7
Screening of Robusta for Resistance to CWD

7.1 Main Findings

- Various methods were evaluated to establish a simple, reliable and cost-effective way of evaluating resistance of coffee germplasm to coffee wilt disease (CWD), so that mass screening activities could be accomplished.
- Two methods were preferred: dipping of roots into a suspension of spores for about 20 min or scraping the stem of seedlings with an infected scalpel.
- Standard concentrations and exposure times were established. Spore concentrations as low as 13 spores per millilitre were sufficient to cause seedling mortality.
- Collections of wild Robusta from Kalangala and Itwara forests showed a high level of resistance to CWD.
- In Uganda, a very wide difference in susceptibility to field-grown clones was found, from 0 to 96% mortality.
- In Tanzania, no variation between CWD strains was found, and all commercial clones released to coffee growers in recent years are highly susceptible to the disease.
- Among the available Tanzanian Robusta material, the highest level of resistance was found in the Maruku germplasm collection but more fieldwork needs to be carried out to further evaluate this.
- At the University of Kinshasa (UNIKIN), studies reveal a range of resistance among the tested material with several genotypes showing substantial levels of resistance with levels of mortality less than 10%, 5 months after inoculation. Overall, the mortality rates found in screening in the Democratic Republic of Congo (DRC) tend to be lower than for similar studies in Uganda and Tanzania.

7.2 Assessment of Resistance

A quick and effective screening procedure is essential to the development of a breeding programme.

Protocols are required to quantify resistance, so that routine assessment of resistance can be carried out on a large scale. If at all possible, a laboratory test should be devised to be used with young plants and seedlings so that time lags can be reduced to the minimum. Although subsequent field testing is essential, much can be done in the laboratory to identify likely resistant material and reject obviously susceptible stock.

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

7.2.1 Artificial inoculation methods

Different infection techniques were developed and compared in order to come up with a simple, effective and repeatable method to reliably infect coffee trees and seedlings with various test strains of the CWD pathogen. These included:

- dipping roots into an aqueous spore suspension;
- syringe injection with a spore suspension into the stem; and
- infection through deliberately wounding seedlings with an infected scalpel.

Other techniques that were also considered but not subsequently used as standard tests included:

- soil infection (planting test plants in CWD-contaminated soil);
- soil drenching (drenching the soils in which test plants were growing with a spore inoculum); and
- soil drenching (as per techniques mentioned above, but after wounding the roots while the plants remain *in situ*).

Root dipping, used at Coffee Research Institute (CORI) in Uganda, involves taking plants between 9 and 12 months old, washing off the soil with tap water and then dipping their naked roots for 30 min into an aqueous solution containing 1×10^6/ml spores of a monoconidial strain of the disease previously isolated from susceptible Robusta clone 257s/53.

After dipping, the plants were replanted in sterilized soil in plastic pots and then maintained in a screen house at ambient temperatures and monitored regularly for signs of disease.

Wounding (carried out at CIRAD in France) involved inoculating 10–12-week-old seedlings with a suspension of 1×10^6/ml monoclonal spores derived from isolate CAB003 collected from Ugandan Robusta. Wounds were made under the cotyledons with a scalpel and applying one to two drops of the inoculum into the wound. The plants were incubated under controlled conditions of 25 °C, 80% RH and 12h/12h lighting and regularly watered and monitored for disease symptoms.

Spore injections (carried out at UNIKIN) used 9–12-month-old plants cultivated in a screen house. It involved injecting 1×10^6/ml spores into the stem above the first internode (the cotyledons).

All the methods of artificial inoculation gave similar results. However, plants inoculated by root dipping developed the disease symptoms earlier than plants inoculated by other methods, had a higher incidence of diseased plants and there was a clear contrast between susceptible and resistant genotypes. This method was, therefore, adopted for large-scale germplasm screening by scientists in Uganda and Tanzania.

However, root dipping required a lot more inputs (polythene pots, soils and manpower) than the other methods, and therefore is more costly. It was also suspected that some of the plants infected by this method could have developed the disease because of extra stress resulting from root damage incurred when stripping off soils from roots in preparation for dipping.

At CIRAD, where labour is costly, stem nicking was adopted. This method can also clearly enable differentiation of resistant and susceptible genotypes. Stem nicking was also adopted for germplasm screening in Ethiopia as it is considered to be less expensive, although the disease levels among plants inoculated by root dip was always higher.

