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Cover photograph. Field 5A in the International Cocoa Genebank, Trinidad.

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Field Assessment of Cacao Germplasm for Resistance to Witches' Broom and Black Pod Diseases: Year one

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Introduction

Witches' Broom and Black Pod diseases are of great economic importance in cocoa producing countries. Worldwide, losses due to BP have been estimated at 20%.WB, however, only occurs in South America and some islands of the Caribbean where it is responsible for 20 to 50% of pod losses. In addition, this disease can also pose a serious problem in the establishment of new plantings.

Current control measures for these diseases, which include chemical and phytosanitary methods, have often proved to be unsatisfactory and very expensive. A genetic solution to the problem is preferable. As a preliminary part of a pre-breeding programme and as part of the CFC/ICCO/IPGRI Project, CRU is currently screening accessions in the ICG,T for BP and WB resistance.

Methods

A total of 228 clones were selected for field evaluation from November 1998 to October 1999. The selection of these clones was based on:

- resistance to BP determined by detached pod inoculation;
- interesting traits (pod index, butter fat content etc.) and inclusion in the list of priority clones (sub-sample A) for the CFC Project Collection;
- known field resistance or susceptibility to WB and BP, and suitability for use as controls.

Depending on the availability of plants, one to five trees per clone were selected and tagged. Observations were made in the ICG,T on a field by field basis (fields 4A, 5A, 5B, 6A, 6B) with independent assessments for the presence of WB and BP. Clones were grouped according to their productivity, using the following classes:

- 0 No pods produced
- 1 Less than 10 pods produced per clone
- 2 Ten or more pods produced per clone but less than 10 pods per tree
- 3 More than 10 pods produced per tree

Vegetative and cushion infections of Witches' Broom

From the selected clones, three branches of approximately 1.5 m in length were selected to represent the canopy. On each branch, the number of healthy shoots and green and dry

brooms was recorded. For cushions, WB incidence was evaluated visually as follows:

• Very resistant (VR) no visible cushion brooms on the tree.

• Resistant (R) 1-2 cushion brooms per tree.

• Moderately resistant (MR) 3-10 cushion brooms per tree.

• Susceptible (S) more than 10 cushion brooms per tree.

These observations were carried out three times a year.

The following classes of resistance were used for vegetative infection of WB:

Very resistant (VR)
 no vegetative brooms observed.

• Resistant (R) <1% of shoots affected.

• Moderately resistant (M) 1.0 < 2.5% of shoots affected.

• Susceptible (S) 2.5 < 5% of shoots affected.

• Very susceptible (VS) ≥ 5.0 % or more of affected shoots.

Pests and diseases of pods

Each tree was observed monthly, ripe pods were harvested and the following variables were recorded as follows:

Ripe pods

- The number of healthy pods.
- The number of pods with Black Pod symptoms, without WB in association with or without other fungi, insects, rodent or bird attack.
- The number of pods with WB symptoms, without BP, in association with or without other fungi, insects, rodent or bird attack.
- The number of pods with both BP and WB symptoms on the same pods, in association with or without other fungi, insects, rodent or bird attack.

A pod was considered healthy if it did not show symptoms of BP and WB, even if symptoms due to other fungi, insects, rodents or birds were present.

Unripe fully grown pods

- The number of healthy pods.
- The number of pods with BP symptoms.

Cherelles

An overall assessment of the cherelles was made to record the extent to which they were rotted or healthy and whether they showed symptoms of BP or WB. Each tree was rated separately for each disease on the following scale:

- 0 No symptoms
- 1 Slightly affected
- 2 Moderately affected
- 3 Heavily affected

The following classes of resistance were used for WB and BP on pods:

- Resistant (R) less than 10% of pods affected
- Moderately resistant (M) 10-25% of pods affected
- Susceptible (S) more than 25% of pods affected

Results

Table 1. Productivity¹ and incidence of BP and WB at the ICG,T between November 1998 and October 1999.

