Epidemiology

Cirad contributed to the elaboration of an experimental design and work method for the epidemiology work that was undertaken in Cameroon.
Also, one Cirad agro-economist took part in the survey of the disease in relation with socio-economic factors in several agro-ecological zones of Cameroon.

Participation in the first General Meeting of the project

Two members of the CIRAD team (Pierre Charmetant and Daniel Bieysse) participated in the first meeting of the project in the Coffee Research Foundation, Ruiru, Kenya (26 February – 1st March, 2002).

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An activity currently given a lot of attention is the transfer of conserved germplasm and various experimental materials from the Oaklands Experimental fields (located on leased private land) to the CRF main station after the lease lapsed. Most of the research materials earmarked for the current project are located in this station and will have to be successfully relocated to a new site to guarantee the continuity of the project components concerned with resistance. These activities were however not taken into account at the time of project conceptualisation and hence may imply major adjustments in the timing of some activities and use of funds.
Another delay was caused by the problems encountered in purchasing durable equipment with the budget allocated for that purpose. The expenditures planned in the Contract were all meant duty free, and, at the end of the year the agreement of the Ministry of Finance for tax exemption had not been obtained yet.

WP1: Breeding for resistance

1.1. Identity sources of resistance

Obtention and screening of "Resistant x Susceptible" F2 progenies for analysis of segregation and search for genes and Quantitative Trait Loci of resistance

Work to characterise new genes of resistance to coffee berry disease continued in the sub-spontaneous population of *Coffea arabica* originating from Ethiopia. This involved the selfing of individual trees and F1 progenies in the germplasm collection and trial fields. The selfed seeds have just been harvested awaiting screening for CBD resistance.

Search for resistance in Coffea eugenioides

The second possible source of new genes for resistance to CBD is the diploid *C. eugenioides,* which is indigenous to the Mt Elgon forest along the Kenya/Uganda border. Prospection missions to the forest were organised in April 2002 to locate the trees in-situ for the purposes of germplasm conservation and to collection of CBD pathogens for diversity analysis. The mission was successful but the trees had just

flowered and the berries were at the pinhead stage. Harvesting of mature-ripe berries is expected during the months of November and December 2002, followed by screening for resistance to CBD. Cuttings were obtained from the trees, which are undergoing propagation in the CRF nurseries.

Based on screening results resistant trees in the two populations will be subjected to an analysis of inheritance of resistance, search for molecular markers and search for resistance QTLs linked to the markers. This work will commence in the second year of the project.

WP2: Nature of the resistance

2.1 Validation of the screening tests

A large number of test seeds were generated by selfing varieties with known resistance/susceptible reaction to CBD. The varieties included Rume Sudan, Catimor, K7, Hibrido de Timor, Pretoria, Padang (all resistant/tolerant) and SL 28 (susceptible).

Field resistance

A survey was also carried out during the months of June and July 2002 coinciding with the peak CBD season in the field to assess the disease incidence on resistant and susceptible varieties growing in farmers' fields.

The infected trees were tagged and will be continuously monitored and scored during each CBD season.

The first laboratory inoculation tests were carried out on the resistant varieties with 30 isolates using SL 28 variety as a susceptible check. The method of inoculation and scoring was as described by Van der Vossen et al. (1976).

Another set of 30 isolates have been selected for inoculation to be done in November 2002.

Upon completion of the inoculation tests a correlation will be established between disease incidence in the field and the reaction of isolates with resistant varieties in the laboratory. Methods of laboratory screening among different laboratories will also be compared.

WP3: The pathogen diversity

3.1 Collection of isolates

Sampling of isolates was extended to include farmers fields and *C. eugenioides* in Mt Elgon forest. The disease is believed to have originated from the forest. Sampling was done by collecting berries with CBD infection. Where no CBD infection was evident like on *C. eugenioides* in the forest, the clean berries and twigs were collected.

C. kahawae was isolated from the berries in farmers' fields and about 80 monoconidial isolates have been obtained. No *Colletotrichum* isolates were obtained from *C. eugenioides* tissues.

Isolates of *Colletotrichum kahawae* were obtained from the infected resistant and susceptible varieties and from as many geographically diverse locations as possible.

The isolates will be used in future studies on pathogen diversity and nature of resistance. More collection will be made in areas where success rate was low and on wild coffee populations.

3.2 Evaluation of aggressiveness

Using results of the first inoculation test (see WP2), some tester isolates were selected and sent to CIRAD for repeat inoculation using different methods and for comparison with other isolates from different origins.

WP4: Disease development

4.1 CBD epidemics

A trip was made to Western Kenya where CBD is high on several varieties including tolerant ones. Plots where disease development can be monitored were identified but they have not been tagged for disease recording because the peak CBD season was over in the area. The work is scheduled to start in March/April 2003.
The assumption is that there will be high disease levels at that time.

4.1 Antagonistic microflora

Coffee berries with CBD lesions were washed and the washing plated on malt extract agar with an antibiotic (chloramphenicol). The cultures that grew were observed for a growth pattern that indicated antagonism. These were isolated and grown as dual cultures with *C. kahawae* to confirm the antagonism.

Means of multiplying the antagonists in mass using low technology will also be evaluated within next project year.

Field application will be by a manual Knapsack sprayer.

Coffee berries and leaves were sent to CIFC (Portugal) for the partners there to try and isolate antagonists.

Reference

Vossen, H A M van der, R T A Cook and G N W Murakaru, 1976. Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum*. (Noack *sensu* Hindorf) in Coffea arabica L. I. Methods of preselection for resistance. Euphytica 25:733-745.

