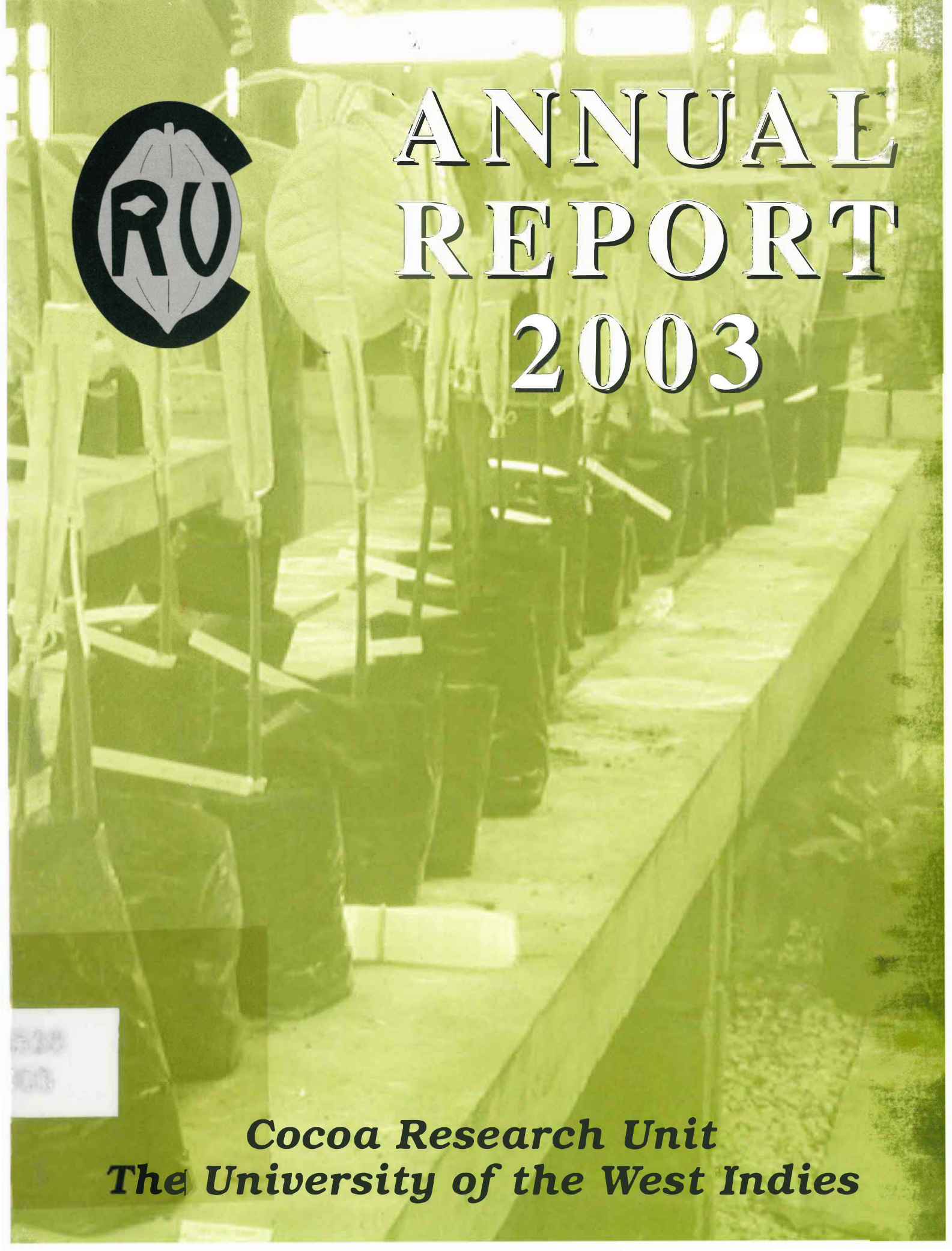

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Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago

Tel. +1 868 662 8788
+1 868 662 4996 Ext. 2115
+1 868 645 3232 2115
Fax +1 868 662 8788
E-mail cru@cablenett.net

Cover photograph. Multiplication of accessions in the Barbados Cocoa Quarantine Station.