Field	Year of planting	Number of clones	Number of trees per	BP incidence	WB incidence	Productivity ¹ (number of clones for each class)				Pods per
			clone	(%)	on pods (%)	0	1	2	3	tree
4A	1993	49	2.4	23.3	9.9	10	17	13	9	6.7
5A	1989	21	3.1	13.6	7.9	1	5	5	10	15.4
6A	1991	11	3.0	21.7	16.0	0	1	1	9	21.9
5B	1988	69	3.5	16.4	14.5	5	16	14	34	14.2
6B	1987	73	3.4	12.1	7.9	4	9	14	46	15.9
Total/ mean		223	3.2	15.3	10.8	20	48	47	108	14.1

¹Classes of productivity

Pods

Of the 228 clones that were selected for observation, five were not included for pod observation because artificial pollinations were carried out on these trees. A further 20 clones (half of which are located in field 4A) were also omitted from the analysis because they produced no pods during the 12-month period of observation. The results are shown in Tables 1 and 2. The number of trees per clone is less in field 4A than in the other fields because the maximum number of trees per plot is four in field 4A compared with 16 in the other fields.

⁰ no pods produced

^{1 &}lt; 10 pods per clone

^{2 &}gt; 10 pods per clone and < 10 pods per tree

> 10 pods per tree.

Productivity

Productivity, given by the number of pods produced per tree, was less in field 4A where the trees were younger (6.7 pods per tree) than in the other fields (14.2 to 21.9 pods per tree). The average productivity was 14.1 pods per tree for the 203 producing clones.

Table 2. Numbers of clones classified by their productivity and disease resistance from field observations between November 1998 and October 1999.

a) Black Pod disease

	Productivity ¹						
Resistance		,					
class	1	2	3	Total			
R	25	23	55	103			
M	9	12	28	49			
S	14	12	25	51			
Total	48	47	108	203			

Classes of resistance to BP and WB diseases R <10% affected pods M 10 < 25% affected pods S $\geq 25\%$ affected pods

b) Witches' Broom disease

	Productivity ¹							
Resistance		т						
class	1	, 2	3	Total				
R	32	24	65	121				
M	11	14	36	61				
S	5	9	7	21				
Total	48	47	108	203				

¹Classes of productivity

0 no pods produced

1 < 10 pods per clone

2 > 10 pods per clones and < 10 pods per tree

3 > 10 pods per tree

Black Pod

The overall incidence of BP was 15.3%. On a field by field basis, the greatest incidence of BP was in field 4A and the least in field 6B. The incidence of BP was less than 10% for 103 clones, 55 of which produced more than 10 pods per tree. Fifty-one clones, 25 of which produced more than 10 pods per tree, were found to be susceptible to BP disease (> 25% BP incidence).

Pods affected by Witches' Broom

The overall percentage of WB was 10.8 %, with the greatest incidence being observed in fields 6A and 5B. The incidence of WB was less than 10% for 121 clones, with half of them producing more than 10 pods per tree. A small number of clones (21) were found to be susceptible with more than 25% affected pods.

Witches' Broom disease on shoots and cushions

The percentage of WB on shoots varied from 0.22 % (field 6A) to 1.16 % (field 5B), with an average of 0.72 % for the total of 228 studied clones (Table 3). Only 15 clones were considered susceptible or very susceptible.

Most of the clones showed a very good level of resistance to cushion infection by *C. perniciosa*, with less than two cushion brooms per tree. However, a small minority of

clones, such as NA 45 and UF 11, were observed to be very susceptible to this form of the disease.

Table 3. Number of clones classified by their resistance to Witches' Broom on shoots from field observations between November 1998 and October 1999.