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Participation in the first meeting of the project

Two members of the CIFC team (Maria do Céu Silva and Vitor Várzea) participated in the first meeting of the project in the Coffee Research Foundation, Ruiru, Kenya (26 February – 1st March, 2002).
In the meeting it was confirmed that the studies to be carried out at CIFC would be:
WP1 (Breeding): Identification of sources of resistance
WP2 (Host x Pathogen interaction):
- Validation of the screening tests (ring test)
- Evaluation of the reaction types

WP3 (Pathogen diversity): Evaluation of isolates aggressiveness
WP4 (Epidemiology): Evaluation of the role of antagonistic micro flora

To evaluate the role of antagonistic micro flora it was decided that:
i) antagonists previously isolated in the CRF (Kenya) and tested with local CBD isolates would be send to CIFC (from May or June) to be tested with other isolates as well as for other complementary studies;
ii) micro organisms previously isolated in IRAD will be sent to CIFC (where the screening of its potential antagonism will be made)
iii) Field tests will be carried out in Kenya to validate the lab results

Activities Carried out at CIFC

WP2 (Host x Pathogen interaction): Validate the screening tests (ring test)

2.1 Material and methods

Seed of 21 progenies of coffee material was received from CIRAD (Table 1) to be tested to CBD isolates from Kenya (KNO 10), Tanzania (TZ005) and Cameroon (CM 732). The progenies ET19-L61-C1, CIRAD (1313), BB1-L175-C3 (CIRAD 1307), CIRAD 1305 and CIRAD 1310 were not inoculated because they did not germinate, so they did not produce hypocotyls.

Table 1. Designation of coffee progenies received from CIRAD

Entry	Station	Tree No.	Cirad No.
ET4 Ethiopia, Bonga, alt. 1730 m	Santa	C3	1238
ET4	Foumbot	C4	1239
ET4	Santa	C5	1240
ET34 - Ethiopia, Mizan Teferi, 1500 m	Foumbot	bc2C3	1242
ET50 - Ethiopia, Bonga, 1700 m	Foumbot	C3	1244
ET54 - Ethiopia, Bonga, 1700 m	Foumbot	C4	1246
ET54	Santa	C5	1247
JAVA Moderately resistant control	Foumbot		1249
Ab1 Abyssinie 1	Santa	L1C2	1303
1305			1305
Bb1 Babadjou 1 (Cameroon)	Santa	L175C3	1307
Co Coorg	Santa	L13C3	1308
Co Coorg	Santa	L13C5	1309
1310			1310
ET14 Ethiopia, Gimma - Limu, 1530 m	Santa	L259C3	1312
ET19 Ethiopia, Gore, 1700 m	Santa	L61C1	1313
ET19	Santa	L61C4	1316
KF1 Ainamba Babaca Kaffa	Santa	L29C1	1322
KF6 Ennarea Limmu Gimma Kaffa	Santa	L46C10	1324
Lb1 Local Bronze 1	Santa	L33C1	1326
CATURRA (Susceptible Control)			1330

The hypocotyls of the germinated seed were inoculated by dipping them in a spore suspension (2 $x10^6$ conidia ml^{-1}). The reactions were evaluated according to the van der Graaff scale.

2.2 Results

The results are presented on Tables 2, 3 and 4.
In these experiments, the coffee progeny CATURRA (CIRAD 1330), used as susceptible control, showed very high levels of resistance when confronted with the three CBD isolates.
The percentage of susceptible hypocotyls in control was 32.5%, 52% and 23.7% when confronted with the CBD isolates from Tanzania, Kenya and Cameroon respectively.
On the other hand, other progenies like Co-L13-C5 (CIRAD 1309), ET54-C4-Foumbot (CIRAD 1246), and ET50-C3-Foumbot (CIRAD 1244) showed more susceptibility than the control.
By this fact it is not possible to draw consistent conclusions and these experiments must be repeated again in the future.

Table 2. Reactions of coffee hypocotyls, according to van der Graaff scale, and number of tested hypocotyls (NTH), inoculated with CBD isolate from Tanzania (TZ005)

CIFC nº	Coffee Designation	0	1	2	3	4	NTH
19239	ET4 - C3 CIRAD 1238			24		11	35
19240	ET4 - C4 - Foumbot CIRAD 1239			15	5	15	35
19241	ET4 - C5 CIRAD 1240			30		10	40
19242	ET14 - L259 - C3 CIRAD 1312			37		3	40
19244	ET19 - L61 - C4 CIRAD 1316			30	2	11	43
19245	ET34 - bc2 - C3 - Foumbot CIRAD 1242			10	3	13	26
19246	ET50 - C3 - Foumbot CIRAD 1244			7		26	33
19247	ET54 - C4 - Foumbot CIRAD 1246			20	3	2	25
19248	ET54 - C5 CIRAD 1247			34	10	11	55
19249	KF1 - L29 - C1 CIRAD 1322		40	7		3	50
19250	KF6 - L.46 - C10 CIRAD 1324			51		14	65
19251	CO - L13 - C3 CIRAD 1308			23		12	35
19252	CO - L13 - C5 CIRAD 1309			1		29	30
19253	Ab1 - L1 - C2 CIRAD 1303		4	30		6	40
19254	Lb1 - L33 - C1 CIRAD 1326			50		5	55
19258	JAVA CIRAD 1249			20		50	70
19259	CATURRA CIRAD 1330			50	4	26	80

Table 3. Reactions of coffee hypocotyls, according to van der Graaff scale, and number of tested hypocotyls (NTH), inoculated with CBD isolate from Kenya (KNO 10)