7.2.2 Inoculum concentration and exposure

Efforts to develop resistant varieties during the historical epidemics of CWD used high concentrations of spores (1.3×10^6/ml) but it was considered that this might be too high for current purposes, since it might lead to useful genetic material being discarded. Hence, experiments were carried out to determine an optimum range for inoculation.

Results of these tests suggested that concentrations between 1.3×10^3 and 1.3×10^5/ml gave the best compromise between avoiding the conflicting problems of rejecting resistant material and accepting susceptible material. Interestingly, the experiments also showed that a concentration as low as 13 spores per millilitre was enough to cause some mortality, albeit with a long incubation period (2+ months).

For root dipping, a series of experiments revealed that a 20 min exposure time was sufficient. Thus, exposure of a low spore concentration for a short time is enough to infect susceptible plants, which attests to the virulent nature of this disease.

7.3 Quantifying CWD Resistance in Artificial Inoculation

It is important to quantify level of resistance found in artificial inoculation experiments. In addition to simply measuring percentage mortality after a given time, a visual scale of disease progression was devised. In Uganda, the plants were graded on a scale of 1 to 5, where 1 = no disease, 2 = curling leaves and stunted growth, 3 = leaf wilting and yellowing, 4 = leaf necrosis, leaf wilting and abscission, and 5 = plants are dead.

Mature trees in the field were also assessed on a scale of 1–5, but the symptoms employed were different: 1 = no disease, 2 = 1–25% defoliation, 3 = 26–50% defoliation, 4 = 51–75% defoliation, and 5 = 76–100% defoliation = dead.

However, this is an inexact science. Studies at both CORI and CIRAD showed that even genetically identical coffee trees succumb at different rates in heavily infected gardens and that the time lapse between the first-observed symptoms and the death of trees varies between genotypes. This is no doubt at least partly due to the different numbers of spores that initially infected the tree as we have seen from the artificial inoculation experiments mentioned earlier.

To identify genotypes that are totally resistant, only plants without any symptoms after a long period of infection (6 months for plants in artificial inoculations and not less than 5 years for plants evaluated in heavily infested fields) could be considered resistant. Where necessary, these plants can be re-inoculated and reassessed for another 6 months to confirm their resistance. The plants which remained healthy after the re-inoculation were considered to have complete resistance and such plants were planted in mother gardens for cloning and further assessments.

7.4 Field Evaluation in Uganda

7.4.1 Survey-collected material

Results showed that the Kalangala and Itwara populations (Chapter 6) were highly resistant. On the other hand, the Nganda and Erecta populations were very susceptible, with Kabale progenies showing intermediate resistance. Arabusta (Robusta × Arabica cross) was also found to be resistant to Ugandan CWD strains.

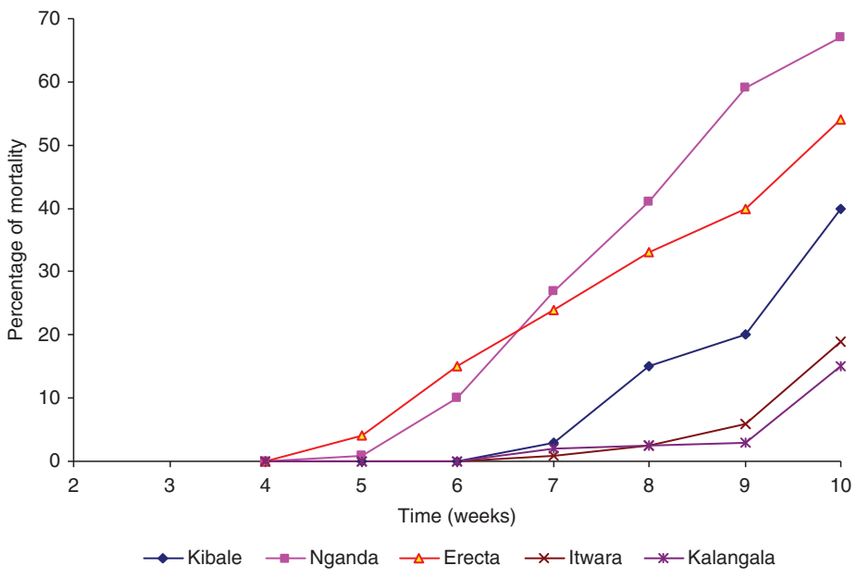
Analysis of variance performed on 1–5 symptom progression data at 10 weeks after inoculation found significant genetic differences for disease levels between sources ($p = 0.0001$) and between progenies across sources ($p < 0.0001$)). Analysis of variance within sources found highly significant ($p < 0.0001$) genetic difference between progenies within Kibale, Kalangala, Erecta and Nganda sources. Differences between Itwara progenies were not significant ($p = 0.08$).

