Resistance of cocoa to *Moniliophthora perniciosa* – progenitors and progenies selection[1]

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ABSTRACT

Witches' broom, caused by *Moniliophthora perniciosa*, is the most important cacao disease in Brazil, and the final objective of this study is increasing the level and durability of resistance to this fungus, through the association of different alleles or genes favorable to the character, for the generation of new cocoa varieties. It was conducted, for 10 years, the evaluation of the number of vegetative brooms and cushions brooms per plant, of progenies from a cross breeding scheme in North Carolina II design, with the clones: Be 4, Cepec 89, CSul 7, EEG 29, ICS 98, Oc 67, RB 39 and Scavina 6, as group 1 of progenitors, and CCN 10, CCN 51, Cepec 86, ICS 9, IMC 76, Na 33, P4b, and SGu 54, as group 2. For both groups there were differences in terms of the general combining ability and specific combining ability. Differences among progenitors and among progenies were also found for the tendencies in the evolution of the number of brooms over the evaluation period. And it was possible to increase the level and durability of resistance through the association of alleles or genes favorable to this character, all the three results confirming the original hypothesis.

Keywords: *Moniliophthora perniciosa*; plant breeding; *Theobroma cacao*.

INTRODUCTION

Witches' broom disease (WBD), caused by *Moniliophthora perniciosa* (Stahel) Aims and Phillips-Mora, is one of the three cacao diseases that accounts for the greatest losses in all cacao-growing regions of South America and Caribbean islands (Gutiérrez *et al.*, 2016; De Souza *et al.*, 2018).

The fungus infects all meristematic tissues: apical buds of leaf flushes – vegetative brooms; flower cushions, that can produce vegetative brooms, abnormal flowers, and parthenocarpic carrot-, strawberry-, custard apple-shaped pods; seedlings and developing pods. Young infected pods suffer hypertrophy, exhibit chlorosis and necrotic lesions (Silva *et al.*, 2002).

For the cacao breeding program developed by the 'Centro de Pesquisa do Cacau' (Cepec) of the "Comissão Executiva do Plano da Lavoura Cacaueira" (Ceplac) the important symptoms to evaluate resistance to WBD in adult plants are: vegetative brooms, cushion brooms and infected pods. And it is known that the proportion of WBD infected pods was positively correlated with the total number of brooms (TB = vegetative plus cushion brooms) in the amount of 0.59 (Pires, 2003).

The breeding program aims to develop new commercial cocoa varieties with high productivity, good general characteristics and greater resistance to diseases, and its central structures are recurrent selection processes for the association of genes and traits of interest. These processes are followed by regional trials of clones selected in the recurrent selection populations, for the final evaluation and selection of the new varieties (Lopes *et al.*, 2011) This article contemplates the evaluation, for vegetative brooms (VB) and cushion brooms (CB), of some of the first generation progenies of the recurrent selection program in progress at Cepec, and similar works have already been presented by Albuquerque *et*

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al., 2009, Yamada *et al.*, 2013, Benjamin *et al.*, 2016, Benjamin *et al.*, 2014. It has the hypothesis that there are differences among progenitors for resistance to witches' broom disease and differences in additive and non-additive effects on the inheritance, differences among progenitors and among progenies for the tendencies in the evolution of the number of brooms over the time, and that it is possible to increase the level and durability of resistance through the association of alleles or genes favorable to this character.

MATERIAL AND METHODS

It was conducted, in Ilhéus, Bahia, the evaluation of the number of vegetative brooms (VB) and cushions brooms (CB), per plant, of progenies from a cross breeding scheme in North Carolina II design, with the clones: Be 4, Cepec 89, CSul 7, EEG 29, ICS 98, Oc 67, RB 39 and Sca 6 (Scavina), as group 1 of progenitors, or paternal progenitors, and CCN 10, CCN 51, Cepec 86, ICS 9, IMC 76, Na 33, P 4B, and SGu 54, as group 2, or maternal progenitors – general information from these clones can be found in International Cocoa Germplasm Database (1991) .

The evaluation of the natural witches' broom infection in field was carried out from 2001 to 2010 (once a year in most of the years and twice a year in 2006, 2009 and 2010), and the progenies were represented by two replications of 20 plants, each. The beginning of the evaluation was at the third year in the field, and the seedlings of the progenies were grafted onto adult plants to accelerate growth. Old plants were left among those under evaluation to keep the amount of inoculum high, and the experiment was taken in an area with high contamination.