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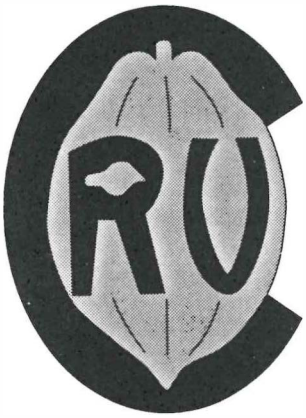

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Evaluation of cacao germplasm for resistance to Witches' Broom disease under controlled conditions

R. Umaharan, J-M. Thévenin, A. Holder and J. Bhola

Introduction

A five-year project with the aim of screening the cacao accessions held in the ICG,T for their resistance to Witches' Broom disease was initiated in July 1998 and was financed by the American Cocoa Research Institute (ACRI), now the World Cocoa Foundation (WCF).
The goal of the project to screen 400 accessions per year was perhaps over-ambitious, but it kept the team motivated in spite of numerous set backs and challenges during the project. Details of steps taken to overcome problems with the methodology have been described in previous reports (Umaharan *et al.* (2000), Umaharan *et al.* in press), and here, it suffices to list the various challenges:

- The need to raise and maintain a large number of rootstocks in order to propagate accessions selected for screening, taking into consideration the frequent high percentage of losses during the grafting exercise.
- The necessity of adapting the inoculation method to match the variable size and weight of grafted plants.
- The need to adjust and fine tune the inoculation method of Purdy *et al.* (1997) in terms of concentration and quantity of basidiospores to be delivered per plant to allow for the conditions in Trinidad to obtain appropriate measurements to indicate the level of resistance to WB.
- The need for the accessions to produce synchronized flushes prior to inoculation.
- The necessity to create incubation conditions close to ideal (temperature of 25-27°C and relative humidity close to saturation) for a good infectious process where the dry season can last up to four months and during which outside temperatures can reach 38°C.
- The need to develop greenhouse conditions favourable to the development of symptoms and to identify the most appropriate variables to measure.

Overall results

In spite of the limitations listed above, five years after the beginning of this project, the total number of accessions grafted reached 1,065. To achieve this, over 22,000 rootstocks were established and utilised.

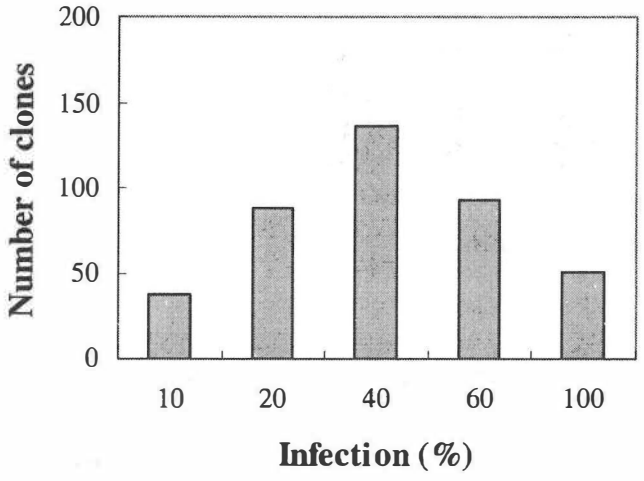
Over 700 accessions were inoculated and screened for WB resistance in green house experiments using spray inoculation techniques (Umaharan *et al.*, in press). The analysis of results was based on the percentage of the total number of inoculated shoots with symptoms and on the broom-base diameter (analysis of variance using the general linear model, MINITAB ver. 13.1 software).