CIFC nº	Coffee Designation	0	1	2	3	4	NTH
19239	ET4 - C3 CIRAD 1238			3	7	25	35
19240	ET4 - C4 - Foumbot CIRAD 1239			20	5	10	35
19241	ET4 - C5 CIRAD 1240			23		17	40
19242	ET14 - L259 - C3 CIRAD 1312			29		11	40
19244	ET19 - L61 - C4 CIRAD 1316			39		4	43
19245	ET34 - bc2 - C3 - Foumbot CIRAD 1242			25		4	29
19246	ET50 - C3 - Foumbot CIRAD 1244			7		27	34
19247	ET54 - C4 - Foumbot CIRAD 1246				2	23	25
19248	ET54 - C5 CIRAD 1247			33		22	55
19249	KF1 - L29 - C1 CIRAD 1322		47			3	50
19250	KF6 - L.46 - C10 CIRAD 1324		50	6		9	65
19251	CO - L13 - C3 CIRAD 1308			22		13	35
19252	CO - L13 - C5 CIRAD 1309			24		6	30
19253	Ab1 - L1 - C2 CIRAD 1303		10	7		17	34
19254	Lb1 - L33 - C1 CIRAD 1326			22		33	55
19258	JAVA CIRAD 1249			15		55	70
19259	CATURRA CIRAD 1330		3	37		40	80

Table 4. Reactions of coffee hypocotyls, according to van der Graaff scale, and number of tested hypocotyls (NTH), inoculated with CBD isolate from Cameroon (CM 732)

CIFC nº	Coffee Designation	0	1	2	3	4	NTH
19239	ET4 - C3 CIRAD 1238			15	5	15	35
19240	ET4 - C4 - Foumbot CIRAD 1239			20	4	11	35
19241	ET4 - C5 CIRAD 1240		7	30		3	40
19242	ET14 - L259 - C3 CIRAD 1312			22	10	8	40
19244	ET19 - L61 - C4 CIRAD 1316			42		1	43
19245	ET34 - bc2 - C3 - Foumbot CIRAD 1242		20	7		2	29
19246	ET50 - C3 - Foumbot CIRAD 1244					34	34
19247	ET54 - C4 - Foumbot CIRAD 1246			4		21	25
19248	ET54 - C5 CIRAD 1247			40	3	12	55
19249	KF1 - L29 - C1 CIRAD 1322			45		5	50
19250	KF6 - L.46 - C10 CIRAD 1324			52	10	3	65
19251	CO - L13 - C3 CIRAD 1308			30	5		35
19252	CO - L13 - C5 CIRAD 1309			10		20	30
19253	Ab1 - L1 - C2 CIRAD 1303			37	2	1	40
19254	Lb1 - L33 - C1 CIRAD 1326		43	10		2	55
19258	JAVA CIRAD 1249			45	4	21	70
19259	CATURRA CIRAD 1330			51	10	19	80

WP4 (Epidemiology): Evaluation of the role of antagonistic micro flora

At CIFC it was evaluated the antagonistic effect of the extract Gal-02 from the fungus *Ganoderma* sp. (collected in rosemary and supplied by Dr. Wagner Bettiol - Embrapa Meio Ambiente, Brazil) on CBD isolates. Previous tests made in Brazil showed that the extract of this fungus basidiomycete have antagonistic effect on different plant pathogens (Bettiol, oral communication).

4.1 Material and methods

Plants and fungus isolates

Susceptible young leaves of Catimor were inoculated with isolates of *C. kahawae* (CIFC Q2) from Kenya, (CIFC Z1) from Zimbabwe, (CIFC Ca1) from Cameroon, (CIFC A6) from Angola, (CIFC M2) from Malawi, (CIFC R1) from Rwanda.

Treatment of coffee leaves with Gal-02

The lower epidermis of detached young leaves was sprayed with a Gal-02 solution diluted 1:10 in 10% ethanol and kept at room temperature. Two hours later the leaves were inoculated with different CBD isolates. Leaves sprayed with 10% ethanol were used as controls.

Inoculation and incubation

The inoculation was done in young leaves according to the technique described by van der Vossen *et al.* (1976) with slight modifications. The young leaves were placed on aluminium trays lying down on a nylon sponge and then inoculated with conidia suspension ($2x10^6$/ml) by means of an atomizer connected to a pressure pump. The trays were covered with a glass plate and put in a phytotron at 22ºC. The incubation period lasted 24h in which the inoculated material was kept in dark conditions. The disease assessment was made according to van der Graaf´s scale (1981).

Light microscopic observations

Conidia germination and appressoria formation in vitro - Glass slides were sprayed with a Gal-02 solution diluted 1:10 in l0% ethanol and kept at room temperature during 2h. Glass slides sprayed with 10% ethanol were used as controls. Germination and appressoria formation "*in vitro*" were evaluated by placing aliquots of 40 µl of the conidia suspensions in the glass slides (previously treated with Gal-02 and with 10% ethanol), which were kept in moist chamber during 17h at 22ºC. After this time the germination was stopped with an aqueous solution of 3% formaldehyde and the fungal structures were stained with a drop of blue lactophenol. The percentage of germinated conidia and appressoria (melanized and hyaline) formed were made on a minimum of 6 microscope fields of 100 conidia each/experiment (Silva *et al.*, 1985)

Conidia germination and appressoria formation in vivo - Germination "*in vivo*" and appressoria formation were evaluated on leaf pieces (±5cm^2), 17h after the inoculation, following the technique described by Silva *et al.* (1985). The leaf pieces were painted with transparent nail polish on the lower surface. About 24h later, the nail polish (leaf replica) was removed with the help of tweezers and stained and mounted with blue lactophenol. Countings of the germinated conidia and appressoria (melanized and hyaline) formed were made on a minimum of 6 microscope fields of 100 conidia each/experiment.