		Res	istance	2,	Witches'Broom		
Field	VR	R	MR	S	VS	Total	(%)
4A	24	20	5	1	0	50	0.41
5A	9	7	4	1	0	21	0.44
6A	10	3	0	0	0	13	0.22
5B	31	18	10	8	3	70	1.16
6B	40	21	11	0	2	74	0.59
Total	114	69	30	10	15	228	0.72

¹VR no vegetative brooms observed

R <1% of shoots affected

MR 1.0 < 2.5% of shoots affected

S 2.5 < 5% of shoots affected

 $VS \ge 5.0$ % or more of affected shoots.

Discussion

The data obtained so far reflect a disparity of productivity and disease pressure from one field to another. There are several possible reasons for this:

- The year of planting: field 4A was planted later than the other fields and is not yet well established in terms of production and development of the canopy.
- The environmental conditions: These can differ from one field to another in terms of shading (bananas, immortelles, old cacao trees), presence of drains and proximity to the river, the position of the fields in the ICG,T relative to other fields, and wind exposure.
- The planting design: the ICG,T was not planted in experimental blocks with replicates. Planting material varies from one field to another with some populations mainly concentrated in a single field.

With these constraints, the aims of these field observations can only be to confirm the level of resistance obtained with laboratory inoculation tests and to detect possible new sources of resistance to WB and BP for further testing, but not to give accurate resistance levels for each clone. Table 4 lists the level of resistance for the 155 clones that produced more than 10 pods per clone. Clones producing less than 10 pods were not included since estimates of levels of resistance to diseases were not considered to be reliable with so few pods.

Though the 12-month period of observation is not enough to give robust data on the resistance levels, it allowed us to identify susceptible clones in the field and eliminate them from further studies. Therefore, for the coming year of observation, the following is planned:

luation

- Observations in field 4A, which did not produce enough pods to give reliable data, will be suspended.
- Fifty clones with levels of resistance to both diseases ranging from very susceptible to very resistant will be observed in subsequent years as controls.
- Forty-five clones showing a good level of resistance to BP disease during the first year will be observed during the second year to confirm the result (BP resistance is a priority in this project).
- An additional 130 clones from sub-sample A will be observed in year 2 to bring the total to 225 clones.

Table 4. Level of resistance of 155 clones to BP and WB, under natural conditions of infection (November 1998 - October 1999).

Clone	Field	Prod.	Re	sistance level			
			BP		WB		
	İ		P	P	C	V	
AGU 3339/12 [CHA]	6B	2	S	M	R	R	
AM 1/73 [POU]**	6A	3	R	M	VR	VR	
AM 2/12 [POU]	5B	3	M	M	VR	VR	
AM 2/38 [POU]**	5B	3	R	M	VR	VR	
AM 2/45[POU]	4A	2	M	S	R	VR	
AM 2/64 [POU]*	5A	3	R	R	VR	M	
AM 2/65 [POU] *	5B	3	R	M	VR	R	
AMAZ 12 **	6B	3	R	R	VR	VR	
AMAZ 6/3	6B	3	R	M	VR	R	
B 5/3 **	6B	3	M	R	VR	M	
B 5/7	6A	3	R	R	VR	R	
B 12/1 **	6B	3	R	R	R	R	
B 13/5 **	5A	3	R	M	R	R	
B 14/13	5B	3	S	M	M	S	
B 23/2	6B	2	R	S	R	M	
CC 71	4A	2	R	S	VR	R	
CL 10/5	5B	3	M	R	R	R	
CL 10/10 **	5A	3	M	R	R	M	
CL 10/15 *	5A	2	R	M	VR	R	
CL 13/41	4A	2	R	R	R	M	
CL 19/10	5B	3	M	R	VR	R	
CL 19/49	5B	3	R	M	R	M	
CL 27/109	4A	2	M	R	VR	VR	
CLEM S/62 *	5B	3	M	R	VR	VR	
CRU 12	5B -	3	M	M	R	R	
CRU 19	5B	3	M	R	VR	R	
CRU 56	5B	3	M	M	R	M	
CRU 72	6A	3	M	R	VR	V	
CRU 96**	5B	3	S	M	R	S	
CRU 101 *	6A	3	R	R	VR	R	
CRU 119 **	5B	3	S	S	R	S	
CRU 124 *	5B	3	R	M	R	M	
DE 52/B	6B	3	R	R	VR	R	
DE 64/B *	6B	2	M	R	VR	VR	
EET 58 *	6B	3	R	R	R	M	
EET 59 **	6B	3	M	S	VR	VS	
EET 162	6B	3	М	R	VR	R	
GS 10	6B	2	M	R	R	M	
GU 175/P	4A	2	R	R	R	R	