Mean separation tests placed sources into three classes in the decreasing order of disease development:

- A, consisting of Nganda;
- B, consisting of Erecta and Kibale; and
- C, consisting of Itwara and Kalangala.

These results indicate that Itwara and Kalangala have high levels of resistance and Nganda the least (Figure 7.1). Mean separation tests for progeny means within sources found large class overlaps in Kibale, Kalangala, Nganda and Erecta, implying resistance to coffee wilt is controlled by many genes that are not equally available among the progenies.

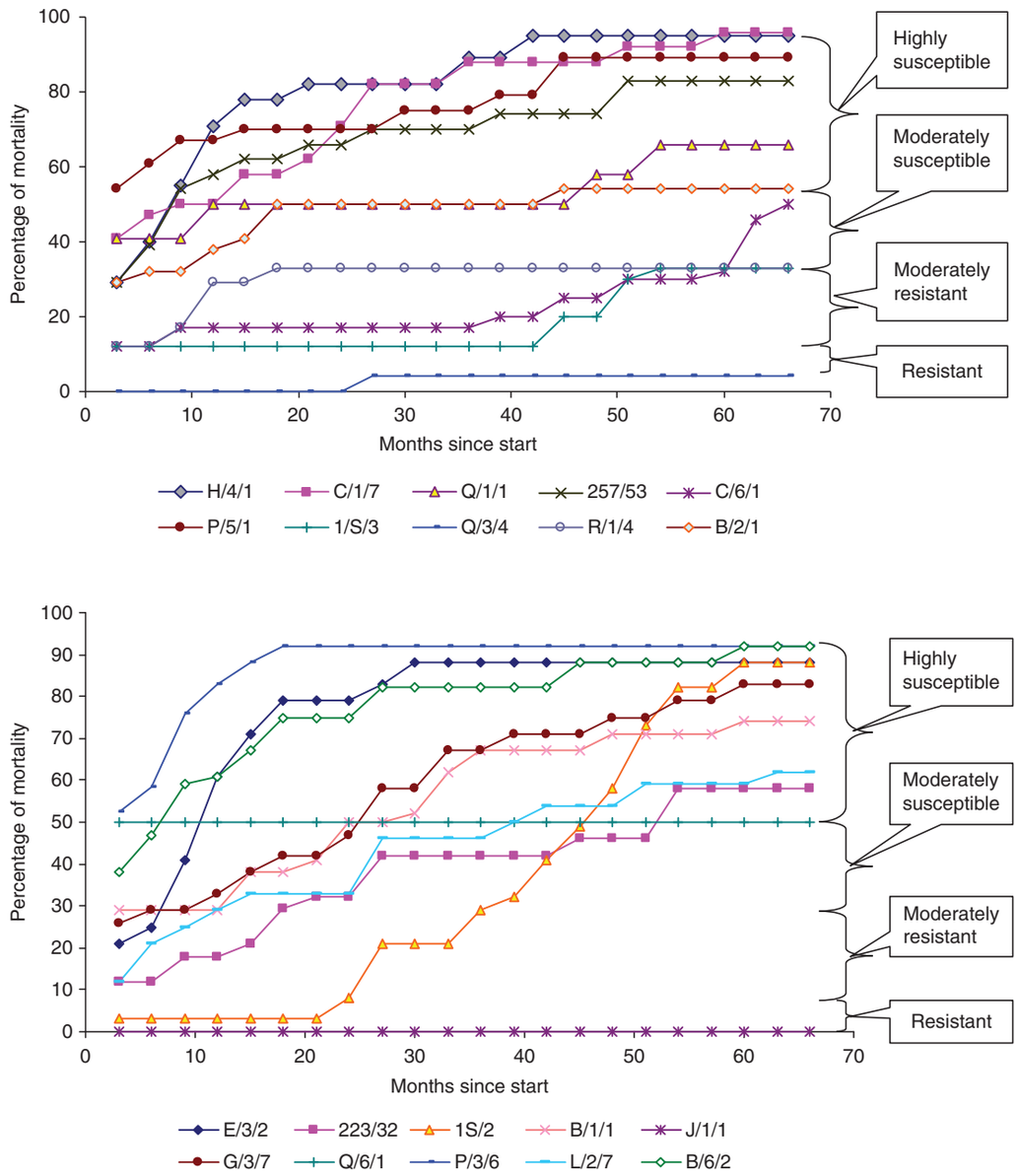
Figure 7.1: Progression of coffee wilt disease (CWD) among open-pollinated progenies of different *Coffea canephora* populations.