Statistical analysis

Arcsine transformed percentages and the Student test were used for statistical analysis.

4.2 Results

Antagonistic studies of Gal-02

As shown in the Tables 5 – 16, the conidia germination of the isolates Z1, M2 and R1 was significantly inhibited *in vitro* by the extract Gal-02, while for the isolates Ca1, Q2 and A6 the results were not conclusive. However, the conidia germination of all the *C. kahawae* isolates tested was inhibited *in vivo*. The differentiation of melanized appressoria of the different CBD isolates was also inhibited by Gal-02, both *in vitro* and *in vivo* (Figs 1 and 2). In what concerns the

percentage of hyaline appressoria, the results of the *in vitro* and *in vivo* studies were not coincident. Even in the *in vivo* studies, contradictory results were obtained for the isolates A6, M2 and R1, in the different experiments. So, apparently it seems that Gal-02 had no effect in the appressoria melanization.

Table 5. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **Z1** (from Zimbabwe) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	74,3	31,3	4,1***	91,8	2,7	18,1***	3,7	27,2	2,8***
II	60,5	1,7	5,9***	74	0	-	16,1	0,5	2,5***

*C= control
***(P 0,01)

Table 6. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **Z1**(from Zimbabwe) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	70,2	46	1,5***	94,2	1	26,4***	2,5	8	3,3***
II	65	50,2	0,9***	88,5	4,2	17,8***	5,5	6	0,1***

*C= control
***(P 0,01)

Table 7. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **Ca1** (from Cameroon) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	37,3	79,3	4,8***	86,7	2,7	26***	4,2	0	-
II	82,2	1	10,6***	64,3	0	-	16,8	1	4***

*C= control
***(P 0,01)

Table 8. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **Ca1** (from Cameroon) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C*	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**

I	80,2	57,3	2,9***	96,8	0	-	0,5	3,5	4,4***
II	94,8	97,3	1,1***	94,2	3	23,2***	3,5	8	2,6***

*C= control
***(P 0,01)

Table 9. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **Q2** (from Kenya) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	17,5	48,3	5,3***	62,3	7,7	7,1***	8,2	12,3	2,1***
II	33,8	62,3	2,4***	93,7	1,3	23***	1	6,5	3,5***

*C= control
***(P 0,01)

Table 10. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **Q2** (from Kenya) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	68,8	60,5	1,7***	75,7	2,5	14,0***	11,5	7	1,6***
II	78,3	70,3	1***	89,2	7,8	11,7***	6,3	3,7	1,1***

*C= control
***(P 0,01)

Table 11. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **A6** (from Angola) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	25,3	3,3	5,8***	88,3	0	-	3,5	0,17	7,3***
II	53,2	54,2	0,2***	94,3	31,3	3,1***	3,5	2,3	1,4***

*C= control
***(P 0,01)

Table 12. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **A6** (from Angola) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	68,8	8,5	4,9***	75,7	0,5	8,1***	11,8	0	-
II	45,8	62	0,6***	94,3	21,5	15,2***	1,8	3,2	0,8***

*C= control
***(P 0,01)

Table 13. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **M2** (from Malawi) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	64,2	21,8	2,9***	78,7	0,3	8,7***	10,7	0,3	2,2***
II	37,5	5,5	9,6***	66,8	0,3	38,1***	10	1	10,5***

*C= control
***(P 0,01)

Table 14. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **M2** (from Malawi) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	68,8	37,3	2,4***	84,8	13,7	8,4***	3	4,7	0,8***
II	40	30,3	1,3***	89,2	0	-	2,7	1,3	1,7***

*C= control
***(P 0,01)

Table 15. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated by the isolate **R1** (from Rwanda) of *C. hahawae in vitro* – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	50,2	1,7	9,4***	75	0	-	14,8	0,7	4,7***
II	30,8	1,3	14,5***	70,3	0	-	5,7	0	-

*C= control

***(P 0,01)

Table 16. Percentages of germinated conidia and appressoria (melanized and hyaline) differentiated at 17 hours after the inoculation, by the isolate **R1** (from Rwanda) of *C. hahawae* on coffee leaves – Experiments I and II

Exp.	Conidia germination (%)			Melanized appressoria (%)			Hyaline appressoria (%)		
	C	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**	**C**	**Gal-02**	**t test**
I	59	44,8	4,4***	85,2	9,2	15,5***	1,8	10,3	5***
II	61,7	36	1,7***	96	41,5	10***	8	6,2	3,3***

*C= control
***(P 0,01)

The reduced percentages of conidia germination and appressoria (melanized) formation in the coffee leaves treated with Gal-02, comparatively to the controls, gave rise to a small number of lesions although the symptoms appeared at the same time in treated and non-treated leaves.
At CIFC it was initiated the study of the modifications induced by Gal-02 in the plant responses (local protection/systemic acquired resistance). The same kind of studies was initiated with the antagonistic bacteria *Bacillus subtilis* (from the CIFC collection).

Isolation, selection and identification of antagonistic micro organisms
At the end of September we received coffee leaves and berries from Dr. Gichuru (Coffee Research Station, Kenya) for bio control studies. The washing technique was used for microflora isolation. It was identified the fungus *Epicoccum* sp..8 The antagonistic effect of this fungus on CBD will be investigated.