The progenitors (clones) were selected for their 'per se' performance, in terms of attributes of interest, and the design of the crosses defined with consideration of the combinations of desired characteristics and genetic distances (Pires, 2003). The performance in relation to natural witches' broom infection was a determining factor in the choices of the Upper Amazon clones: CSul 7, RB 39, Sca 6, IMC 76, Na 33 and P 4B; Lower Amazon Be 4 and Cepec 86; Trinitarians CCN 10 and CCN 51; Criollo Oc 67 and Hybrid of unknown origin Cepec 89 (Pires, 2003).

The 12 broom removals and counting periods were considered as repeated measures, and the data analysis was conducted as a multivariate analysis to determine the effects of progenies, or of father, mother, and father x mother interaction; and as repeated measures for the effects of removal period and interactions of progenitors or progenies with removal period (PROC GLM / MANOVA; PROC GLM / REPEATED - SAS Institute, 1988). To simplify the interpretations, the results are presented in the form of proportions between the average of brooms of each progenitor or progeny and the general average of the experiment.

RESULTS AND DISCUSSION

Highly significant effects, regarding the number of vegetative brooms (VB) and the number of cushion brooms (CB) per plant, for fathers, mothers and for the interaction fathers x mothers, were found (probability of error by the Wilks' Lambda test - $p < 0.0001\%$ - not shown). Thus, there are additive effects on the characters inheritance, or differences among clones in terms of general combining ability, and non-additive effects, or differences in the specific combining abilities.

Highly significant differences were also found with both types of brooms for the year or removal period, and for the interactions of this removal period with father and mother (probability for Wilks' Lambda - $p < 0.0001$). Therefore, there were differences for the tendencies in the evolution of the number of brooms over time among fathers and among mothers.

Disregarding the parents and directly analyzing the differences among progenies, highly significant effects were also found for progeny, removal period, and removal period x progeny interaction, for both types of broom (probability for Wilks' Lambda – $p < 0.0001$).

For the progenies general averages, the two variables had a correlation of 0.52, significant at $p < 0.0001$. Both variables, together (TB), had already showed a correlation of 0.59 with the proportion of WBD infected pods, in an evaluation of Cepec's germplasm collection (Pires, 2003). Thus, genetic gains for the numbers of vegetative and cushion brooms should lead to less wear out on the plants, and consequent reduction in production loss; decrease in inoculum, which can also reduce fruit loss; and resistance gain in fruits, by indirect selection.

For vegetative brooms, and considering the total evaluation period, clone Sca 6 had the best performance for the group 1 of progenitors, being distinct from all the others - Table 1, which shows the ratio between the average of each progenitor and the general average of all progenitors, for each counting, and the significance for the differences between sets of broom averages, by the Wilks' Lambda test. This clone, collected in Peru, is the most traditional source of resistance to WBD (Bartley, 1994), and has an inheritance that indicates the presence of two dominant alleles of great effect for this characteristic (Pires *et al.*, 2012).

However, the averages of this progenitor passed from very different in the first years, to very close to the general averages, in the last years (Table 1). And this change in behavior was even clearer for CB, for which Sca 6 is

not distinguished from Be 4, which had the lowest overall numerical value, and ICS 98 (Table 2). Reductions in resistance to WBD from Scavina's descendants, with the evolution of the pathogen populations, have already been reported (Pires, 2003; Albuquerque *et al.*, 2009; Pires *et al.*, 2012; Gramacho *et al.*, 2012), and occurred concomitantly with the intensification of planting of the first commercial varieties indicated in the state of Bahia as resistant, all descendants of this clone.

For VB, the progenies of Sca 6 that best maintained the resistance, from the beginning of the evaluation to the final period, were: Sca 6 x P 4B, Sca 6 x Cepec 86 and Sca 6 x IMC 67 (Table 3), the latter ones not being different from the first by Wilks' Lambda test ($p > 0,05$ - not shown). The worst performances were from Sca 6 x CCN 10 and Sca6 x ICS 9 progenies (Table 3), both statistically different from Sca 6 x P 4B ($p < 0,05$ - not shown).