Clone	Field	Prod.	Res	istar	istance level			
			BP		WB			
	İ		P	P	C	V		
GU 241/P	4A	2	R	R	R	VR		
GU 300/P	4A	2	S	M	VR	R		
ICS 1 **	6B	3	S	M	M	M		
ICS 10	6B	3	R	R	VR	R		
ICS 40 *	6B	3	M	R	VR	VR		
ICS 62	4A	2	S	R	VR	R		
ICS 70**	6B	3	S	S	VR	R		
ICS 80 **	6A	3	S	M	VR	VR		
ICS 84 **	6B	3	R	R	M	M		
ICS 95 **	6B	3	R	M	VR	R		
IMC 2 **	6B	3	S	R	VR	R		
IMC 6 *	6B	3	R	R	VR	VR		
IMC 16 *	6B	3	R	R	VR	R		
IMC 20	6B	3	R	R	VR	М		
IMC 47 *	6B	3	R	R	VR	R		
IMC 57 **	6B	3	R	R	VR	VR		
IMC 58 **	6B	3	S	R	VR	R		
IMC 66	6B	2	S	R	VR	VR		
IMC 103**	6B	2	S	M	VR	VR		
IMC 104	6B	2	R	S	VR	VR		
IMC 105	6B	2	M	M	VR	R		
JA 1/21 [POU]*	5B	2	R	R	R	R		
JA 5/19 [POU] **	5B	3	M	M	VR	S		
JA 5/25 [POU] **	5B	3	R	M	R	R		
JA 5/41[POU]	5B	2	R	S	M	VS		
JA 6/4 [POU] **	5B	2	R	S	R	VS		
LP 1/45 [POU] **	5B	3	R	M	VR	VR		
LP 3/5 [POU] *	5B	3	R	R	VR	VR		
LP 4/24 [POU] **	5B	3	R	R	R	R		
LP 4/32 [POU] **	5B	2	S	S	R	M		
LX 25 **	6A	2	S	S	VR	R		
LX 31 *	5B	~2	R	M	R	R		
MAN 15/60 *	6B	3	R	R	VR	VR		
MATINA 1/7 **	6B	3	M	R	VR	VR		
MOQ 4/20 *	5B	2	R	R	VR	VR		
MOQ 5/34	4A	3	M	R	R	R		
MOQ 5/5 **	6A	3	S	M	VR	VR		
MOQ 6/102	5B	2	M	M	VR	VR		

Resistance levels : P : on pods ; C : on flower ccushions ; V : vegetative, on shoots