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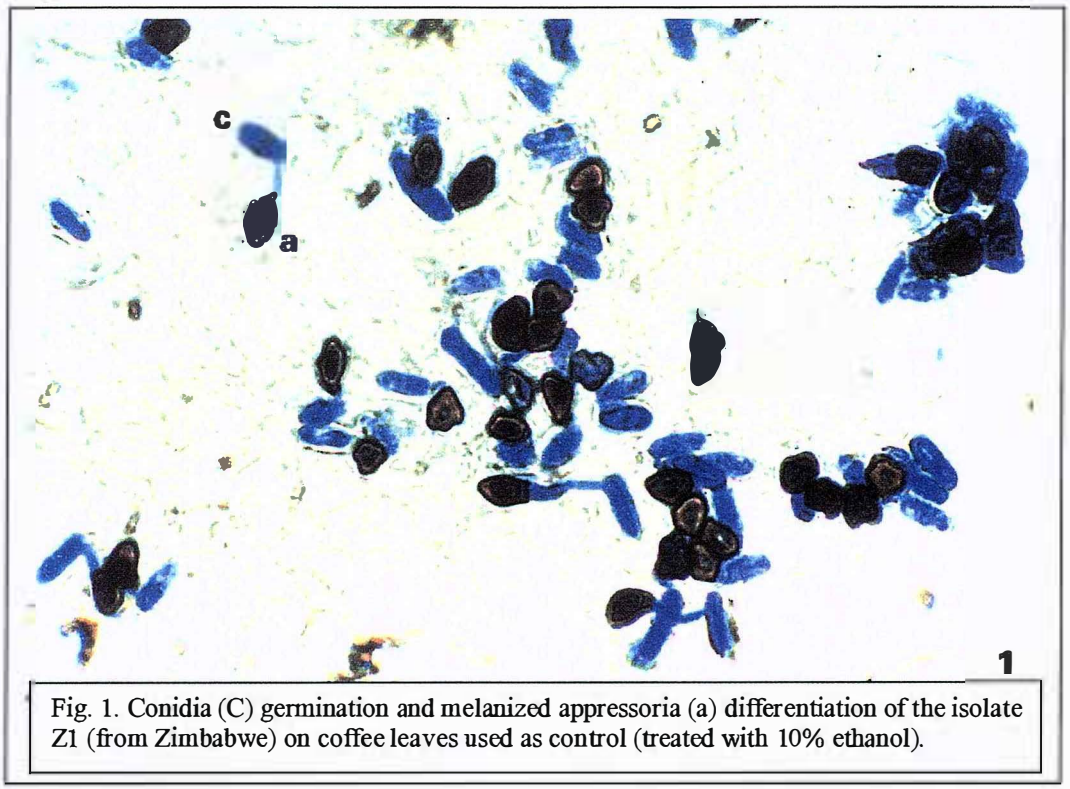

Fig. 1. Conidia (C) germination and melanized appressoria (a) differentiation of the isolate Z1 (from Zimbabwe) on coffee leaves used as control (treated with 10% ethanol).

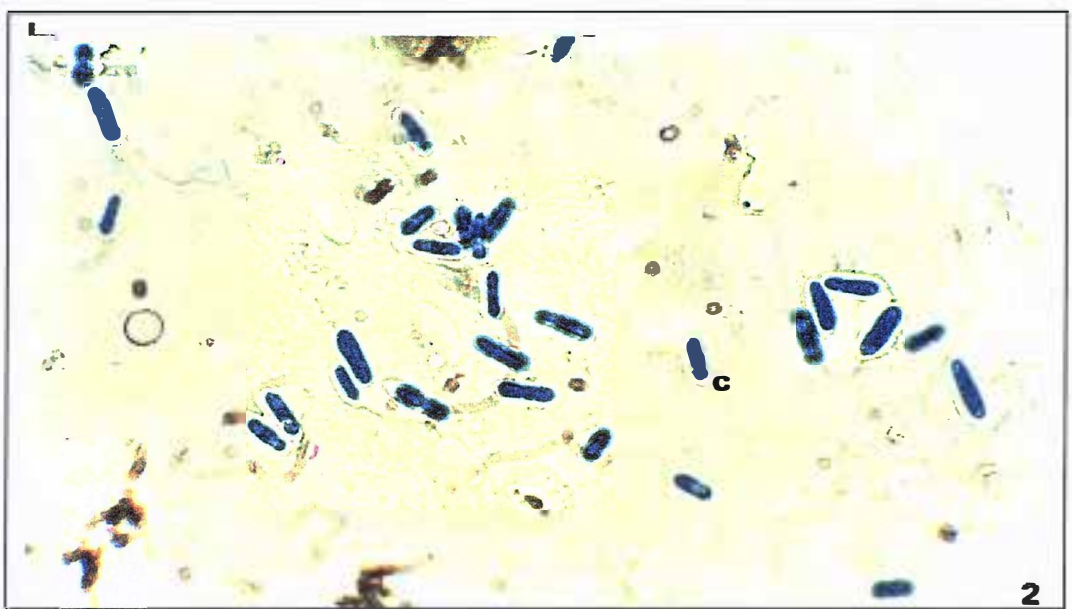

Fig 2. Inhibiton of conidia (c) germination of the isolate A6 (from Angola) on coffee leaves treated with Gal-02.

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CONTENTS

WP1: BREEDING FOR RESISTANCE

Rehabilitation, maintenance and inventory of collections and variety trials have been the main activities in year 1. Weeding, pruning, and regeneration were made:

- On Foumbot Station in the germplasm collection, the Java seed garden and diallel trials established in 1986 and 1989
- On Santa Coffee Estate in the collection, and variety trials 1973, 1974, 1975, 1976A, and 1976B.