7.4.2 Cloned materials

A study of various field-grown *Coffea canephora* clones at CORI revealed a range of responses to CWD infection (Figure 7.2). Clone J/1/1, which did not succumb to the disease throughout the assessment period, was considered resistant. Clones Q/3/4, R/1/4 and 1S/3, whose disease levels were very low, were also considered resistant. Statistical analysis using analysis of variance showed very significant differences between the clones.

Figure 7.2: Tree mortality of 20 *Coffea canephora* clones evaluated for coffee wilt disease (CWD) resistance in a field at Kituza.



Clone 1s/2 appeared to be resistant for the first year of the study, after which the trees began dying in large numbers. Hence, for field screening there is a need to assess the plants for many years, to allow the inoculum build-up or the disease to overcome the plant's resistance.

Field resistance studies of *C. canephora* at CORI displayed mortality ranging from 0 to 96%. The disease period (period from appearance of first symptoms to death of the three) ranged from 1 to 16 months. Generally, resistant genotypes had long disease periods, but certain resistant genotypes had exceptionally long disease periods while some had very short disease periods.

7.4.3 Comparing resistance in the field and in artificial inoculations

The field results confirmed the general pattern of resistance revealed in the artificial tests (i.e. similar indicators such as percentage of dead plants or percentage of plants defoliated).

There was a high correlation (p = 0.006) between field and laboratory mortalities. However, the measured levels of disease varied somewhat between laboratory and field tests.

One Robusta clone (B2/1) showed differential resistance, with a different response between root dipping and stem wounding, suggesting that there might be different types of resistance. This state of affairs has been reported previously in cotton for resistance against *Fusarium oxysporum* f. sp. *vasinfectum* (Bugbee and Sappenfield, 1968).

Rooted cuttings and seedlings of some of the clones studied in the field were also evaluated by artificial inoculations in a screen house at CORI. The final percentage of tree mortality was correlated with the field mortality. It was discovered that even clone J/1/1, which had no diseased trees in the field, had 15% of mortality through artificial inoculation (Table 7.1).

In contrast, clone B/2/1 had 54% mortality in the field and 0% mortality after artificial inoculation. Nevertheless, there was a highly significant correlation (p = 0.002) between the mortality in the field and the mortality of cuttings in the screen house, suggesting that the artificial inoculation protocol developed is an acceptable way to screen germplasm for resistance. This means that large numbers of open-pollinated seedlings of resistant material can be quickly screened to eliminate non-resistant progeny.

7.4.4 Inheritance of CWD resistance

An understanding of the nature of the coffee tree's resistance to CWD is necessary to breed resistant varieties. The statistical analysis of the disease symptom severity data from the field, as well as the inoculation tests found highly significant ($p < 0.0001$) genetic differences between the clones (Table 7.1).

The range of results and the responses of the different *C. canephora* populations suggest that the CWD resistance in *C. canephora* is controlled by many genes, which are variably distributed among the genotypes and populations.

Table 7.1: Mortality of *Coffea canephora* clones in the field and their rooted cuttings and progenies in artificial inoculation.