^{*} resistance level to be confirmed in year 2

^{**} clones used as controls for the whole period of field observations

Table 4. continued

Clone	Field	Prod.	Re	esistar	ice lev	el
			BP		WB	
			P	P	C	V
NA 8	5A	3	М	R	VR	VR
NA 12	6A	3	S	R	VR	VR
NA 32	6B	3	М	R	VR	VR
NA 43	5A	3	М	S	R	VR
NA 45**	5B	3	S	S	S	S
NA 61	4A	2	S	R	VR	VR
NA 141	5B	3	S	R	R	R
NA 142 **	6A	3	R~	M	VR	R
NA 149 **	5B	3	S	M	VR	VR
NA 159 *	5B	2	R	R	VR	R
NA 170 **	5B	3	R	M	VR	VR
NA 176	5B	3	M	R	VR	R
NA 178 **	5B	3	S	M	VR	VR
NA 187	6B	2	R	R	VR	VR
NA 226 **	6B	3	S	M	VR	VR
NA 230	6B	3	M	R	VR	VR
NA 268	6B	3	S	R	VR	R
NA 337	5B	2	M	M	VR	R
NA 342 **	6B	3	R	R	R	VR
NA 387 **	5A	3	R	R	VR	R
NA 423	4A	3	S	R	VR	VR
NA 432 *	6B	3	S	R	VR	VR
NA 670	5A	3	М	R	VR	VR
NA 672 **	5B	3	S	S	VR	M
NA 680 *	5A	2	R	R	VR	R
NA 739	5A	3	М	R	VR	VR
NA 753	6B	2	R	R	VR	R
NA 756 **	6A	3	М	S	VR	VR
NA 763	5B	2	S	R	R	R
NA 794	6B	3	R	М	VR	VR
NA 807*	5A	2	R	R	VR	R
NA 824	5B	3	R	М	VR	VR
OC 61	4A	2	R	R	VR	VR
POUND 7/A	6B	3	R	R	VR	R
POUND 10/B *	6B	3	R	R	VR	R
POUND10/C	6B	2	R	M	VR	R
POUND 26/C	6B	2	M	R	VR	R
POUND 31/A	6B	2	M	M	R	VR
POUND32/A *	6B	2	R	R	VR	VR

Clone	Field	Prod.	Res	sistanc	e leve	1
		İ	BP		WB	
			P	P	C	V
PLAYA.ALTA 2 *	6B	3	R	R	VR	VR
PA 4 **	5B	2	S	R	VR	VR
PA 12	6B	3	S	R	VR	VR
PA 34 *	5B	3	R	R	VR	R
PA 39 *	5A	2	R	R	VR	R
PA 46 **	6B	3	R	M	VR	VR
PA 67 *	5B	3	R	R	VR	VR
PA 70 **	5B	2	М	M	R	S
PA 84	5B	2	R	M	R	VR
PA 88 **	5B	3	R	R	M	S
PA 118	5B	3	M	M	VR	VR
PA 120 *	6B	3	R	R	VR	R
PA 121 *	6B	3	R	R	VR	VR
PA 136	5B	3	М	M	VR	M
PA 137	5A	3	S	R	VR	VR
PA 151 **	5B	3	S	R	VR	VR
PA 156 *	5A	3	R	R	VR	VR
PA 169 *	6B	3	R	R	VR	VR
PA 195 *	6B	3	R	R	VR	VR
PA 202 *	5A	2	R	R	VR	M
PA 218 *	6B	3	R	M	VR	VR
PA 289 *	5B	3	R	M	VR	VR
PA 296 *	6B	3	R	R	VR	VR
PA 299 *	5B	3	R	M	VR	VR
PA 303 *	6B	3	R	R	VR	VR
RIM 2	4A	3	S	3	S	VR
RIM 13	4A	3	M	R	R	R
RIM 24	4A	3	S	M	R	VR
RIM 41	4A	2	S	S		R
SC 6 [COL]	4A	3	R	R	VR	VR
SC 20 [COL]	4A	3	S	M	R	R
SCA 6 **	6B	3	M	R	VR	VR
SM 10	4A	2	R	R	R	M
SPA 18	4A	3	R	M	VR	R
SPEC 160/9	6B	3	R	R	VR	VR
SPEC 185/4 **	6B	3	R	R	R	M
TRD 44	4A	2	M	M	R	M
TRD 45	4A	3	R	R	R	R
UF 11 **	6B	3	M	M	S	VS

^{*} resistance level to be confirmed in year 2

^{**} clones used as controls for the whole period of field observations Resistance levels: P pods; C flower cushions; V vegetative (on shoots)