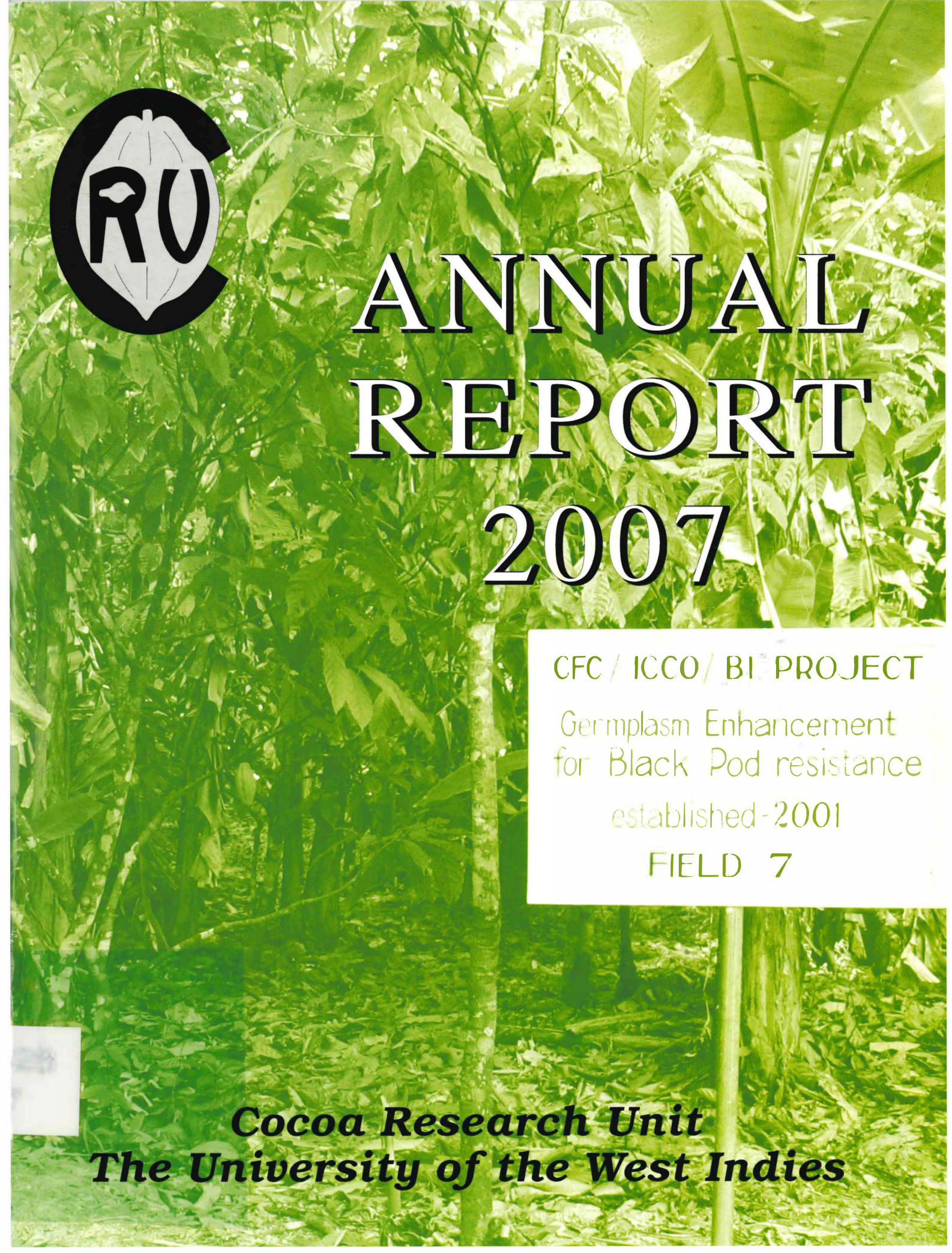

ANNUAL REPORT 2007

CFC / ICCO / BI PROJECT

Germplasm Enhancement for Black Pod resistance

established - 2001

FIELD 7

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The University of the West Indies

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Cover photograph. Field trial planted in 2001 with enhanced germplasm for Black Pod disease resistance