Clone	Field mortality	Rooted cuttings	Open-pollinated progenies
J/1/1	0.0 a	15.0 b	-
Q/3/4	4.2 b	20.0 b	-
1S/3	33.3 c	-	35.0 abcd
R/1/4	33.3 c	44.4 bc	35.0 abcd
C/6/1	50.0 cd	-	65.0 def
Q/6/1	50.0 cd	-	80.0 fg
B/2/1	54.2 cd	0.0 a	10.0 a
223/32	58.3 cde	90.0 d	65.0 def
L/2/7	62.5 def	50.0 bcd	25.0 abc
Q/1/1	66.7 defg	-	53.0 cde
B/1/1	75.0 defgh	-	85.0 fg
257/53	83.3 efgh	25.0 b	40.0 bcde
G/3/7	83.33 efgh	80.0 cd	50.0 cde
P/5/1	87.5 fgh	-	-
E/3/2	87.5 fgh	-	25.0 abc
1S/2	87.5 fgh	100 e	20.0 ab
P/3/6	91.7 gh	-	69.0 efg
B/6/2	91.7 gh	-	68.0 efg
H/4/1	94.4 gh	70.0 cd	60.0 def
C/1/7	95.8 h	63.6 cd	95.0 g

Means separated by the Student-Newman-Keuls means separation test.
Figures represented by different letters are significantly different.

The inheritance of CWD resistance in *C. canephora* was calculated by using disease data recorded on mature trees of full sib progenies. The data were analysed using Diogene quantitative genetics software with a Garretsen and Keuls random model adapted for incomplete or half diallel (Keuls and Garretsen, 1977). This model estimated broad sense heritability as 0.329 and narrow sense heritability as 0.112. These rather low values suggest that CWD resistance is heritable but its transmission from parent to progeny is only about 33%. This means that the advantage of using progeny of crosses between resistant parents as a source of planting materials is low. Commercial CWD-resistant Robusta coffee varieties should therefore be cloned (vegetative propagation) in order to retain resistance.

7.5 Tanzanian Studies

Commercial Robusta clones MS1, MS2, MS3, MS4 and MS5 released to coffee growers in recent years are all susceptible to CWD to varying degrees. Hence, scientists at the Tanzania Coffee Research Institute (TaCRI), led by DL Kilambo, carried out a range of tests to detect: (i) the variation in aggressiveness of CWD strains; and (ii) the presence of resistance in Tanzanian coffee accessions.

7.5.1 Variation among CWD isolates

A total of 14 isolates originating from CWD-infected coffee trees in Muleba, Bukoba and Karagwe districts (Table 7.2) were selected based on pigmentation, growth, size and shape of conidia.

Table 7.2: Pathogenicity test results of *Fusarium xylarioides* on Robusta varieties MS1 and MS2.

CWD isolate accession number		Location collected		Number of dead seedlings	
TaCRI	CAB International UK	District	Altitude (m)	MS1	MS2
2004/10	T 1	Muleba	1547	9	9
2004/13	T 2a	Muleba	1545	10	9
2004/07	T 3a	Muleba	1395	9	10
2004/08	T 4	Muleba	1510	10	9
2004/02	T 5a	Muleba	1287	10	9
2004/06	T 8a	Bukoba	1189	10	10
2004/01	-	Bukoba[a]	1200	10	9
2004/12	T 9a	Bukoba	1256	10	9
2004/14	T 12a	Kragawe	1424	9	9
2004/03	T 13a	Kragawe	1317	10	9
2004/05	T 14a	Kragawe	1659	9	9
2004/09	T 15a	Kragawe	1354	9	9
2004/09	T 15b	Kragawe	1354	9	9
2004/09	T 15c	Kragawe	1354	10	10
Mean	-	-	-	9.57	9.21
SE±	-	-	-	0.14	0.11
CV	-	-	-	5.30	4.50
LSD	-	-	-	0.30	0.23

[a] Isolate 2004/01 was used for the CWD resistance evaluation.

Table 7.3: Eight best-performing Robusta lines resistant to coffee wilt disease (CWD) and coffee leaf rust (CLR).

Robusta line/cultivar	CWD resistance[a]	Survivors (%)	CLR resistance[a]
Muleba collection number 26	R	62.5	R
Ngara collection number 10	R	87.5	R
Germplasm collection number 1/62	R	100.0	R
Ngara collection number 13	R	80.0	R
Bukoba collection number 27	R	80.7	MR
Maruku collection number 10	R	56.0	MR
Muleba collection number 2	R	80.0	R
Maruku selection number 2	S	20.0	R

[a]Commercial cultivar, R = Resistant, MR = Moderately Resistant and S = Susceptible.