An inventory of germplasm available was made in all these fields. The results show that the Ethiopian material is slowly disappearing.

WP2 Nature of Resistance – Host/pathogen interaction

Validation of tests, correlation with field resistance

IRAD received from CIRAD seeds of Catimor and Sarchimor lines that appeared to have some resistance in earlier hypocotyls tests made by CIRAD. They have been germinated and transplanted; they are currently in the nursery (Table 1).
They will be established in zones with a high parasitic pressure in order to confirm their field resistance and to correlate it with the results of hypocotyls tests.

Ring test

Seeds of various genotypes as listed in Table 2 and 3 were collected and dispatched to Cirad for hypocotyls tests to be made at Cirad and CIFC (Ring Test).

Table 1: List of seed samples received from Cirad for field-testing

Variety	Sample	Line	Seeds received, Weight (g)	Plants obtained (Nkolbisson nursery, October 2002)
Colombia	R1	T17924	71	90
Colombia	R2	T17925	92	79
Colombia	R3	T17926	80	200
Colombia	R4	T17927	81	59
Colombia	R5	T17928	79	8
Colombia	R6	T17929	81	61
Colombia	R7	T17930	84	78
Colombia	R8	T17931	85	111
Colombia	R9	T17932	75	79
Colombia	R10	T17933	81	5
Colombia	R11	T17934	78	72
Colombia	R12	T17935	83	24
Colombia	R13	T17936	83	64
Colombia	R14	T17937	86	40
Colombia	R15	T17938	85	19
Colombia	R16	T17939	83	59
Colombia	R17	T17940	84	52
Colombia	R18	Villa Sarchí	94	41
Colombia	R19	Catuaí Regional	79	51
Colombia	R20	Caturra	86.6	53
Sarchimor	R1	T17924	95	97
Sarchimor	R2	T17925	86	10
Sarchimor	R3	T17926	82	10
Sarchimor	R4	T17927	79	30
Sarchimor	R5	T17928	89	70
Sarchimor	R6	T17929	90	6
Sarchimor	R7	T17930	86	69
Sarchimor	R8	T17931	78	0
Sarchimor	R9	T17932	79	56
Sarchimor	R10	T17933	95	100
Sarchimor	R11	T17934	92	103
Sarchimor	R12	T17935	71	76
Sarchimor	R13	T17936	83	50
Sarchimor	R14	T17937	83	60
Sarchimor	R15	T17938	77	80
		TOTAL	2915.6	2062

Table 2: List of seeds samples collected on Foumbot Station in 2001 for dispatch to CIRAD and CIFC for ring test
Dispatch prepared 30/11/01 at Nkolbisson

Field	Variety	Tree	Total Parchment Weight (g)	Nkolbisson	Cirad	CIFC
Collection	Et02	4	39.95	40	0	0
Collection	Et02	6	94.92	47	48	0
Collection	Et03	2	48.30	48	0	0
Collection	Et03	3	15.57	16	0	0
Collection	Et04	1	33.74	33	0	0
Collection	Et04	2	52.38	52	0	0
Collection	Et04	3	143.65	48	48	48
Collection	Et04	4	162.37	62	50	50
Collection	Et04	5	273.30	133	70	70
Collection	Et06	2	34.25	34	0	0
Collection	Et06	3	32.50	32	0	0
Collection	Et11c	5	64.87	65	0	0
Collection	Et17	5	52.30	52	0	0
Collection	Et17	6	27.89	28	0	0
Collection	Et19	1	62.38	62	0	0
Collection	Et19	1	36.26	36	0	0
Collection	Et19	3	63.74	63	0	0
Collection	Et33	4	92.55	47	46	0
Collection	Et34	4	105.04	55	50	0
Collection	Et34bc1	1	25.56	25	0	0
Collection	Et34bc2	3	128.98	45	42	42
Collection	ET34bc5	1	46.72	47	0	0
Collection	Et34bc5	2	32.93	33	0	0
Collection	Et40	2	67.06	67	0	0
Collection	Et50	3	160.92	61	50	50
Collection	Et54	2	87.79	44	44	0
Collection	Et54	4	128.17	42	43	43
Collection	Et54	5	213.43	73	70	70
Collection	Et55	2	74.07	37	37	0
Collection	Et55	5	70.38	35	35	0
Collection	Et59	3	16.80	17	0	0
Seed Garden	**Java**	**-**	**7500**	**2500**	**2500**	**2500**

Table 3: List of seeds samples collected on Santa Estate in 2001 for dispatch to CIRAD and CIFC for ring test

Prepared and sent to Cirad in February 2002

Variety	Field	Row	Tree	Qty (1)	Total Parchment weight (g)
Ab1	Collection	1	1	-	21
Ab1	Collection	1	2	++	243
Ab1	Collection	1	3	-	19
Bb1	Collection	175	1		518
Bb1	Collection	175	2		27
Bb1	Collection	175	3		231
Caturra	x		x	++	1097
Co	Collection	13	3	++	210
Co	Collection	13	5	++	150
Et02	Collection	275	2	+++	445
Et04	Collection	210	2	-	18
Et14	Collection	259	3	++	145
Et19	Collection	61	1	++	414
Et19	Collection	61	2	+	99
Et19	Collection	61	3	++	110
Et19	Collection	61	4	++	150
Et19	Collection	61	5	?	39
Et26	Collection	220	1	?	23
Et50	Collection	66	2	+	82
Il3	Collection	21	6	+	43
Jm1	Collection	25	6	-	17
Kf1	Collection	29	1	+++	418
Kf6	Collection	46	2	-	55
Kf6	EV73	46	10	+++	511
Kf9	Collection	32	3	+	94
Lb1	Collection	33	1	++	303
Lb1	Collection	33	5	?	29
Mot	EV73	62	1	+++	146
MoT	EV73	62	3	++	226

(1) drying, 11/02

WP4 DISEASE DEVELOPMENT – EPIDEMIOLOGY

Two main tasks have been undertaken in the field.