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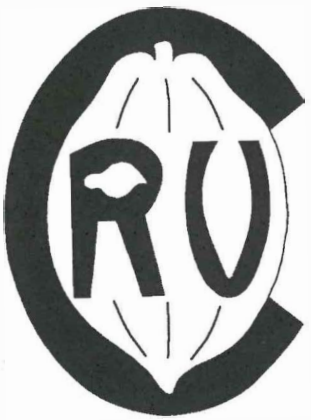

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Focusing on early-screening methods of Witches' Broom resistance

P. Deberdt, A. Holder, V. Jadoo and J-M. Thévenin

Introduction

A component of the CFC/ICCO/BI project *Cocoa productivity and quality improvement: a participatory approach* was to investigate and develop the methods of screening for WB resistance. Three experiments were performed either in the greenhouse or in the field. The experiments performed in the greenhouse were also scheduled to be compared with similar experiments in two others countries (Brazil and Ecuador) participating in the project.

Materials and methods

Experiment 1

Experiment 1 was a comparative study between two inoculation methods, spraying and agar droplet (Surujdeo-Maharaj *et al.*, 2003), and three types of planting material (open-pollinated seedlings, hand-pollinated seedlings and clonal material). Clones studied were SCA 6 and NA 289 (resistant to WB), MAN 15/2 [BRA] and UF 668 (susceptible to WB) and LX 25 (moderately resistant to WB). To obtain the hand-pollinated seedlings, the clone BE 10 was used as the male donor which is homozygous and susceptible to WB. The experiment was performed in the greenhouse. Seven-month-old seedlings were inoculated using an inoculum concentration of $3x10^5$ basidiospores/mL. About 6 grafted plants, 30 open-pollinated seedlings and 30 hand-pollinated seedlings were inoculated, the exact number depending on the availability of plants. To assess the WB resistance level, the variables recorded were the time to the appearance of first symptoms (TFS) and the maximum broom diameter (MBD).

Experiment 2

Experiment 2 was a ring test between Trinidad, Brazil and Ecuador to assess the degree of resistance to WB of genotypes in the International Clone Trial (ICT) and to compare the level of resistance obtained in 3 different sites. Open-pollinated progeny populations were generated from ten ICT clones EET 59 [ECU], LCTEEN 46, MXC 67, PA 150 [PER], PA 120 [PER], NA 33, AMAZ 15/15 [CHA], PLAYA ALTA 2, GU 255/V and IMC 47. These clones displayed various level of resistance in the field under natural conditions of infection. The experiment was performed in the greenhouse. Seven-month-old seedlings were inoculated by the agar droplet method using an inoculum concentration of $3x10^5$ basidiospores/mL. One replication contained between 19 and 30 open-pollinated seedlings per progeny population. The seedlings were inoculated using the agar droplet method, and two replications were performed. To assess the WB resistance level, the variables recorded were TFS and MBD.

Experiment 3

Experiment 3 focused on developing a method of screening for resistance to WB on attached pods in field. Three clones were selected in UWI Campus fields according to their different level

of WB resistance: ICS 95 (susceptible to WB), IMC 67 (resistant to WB) and LCT EEN 162/S-1010 (resistance level unknown). Open-pollinated pods were generated from each clone. Young growing pods were inoculated when they were estimated to be 8 weeks old. The length of each cherelle was measured at inoculation time. Two inoculation methods were performed: i) the spray method (1 mL of suspension of inoculum manually sprayed on the whole pod) and ii) the agar droplet method (30 µl of the inoculum suspension in 1% agar placed on the convex surface of the pod). The inoculum concentration was 10^6 basidiospores/mL. A control treatment was also performed with sterile water. For each inoculation method including the control treatment (3 treatments performed), about 15 cherelles were inoculated. Filter paper plus tape were used to cover the site of inoculation on cherelles inoculated with the agar droplet method. After inoculation, cherelles were enclosed in a plastic bag containing wet tissue paper. The bags, filter papers and tapes were removed after different incubation times of 72, 36 or 18 hours. The status of the pods was recorded weekly as "Wilt", "Black Pod disease", "WB disease", "other damage (damage due to rodent or to human influence)" and "No Symptom". After 3 months, internal symptoms were recorded as the proportion of necrosis in pods showing external WB symptoms.