For CB the results were similar, but with greater distinction (Table 4). The Sca6 x ICS 9 progeny had the worst performance, with loss proportions greater than those of all other combinations (Wilks' Lambda, $p < 0.01$ - not shown), and was followed by the progenies with CCN 10 and CCN 51, with the second and third worst performance. This distinction between the progeny with ICS 9 and those with CCN 10 and CCN 51, was expected, due to the expected absence of resistance genes in ICS 9 and presence in the CCNs. Clones CCN 10 and CCN 51 had already shown resistance factors different from those of Scavina (Pires *et al.*, 2012), and are originating from selections conducted in Ecuador.

There was also, for CB, a significant difference between the progeny with CCN 10 and the progeny with CCN 51, and that with CCN 10 was still significantly different from all the other progenies of Sca 6 (Wilks' Lambda, $p < 0.01$ - not shown). Thus, both CCNs would have factors supporting the resistance, but with different effects.

The performance of the Sca 6 x SGu 54 progeny was surprising as for VB as CB, because resistance factors were not expected in SGu 54. But SGu 54 has a general average as a parent close to the general average for CB (Table 2), although it has, as expected, one of the worst averages for VB (Table 1).

For CB, the progenies of Sca 6 that best maintained the resistance were Sca 6 x Cepec 86, Sca 6 X P 4B and Sca6 x IMC 67.

These results on the change in the inheritance of the most important and most used source of resistance to witches' broom, and the effects on sustaining resistance by inheritances from other sources (also indicated in Pires *et al.*, 2012a), show the importance of processes of recurrent selection for the improvement of the level and durability of this character.

If considered only the last seven counting periods, during which the two groups of Scavina progenies were already separated (Tables 3 and 4), the additive effect of Scavina 6 for VB is no longer distinguishable from the effect of RB 39, which now has a lower overall average proportion (overall average not shown, averages for all counting periods in Table 1, Wilks' Lambda, $p > 0.05$ - not shown). And this is the only significant change. As for CB, the change is very big. Scavina now has a general average as a parent lower only than that of Oc 67 (Wilks' Lambda, $p < 0.01$- not shown). Its seven averages (Table 2) are not different from those of Be 4, EEG 29, ICS 98 and RB 39, and are different from those of Cepec 89 and CSul 7, both with lower overall averages than those of Scavina (Wilks' Lambda, $p > 0.05$, not shown). The behavioral change of Scavina's descendants has already been reported as more noticeable for CB than for VB (Pires, 2003; Pires *et al.*, 2012)

For VB, two others prominent parents from group 1 were CSul 7 and RB 39, not distinct from each other and distinct from all the others, with the exception of Be 4 (Table 1). Both had no increase in the proportion of brooms over time, as happened with Sca 6, and this was also observed in the evaluation of the Clones 'per se' - both were different from clones descending from Scavina 6 and not distinct from each other (Pires *et al.*, 2012). These clones come from the State of Acre, and their best combinations were with clones P 4B and Na 33 (Table 3). CSul 7 x P 4B was statistically different from CSul 7 progenies with CCN 51, CCN 10, ICS 9 and IMC 76; CSul 7 x Na 33 was different from CSul 7 progenies with CCN 51 and CCN 10; RB 39 x P 4 B and RB 39 x Na 33 were, among the RB 39 progenies, only statistically different from the one that included the clone SGu 54 (Wilks' Lambda, $p > 0.05$ - not shown). Eleven of the 16 progenies of these two clones had a general proportion of VB that were numerically smaller than the general average of the experiment (Table 3).

The worst results for VB were the ones of the clone EEG 29, selected in Espirito Santo State, ICS 98, from Trinidad Tobago (both also did not show good performance 'per se' for the character - Pires, 2003) and OC 67, from Venezuela (Table 1).