4.1 Task 1: Evaluation of crop losses due to CBD in various ecological zones, evolution of the disease in the space and in the time

4.1.1 Objectives

- Describe the evolution in the time of the disease within the coffee bush and within the field in various agro-ecological conditions.
- Evaluate the influence of cultural practices on the development of the disease.
- Propose a model that takes into account these factors, and strategies specific to each agro-ecological context.

4.1.2 Localisation

Three Arabica coffee farms were chosen in the highlands of West Cameroon where the parasitic pressure is high, namely:

- Dutsitsa (Bafou): > 1800 m a.s.l.
- Santa: between 1600 and 1800 m.a.s.l.
- Babadjou: around 1600 m a.s.l.

The cultural practices in each location are different.

4.1.3 Material and methods

Planting material

At Dutsitsa and Santa, the fields are planted with homogeneous local Arabica varieties.

At Babadjou, two fields are used. One is planted with local variety ("Jamaïque"), the other one with Java (resistant selection).

Method

200 vigorous and good yielding coffee trees were earmarked in each trial field. Each field was divided into 2 experimental plots of 100 earmarked trees of which 50 were shaded by various fruit trees (mango, avocado, banana, cola nut etc.), and 50 under full sunshine.

Each experimental tree was labelled with a number and a coloured tag according to shade: blue for shade, yellow for full sunshine.

It had been planned to have one sub-plot with recommended practices, however due to the late start of the experiment in the season this treatment was not included.

Experimental design

Multilocational trial with 3 sites
Two cultural practices per site, in two adjacent fields.
(in 2002 these two fields are blocks)
Two treatments: shaded/not shaded
Randomisation tree by tree in each field (50 trees per treatment)
Adjacent control in one site: variety Java, 2 blocks/cultural practices.

Records

Disease evolution

Three branches per coffee bush were randomly selected at the pinhead stage of the fruits. Assessments were made from 29 May to 10 October 2002 as follows;

a) First assessment: all fruits counted
b) Weekly: census and labelling of diseased cherries
c) From the second week onwards: census of cherries labelled on the previous week.

Cause of fruit fall

Under one of the 3 branches earmarked an aluminium foil was placed. Fallen fruits were examined in order to assess the cause of fruit drop (physiology, pests etc.).

22 assessments were made in each site (plus 22 in Java blocks) thus 88 assessments in total. Two assessments remain to be done:

- weight of fresh cherries harvested on each experimental branch, and on each experimental tree. Picking will start 28/10/02.
- Map of each experimental block (precise location of each tree) to be done in December 2002.

4.1.4 Results

All data recorded in this experiment have already been entered under Excel. After crosschecking the data a detailed analysis will be made at the beginning of 2003. It is too early at this stage to give any indication of the level of disease this year as the data have not been compiled yet. Table x gives some meteorological data for the season in two locations. No records could be made in Bafou as nobody was available there for follow up.

Meteorological Data over two of the sites during the CBD season

Site	Shaded Coffee		Unshaded Coffee		Rainfall (ml)
	Min. T° (°C)	Max. T° (°C)	Min. T° (°C)	Max. T° (°C)	
Babadjou	14.6	25.2	15.4	36.2	196.9
Santa	14.2	25.5	13.5	32.2	201.1

4.1.5 Perspectives

Next year the same assessments will be made on the same trees using the same protocol. Therefore:

- experimental trees will be checked and re-labelled the same way in December 2002.
- Recommended cultural practices will have to be applied in one sub-field per site starting at the end of picking. All maintenance activities will have to be done at the same time in all sites.
- The choice of experimental branches and the first assessment have to be done at the pinhead stage. In the coming season the presence of leaves a each node will be noted as well in order to connect the fruit fall to the branch's physiology.

4.2 Task 2: CBD evolution in the time and in the space in two agro-ecological zones and under various farming systems in West Cameroon

4.2.1 Objectives

To assess the influence of farming systems (shade, intercropping practices) over 4 years, in 2 different agro-ecological zones, and at two altitudes.

4.2.2 Achievements

Choice of sites

Five trips were made to the Arabica zone in this first year.

The first one was made at the end of November 2001 with the Project Co-ordinator (P. Charmetant). Several farms were visited around Santa and Bamenda. Preliminary observations were made on the farming systems prevailing in this region.

In February 2002 the second trip was aimed at identifying production zones situated at various elevations that could be earmarked for the project. Many farms were visited around Santa, Dschang, and Kumbo. This allowed us to update our knowledge of the cultural practices used. Interviews took place with the University of Dschang to discuss the possibility of welcoming trainees, and with a major stakeholder of the Coffee Sector (Caplame co-operative). Kumbo zone was not considered useful for the study.

Three more trips were made in June, July, and October 2002. The first aim was, in the light of the first observations, to select some farmers as partners of this research task. Then the experiment was put in place, and the characterisation of each site was started (soil samples, detailed observation and recording of cultural techniques etc.). The follow up of the farms started.