For the purposes of this report, results from 553 accessions were selected and assessed for percentage infection and broom-base diameter, since they fulfilled the criteria of having at least 3 replications with four shoots per replicate. These accessions belong to 53 accession groups and represent a good cross-section of the accessions held in the ICG,T.

Percentage infection

Overall, a normal distribution (Figure 1) of resistance to WB was obtained (r = 0.99, P <0.01, Ryan-Joiner test for normality). Approximately 32% of the clones assessed showed less than 20% symptoms after being inoculated with *Crinipellis perniciosa*.

Figure 1. Distribution of resistance to Witches' Broom disease for 533 clones from the ICG,T, based on percentage infection.

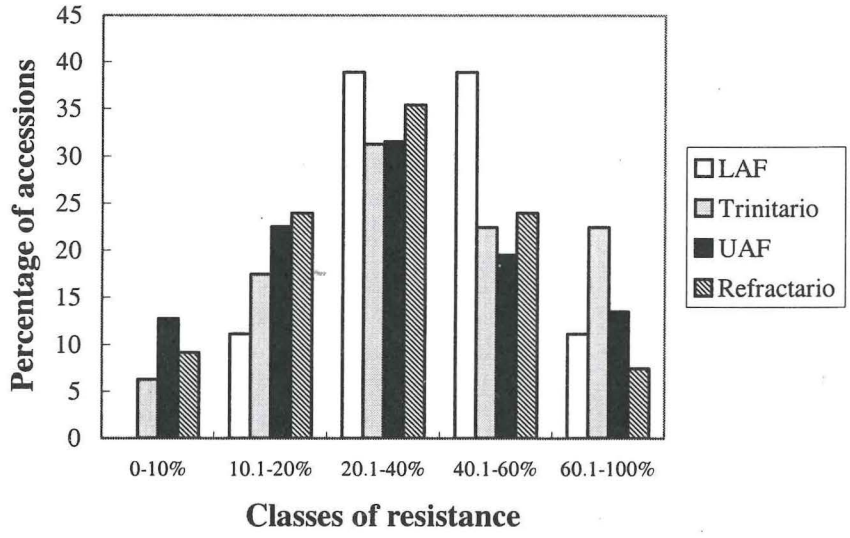


The results were further analysed according to the main genetic groups, the Forastero (subdivided into Upper and Lower Amazon types), Trinitario and Refractario types (Figure 2). When the Forastero group was evaluated, the Upper Amazon Forastero (UAF) had approximately 35% of the accessions showing less than 20% symptoms. The Refractario group also showed a similar high percentage of accessions (33%) showing less than 20% infection. In contrast, the Lower Amazon Forastero (LAF) and Trinitario populations had 11% and 24%, respectively.

This indicates that a large percentage of resistant clones screened from the ICG,T belong to the UAF group, which concurs with Pound's observation (Pound, 1938), that resistant trees appeared frequently in the Upper Amazon area around and to the west of the Iquitos. Furthermore, the accession groups showing the largest percentage of clones (58.8%) with less than 20% symptoms was IMC (Iquitos Mixed Calabacillo), which is a wild UAF type from Peru, originally selected for lack of symptoms to WB. In addition, while the UAF accessions were predominantly collected from Peru, the Trinitario types are more diverse in their origin and were not collected/selected solely for their reaction to WB but for other traits such as yield and/or resistance to BP, as well as for the uniqueness or diversity of their germplasm.

Some individual accession groups within the Refractario genotypes also yielded especially promising results e.g. the B [POU] (47.1%), LX & LV [POU] (41.2%) and LP [POU] (45.2%) accession groups. This agrees with the observation of Pound (1938), that there were trees showing little or no disease symptoms from highly diseased areas in Ecuador.

Figure 2. Distribution of resistance to Witches' Broom disease for Forastero, Trinitario and Refractario groups from the ICG,T, based on percentage infection.