Data analysis

The analysis of variance was performed using the general linear model (GLM) procedure (SAS Software).

Results and Discussion

Experiment 1

Time to first symptoms (TFS, days)

Analysis of variance showed that there was a significant interaction between the method of inoculation and the planting material ($F = 12.82$; $P < 0.0001$). There was also a significant interaction between the method of inoculation and the clone tested ($F = 3.79$; $P = 0.005$), therefore data analysis was performed for each inoculation method:

(i) *Agar droplet method.* No interaction was found between planting material and clone ($F = 1.30$; $P = 0.27$). The analysis of variance showed that there were significant differences among the clones tested ($F = 4.52$; $P = 0.0017$) and among the planting material ($F = 48.69$; $P < 0.0001$). According to the TFS measure, the clones tested were separated into 2 statistical groups with the period for NA 289 being significantly shorter than all the other clones (Table 1). The planting materials were also separated in 2 statistical groups, with significantly longer TFS in the clonal material and than either of the seedling treatments (Table 2).

(ii) *Spray method.* No interaction was found between planting material and clone ($F = 0.16$; $P = 0.9772$). The analysis of variance showed that there were no significant differences among the clones tested ($F = 1.14$; $P = 0.3393$; Table 1), but significant differences were found among the planting materials ($F = 15.55$; $P < 0.0001$). According to the TFS measure of resistance, the planting materials were separated into 3 statistical groups showing the longest TFS in the clonal material and the shortest one in the hand-pollinated seedlings (Table 2).

Maximum broom diameter (MBD, mm)
Analysis of variance showed that there was no interaction between the method of inoculation and the planting material (F = 0.20; $P = 0.82$) or between the method of inoculation and the clone tested (F = 2.32; $P = 0.057$). There was also no interaction between the clone tested and the planting material (F = 1.37; $P = 0.23$). No significant differences were found among the two methods of inoculation (F = 3.14; $P = 0.066$) while significant differences were found among the planting materials (F = 33.95; $P < 0.0001$) and among the clones tested (F = 5.57; $P = 0.0002$). According to the MBD measure of resistance, the clones tested were separated in 2 statistical groups the value for UF 668 (known to be susceptible to WB) being statistically greater than the other clones (Table 1). The planting materials were separated into 3 statistical groups with the clonal material having the smallest MBD and the hand-pollinated seedlings having the largest one (Table 2).

Table 1. Measures of symptom severity in five cocoa genotypes following inoculation by the agar droplet and spray methods.

	TFS (days)				MBD (mm)	
	Agar droplet method		Spray method			
Genotype	Mean*	SE	Mean*	SE	Mean*	SE
UF 668	13.6 a	0.39	14.7 a	0.38	13.8 a	0.43
NA 289	12.1 b	0.25	13.6 a	0.35	12.4 b	0.35
LX 25	13.4 a	0.72	15.3 a	0.74	12.1 b	0.61
MAN 15/2 [BRA]	14.6 a	0.47	15.5 a	0.53	11.7 b	0.47
SCA 6	14.6 a	0.52	14.8 a	0.41	11.0 b	0.42

Table 2. Measures of symptom severity in three types of plant material representing cocoa genotypes.

	TFS (days)				MBD (mm)	
	Agar droplet method		Spray method			
Plant material	Mean*	SE	Mean*	SE	Mean*	SE
Grafted	17.2 a	0.22	16.7 a	0.31	8.9 c	0.38
Open-pollinated	12.9 b	0.22	14.7 b	0.26	12.3 b	0.23
Hand-pollinated	12.5 b	0.37	12.8 c	0.32	14.5 a	0.43

TFS = incubation period; MBD = maximum broom diameter; SE = standard error.
*Mean scores followed by the same letter are not significantly different according to the Student Newman-Keuls test at 5% probability.