Location of the sites

The network is made of 11 farmers spread over Dschang and Santa zones, at various elevations (Table 4)

Beside differences in soils and climate, the cultural practices vary between the farmers, namely weeding, chemical spraying, intercropping.

Table 4: Identity and localisation of the farmers included in the study

Zone	Village	Farmer	Name	Position	Altitude
Dschang	Bafou	1	DJEUFACK Janvier	05°33.540 N 010°04.520 E	1,815 m
		2	SATSA Edouard	05°33.446 N 010°04.998 E	1,820 m
		3	GUIFO Gérard	05°33.467 N 010°04.849 E	1,817 m
	Penka-Michel	4	FONGANG Jean-Louis	05° 25.727 N 010° 14.600 E	1,444 m
		5	KOLLA Isidore	05° 27.095 N 010° 14.105 E	1,454 m
		6	TAKOUGARY Janvier	05° 27.095 N 010° 14.105 E	1,454 m
Mbouda	Babadjou	7	TOMBOU Mathieu	05° 43.771 N 010° 08.118 E	1,784 m
Santa	Mbu	8	TEMBON Elias	05° 47.190 N 010° 09.672 E	1,738 m
		9	DJITA Godfrey	05° 47.113 N 010° 09.557 E	1,753 m
		10	PA'ALLO John	05° 46.989 N 010° 09.581 E	1,757 m
		11	CHE Africa	05° 46.999 N 010° 09.509 E	1,733 m

Experimental design

In each coffee farm 3 micro-plots of 4 coffee trees were earmarked, depending on shade intensity. Each micro-plots is a replication.

This defines 3 treatments:

- unshaded coffee (most of the time inter-cropped with maize, macabo (banana), potato, beans etc.).
- light shade (bananas ,with more or less intercropping)
- dense shade (safou, or various fruit trees).

The shade intensity was determined according to the type of shade; it will be precisely measured in 2003 using a lux-meter.

In each micro-plot two branches, at the top and in the middle of the coffee tree respectively were earmarked and were assessed at regular time intervals.

Sites, treatments and replications are summarized in Table 5.

Table 5: Number of replications (micro-plots of 4 trees) in the study

		Treatment: shade			
Localisation	**Farmer No.**	**Unshaded**	**Light shade**	**Dense shade**	**Total**
Dschang/Bafut	1	7	6	-	13
	2	5		5	10
	3	5		4	9
Sub-total	**-**	**17**	**6**	**9**	**32**
Dschang/Penka Michel	4	5	5	5	15
	5	5	5		10
	6			5	5
Sub-total	**-**	**10**	**10**	**10**	**30**
Santa/Babadjou	7	5		5	10
Santa/Mbu	8	5		5	10
	9		5	5	10
	10	5		5	10
	11	5		5	10
Sub-total	**-**	**20**	**5**	**25**	**50**
Grand Total	**-**	**47**	**21**	**44**	**112**

Assessments were made from July to October 2002 every fortnight thus six times. The numbers of healthy fruits and of fruits attacked per recorded branch were assessed.

Assessments on % losses will be transformed into 6 classes (0 = no losses, 1 = .1 to 10%, 2 = 10.1 to 20% etc.).

Soils samples have been taken from each farm. 10 topsoil samples (10-20 cm) were taken, and mixed, for each farm.
A questionnaire is currently being elaborated, in order to identify the techniques used by the farmers in 2002.

4.2.3 Perspectives (2003)

- soils analyses
- Enter and analyse data collected from July to October 2002.
- Survey: characterise the farms and identify the techniques developed by the farmers in 2002, enter and analyse data.
- Extend the network. Because of the late start of the disease assessments 4 fields identified in the Santa zone[1] have not been assessed in 2002 as the losses due to CBD were already quite important in July. Two other farms located at Babajou (1660 m a.s.l.) and at Awing (1550 m a.s.l.) will be added to the sites. The total number of farms assessed will then be 17.
- Assessment of shade intensity using a lux-meter. Each time the field will be visited a measure will be taken in each micro-plot. A mean value will be calculated for statistic analvses.

List of scientists involved

Jagoret Patrick (Agronomy, Farming Systems)
Mouen Bédimo Joseph (Phytopathology, Epidemiology)
Deumeni Jean-Pierre (Phytopathology, assessment of epidemiology trials)
Bella Manga (Phytopathology, genetic resistance, scientific supervisor)
Nyassé Salomon (Phytopathology, Administrative Co-ordinator)
Snoeck Didier (Agronomy and Breeding, Assistant Co-ordinator)

Annexe 2: DATA SHEET FOR ANNUAL REPORT

Contract number : ICA4-CT-2001-10008

Year 1

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 (cumulative)	Totals
Number of communications in conferences (published)	0
Number of communications in other media (internet, video,)	1
Number of publications in refereed journals (published)	0
Number of articles/books (published)	1
Number of other publications	1
2. Training	
Number of PhDs	0
Number of MScs	0
Number of visiting scientists	0
Number of exchanges of scientists (stays longer than 3 months)	0
3. Achieved results	
Number of patent applications	0
Number of patents granted	0
Number of companies created	0
Number of new prototypes/products developed	0
Number of new tests/methods developed	0
Number of new norms/standards developed	0
Number of new softwares/codes developed	0
Number of production processes	0
4. Industrial aspects	
Industrial contacts	~~yes~~ no
Financial contribution by industry	~~yes~~ no
Industrial partners : - Large	~~yes~~ no
- SME [1]	
yesno	

S. Comments

Other achievements (use separate page if necessary)

[1] Less than 500 employees.