In addition, 3- and 9-month-old seedlings of the supposed CWD-susceptible and CWD-tolerant varieties (MS1 and MS2) were challenged by dipping their roots in spore suspensions of the 14 isolates (1.3×10^6 spores per millilitre). Ten seedlings per isolate and variety were thus treated and then repotted with fresh soil. The inoculated seedlings were monitored for 9 months.

The results showed that all the isolates were highly aggressive, and there was no detectable difference in the aggressiveness of the various CWD strains tested. Both Robusta varieties (MS1 and MS2) succumbed.

7.5.2 Variation among Robusta clones

Approximately 175 clones (+MS2) were evaluated for resistance using one CWD isolate (2004/01) employing both root-dipping and stem-nicking inoculation techniques (ten seedlings per treatment).

The results showed that out of the 1000 individual coffee trees tested, only 273 survived. The results suggest that some Robusta accessions from the Maruku germplasm collection have the highest potential resistance against CWD (Table 7.3). Most of the survivors were found from MR 10, ML 2, NG 10, 1/62 and ML 26 accessions which are also good in terms of yielding ability, bean sizes and fair in out-turn ratio. However, more work is needed to evaluate their performance under high disease pressure in different agro-ecological zones.

7.6 DRC

Scientists at the UNKIN carried out a study on screening of varietal resistance under field conditions at Beni in North-Kivu. To date, results obtained (based on the mortality rate recorded on different genotypes 5 months after inoculation) show only a moderate level of mortality (Table 7.4) with a statistically significant difference between the extremes of mortality (6.6 to 16.1%), as measured by the Tukey test ($p < 0.05$).

Table 7.4: Percentage of mortality of inoculated plants of various Robusta genotypes tested for resistance to coffee wilt disease (CWD).

Number	Genotype	Plants dead (%)	*F*-test group		
1	KR16/13A	6.6	a	-	-
2	KR19/1B	7.5	a	-	-
3	KR8/10	7.5	a	-	-
4	KR19/11	7.6	a	-	-
5	KR20/51	7.8	a	b	-
6	KR19/18B	8.1	a	b	-
7	KR17/55	8.2	a	b	-
8	KR10/7A	8.4	a	b	-
9	KR18/10	8.49	a	b	-
10	KR19/28	8.9	a	b	c
11	KR19/26	9.1	a	b	c
12	KR6/6	9.3	a	b	c
13	KR18/30	9.3	a	b	c
14	KR19/1A	9.5	a	b	c
15	KR19/55	9.7	a	b	c
16	KR20/50	10.2	a	b	c
17	KR19/31	10.3	a	b	c
18	KRA/6	10.6	a	b	c
19	KR16/55	11.0	a	b	c
20	KR17/47	112	a	b	c
21	KR1/3	11.2	a	b	c
22	KR16/13B	11.2	a	b	c
23	KR18/10A	11.4	a	b	c
24	KR1/1	11.5	a	b	c
25	KR19/18A	11.8	a	b	c
26	KR10/7B	12.1	a	b	c
27	KR19/12	12.3	a	b	c
28	KR20/31	12.4	a	b	c
29	KRC/3	12.5	a	b	c
30	KR3/5	12.6	a	b	c
31	KR12/6A	12.9	a	b	c
32	KR8/8	13.3	-	b	c
33	KR20/10	14.4	-	b	c
34	KR9/8	15.0	-	b	c
35	KR2/5	16.1	-	b	c

7.7 Conclusions

A range of inoculation methods were studied, with root dipping or stem nicking proving to be the most reliable and cost-effective. The tests showed how virulent the disease can be, with a very low spore concentration of 13/ml being sufficient to induce some mortality.

Most encouragingly, many genotypes display resistance to CWD, giving hope that these can be used to form the basis of durable resistant lines for commercial release. Much further work needs to be carried out, however; as many resistant lines as possible are needed to supply a population of strains that can be employed under a range of conditions and that can stand the maximum chance of maintaining resistance in the long term.

The rather low level of mortality found in several DRC genotypes is also encouraging, perhaps reflecting the country's previous experience with the disease.