Experiment 2

Time to first symptoms (TFS)
The analysis of variance showed that there were no significant differences among the clones tested (F = 1.86; $P = 0.057$). Mean values varied between 14.38 days for PA 150 [PER] and 16.84 days for GU 255/V (Table 3)

Maximum broom diameter (MBD)
The analysis of variance showed that there were highly significant differences among the clones

tested (F = 4.94; $P < 0.0001$). According to the MBD measure of resistance, the clones tested were separated in 4 statistical groups showing PA 120 [PER], IMC 47 and GU 255/V clones with the smallest MBD and EET 59 [ECU] and LCT EEN 46 clones with the largest ones (Table 3). These results confirmed both a recent study on the evaluation of the WB resistance of ICT clones on grafted plants (collaboration between MALMR and CRU) and results obtained under natural conditions of infection in the UCRS, both conducted within the frame of the first CFC/ICCO/BI project.

Table 3. Measures of symptom severity in 10 open-pollinated progeny populations from genotypes in the International Clone Trial.

	TFS (days)		MBD (mm)	
Genotype	Mean*	SE	Mean*	SE
EET 59 [ECU]	15.2 a	0.41	12.1 a	0.37
LCT EEN 46	16.7 a	1.12	11.4 ab	0.42
MXC 67	14.5 a	0.39	11.3 abc	0.46
PA 150 [PER]	14.4 a	0.35	10.9 abc	0.37
NA 33	15.0 a	0.50	10.9 abc	0.47
AMAZ 15/15 [CHA]	15.0 a	0.43	10.7 abc	0.37
PLAYA ALTA 2	16.3 a	0.91	9.9 bcd	0.50
GU 255/V	16.8 a	0.82	9.8 cd	0.39
IMC 47	15.5 a	0.51	9.6 cd	0.52
PA 120 [PER]	14.4 a	0.56	9.0 d	0.38

TFS = incubation period; MBD = maximum broom diameter; SE = standard error.
*Mean scores followed by the same letter are not significantly different according to the Student Newman-Keuls test at 5% probability.

Experiment 3

Eighty pods were inoculated and compared with the control treatment (sterile water as the inoculum suspension). No WB symptoms were recorded on pods inoculated with sterile water (control pods). A high proportion of cherelles died because of wilt and other damage and few due to the black pod disease.

Only one pod showed WB disease-like symptoms on the susceptible clone ICS 95 inoculated with the agar droplet method: one month after inoculation, multiple necrotic spots covered a large area of the pod; three months after inoculation, distortion of the pod was recorded; the pod then stopped growing (the ratio of initial to final pod length over 3 months was 1.25 for the inoculated pods and 2.58 for control pods) and 50% of the internal fruit was necrosed.

Conclusion and perspective

The results of the experiments 1 and 2 revealed that for both TFS and MBD, the effect of plant material was greater than the effect of clones. This is true for both methods of inoculation. This provides evidence that results of inoculation can only be compared if the planting material is the same throughout, it may explain some anomalies in results from earlier work. For TFS, the agar droplet inoculation method was able to discriminate between clones, but no clone effect was found with spray inoculation, suggesting that agar droplet may be a better method for screening. The results also showed that measurements of MBD gave better discrimination between

susceptible and resistant clones than TFS. This finding is in contrast to the results of Surujdeo-Maharaj *et al.* (2004) which suggest that TFS is the most discriminatory measure of resistance. The most apparent difference between the experiments described here and those of Surujdeo-Maharaj *et al.* was the age of the plants at the time of inoculation. Surujdeo-Maharaj *et al.* (2004) used seedlings that were at least 12 months old,

The most promising ICT clones showing a high level of WB resistance were PA 120 [PER], IMC 47 and GU 255/V. It will be interesting to compare the results of experiments 1 and 2 with those from similar experiments carried out in Ecuador and Brazil.

The results of the experiment 3 performed in Campus field revealed that it was possible to induce WB like symptoms on attached cherelles, however Koch's postulate should be verified if there is any doubt whether the symptoms are characteristic of the disease. Previous work reported a weak correlation (Thévenin *et al.*, 2005) between vegetative brooms and WB symptoms on pods ($r = 0.39$). We need therefore to focus on the development of a screening test for pods. The next step will be to get the right conditions to repeatedly obtain symptoms. It may therefore necessary to work in UCRS where susceptible clones can be more easily found.

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