Symptom severity

Due to the problems of repeatability and disease escapes, we endeavoured to conduct experiments where most of the inoculated plants developed symptoms, including the resistant controls. This was a reliable indicator that the experiment was successful, and permitted resistance/susceptibility to be assessed according to symptom severity, in addition to percentage infection.

Broom-base diameter was found to effectively discriminate between levels of resistance (Surujdeo-Maharaj *et. al.*, 2003) regardless of screening techniques used (spray inoculation using Preval Sprayer and agar droplet technique). However, no significant correlation ($P > 0.05$) was found between percentage infection and broom-base diameter since accessions with large broom-base diameters did not necessarily have high percentage infection and vice versa. This suggests that there may be more than one mechanism of resistance influencing infection and disease development. Additionally, percentage infection is influenced by the number of shoots inoculated, which is highly variable in clonal plants and is difficult to standardise.

Analyses of broom-base diameter was found to be highly significant (Table 1), indicating that there were useful differences among the accessions screened and therefore selections could be made based on this variable.

Table 1. Analysis of variance for broom-base diameter.

Source	Degrees of freedom	Seq SS	Adj SS	Adj MS	F	*P*
Clone	138	1428.1	1428.1	10.35	2.87	<0.001
Error	300	1080.9	1080.9	3.60		
Total	438	2509.0				

From the results, a total of 90 accessions (Table 2) from the 553 accessions analysed were selected for further confirmation using the agar droplet screening technique. These accessions either developed few symptoms (total percentage of symptoms less than 20% or absence of brooms) or when brooms developed, they were small and thin, with an average diameter less than 6 mm, which is the average broom-base diameter observed in resistant controls.

Table 2. Accession groups with promising clones.

Accession group	No. of clones	Accession group	No. of clones
AM [POU]	4	LV [POU]	2
B [POU]	10	MATINA	1
CL	2	MO	1
CRU	3	MOQ	3
CRUZ	1	NA	4
CBO [VEN]	1	POUND [POU]	2
DOM	1	PA [PER]	11
EET [ECU]	2	SCA	3
GU	5	SJ [POU]	3
ICS	8	SLA	1
IMC	10	SLC	1
LCT EEN	2	SPA [COL]	1
LP [POU]	6	UF	2

Continuation of the project

Before the project came to an end in July 2003, a proposal was submitted to WCF to continue the activities with a change of emphasis, giving particular attention to the confirmation and quantification of the level of resistance of clones found to be putatively resistant during the preliminary screening exercise. Revised targets were set, based on the projected level of financing and the technical difficulties encountered in the first phase of the project: screening of 60 accessions per year and confirmation of resistance of 30 accessions per year. The first year of this proposal was approved with the possibility of extension and the new phase of the project started in August 2003.

A total of 33 clones were screened in the period August to December 2003, out of which 17 were selected for further confirmation.

From the list of 90 accessions selected for confirmation during the first phase of the project (Table 2), 50 clones have been grafted, 10 of them have been inoculated using the agar droplet technique and they are currently undergoing symptom evaluation.

Conclusions

Results obtained from screening have shown that there is considerable variation for resistance to WB within the collection of cacao held at the ICG,T. Apart from the selection of promising types, the project has identified those accessions which are definitely susceptible to the disease. Also inferred from the observations, was the need for additional criteria to that of percentage infection, as measures of resistance or susceptibility to WB.

The results also suggest that a suitable protocol to screen for resistance to WB is a two-tiered system. In this, initial mass screening is carried out by spray inoculation, which is convenient for screening a large number of plants at the same time, followed by confirmation of selected clones and quantification of their level of resistance using the agar droplet technique.

The results from this screening for WB resistance, after being cross-checked with data on natural infection in the field, will be combined with data on other traits like BP resistance, pod index etc. This information will provide a comprehensive list of accessions from the ICG,T which will be used as a guide for the selection of parents for germplasm enhancement and breeding work in the future.

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