For CB, the worst performance was that of Oc 67, which distinguished itself from all the others, as for the set of 12 periods (Table 2) as for the set of the last seven periods (Wilks' Lambda, $p > 0,05$ - not shown). For the 12 evaluations periods, the best average proportions, in addition to that of Scavina 6, were from Be 4 and ICS 98, both not different from the ones of Scavina, and from EEG 29 (Table 2). The best combination of Be 4, in numerical value, was with Cepec 86 (Table 4), but this

did not differ significantly from the others (Wilks' Lambda, p > 0.05 - not shown). For ICS 98, the best combinations were with CCN 10 and IMC 76, which differed significantly from the worst combination, with Cepec 86 (Wilks' Lambda, p > 0.05 - not shown). For EEG 29, the combination of lower numerical values was that with ICS 9, and there were no significant differences among EEG 29 progenies (Wilks' Lambda, p > 0.05 - not shown). For the last seven evaluation periods, Be 4, EEG 29 and RB 39 had the best performance in numerical values, and only EEG 29 did not differ significantly from the worst parent, OC 67 (Wilks' Lambda, p < 0.05 - not shown). The best progeny of RB 39 was the one with NA 33, which was significantly different from progenies with CCN 51, IMC 76 and SGu 54 (Wilks' Lambda, p < 0.05 - not shown).

Table 1: Proportion between the average of Vegetative Brooms of each parent in group 1 and the general average of the eight parents, for each counting period, and significance for the differences between means, by the Wilks' Lambda test *Moniliophthora perniciosa /Theobroma cacao* pathosystem, Ilhéus, Bahia

Progenitor	2001	2002	2004	2005	2006	2006	2007	2008	2009	2009	2010	2010	Mean
Be4	1.18	0.90	0.79	0.80	1.01	1.19	0.87	1.07	1.20	1.00	1.17	0.93	1.01
Cepec89	0.78	1.19	0.81	0.97	1.20	1.49	1.19	1.01	1.26	1.09	1.20	0.98	1.10
CSul7	0.76	0.74	0.87	0.50	0.82	0.66	0.73	1.01	0.79	0.43	0.72	1.05	0.76
EEG29	0.84	1.46	1.84	2.16	1.55	0.96	1.53	1.07	1.01	1.02	0.92	0.97	1.28
ICS98	1.49	1.22	1.28	1.12	1.42	1.41	1.69	1.53	1.47	1.70	1.37	1.11	1.40
Oc67	2.22	1.61	1.16	1.31	1.23	1.34	0.99	0.85	0.82	1.35	0.69	1.36	1.24
Rb39	0.82	0.85	1.21	1.02	0.50	0.51	0.52	0.70	0.71	0.59	0.72	0.78	0.74
Sca6	-0.08	0.05	0.03	0.12	0.28	0.44	0.48	0.75	0.75	0.83	1.22	0.82	0.48

Progenitor	M		1	2	3	4	5	6	7
Be4	1,01	1							
Cepec89	1,10	2	ns						
CSul7	0,76	3	ns	**					
EEG29	1,28	4	**	**	**				
ICS98	1,40	5	ns	*	**	**			
Oc67	1,24	6	*	**	**	**	**		
Rb39	0,74	7	ns	**	ns	**	**	**	
Sca6	0,48	8	*	**	**	**	**	**	**

ns, *, ** - not significant, significant at 5% and significant at 1% of probability of error.

Table 2: Proportion between the average of Cushion Brooms of each parent in group 1 and the general average of the eight parents, for each counting period, and significance for the differences between means, by the Wilks' Lambda test - *Moniliophthora perniciosa / Theobroma cacao* pathosystem, Ilhéus, Bahia

Progenitor	2001	2002	2004	2005	2006	2006	2007	2008	2009	2009	2010	2010	Mean
Be4	-0.15	0.02	0.16	0.46	0.94	1.43	1.04	0.91	1.05	0.65	1.13	0.36	0.67
Cepec89	0.08	1.06	0.32	0.86	1.29	1.38	0.64	0.65	0.93	1.16	1.05	1.34	0.90
CSul7	0.69	2.03	1.07	1.05	1.04	0.93	1.29	0.85	0.96	0.73	0.99	1.20	1.07
EEG29	-0.22	0.69	0.81	1.75	1.30	0.60	0.91	0.96	0.82	0.87	0.72	0.94	0.85
ICS98	0.11	0.85	0.40	0.37	1.24	0.98	1.54	1.14	1.31	0.77	0.81	0.89	0.87
Oc67	6.31	1.74	3.05	1.84	0.91	1.18	0.72	1.22	1.02	1.98	0.76	1.40	1.84
Rb39	1.27	1.58	2.23	1.41	0.84	0.64	0.87	0.86	0.80	0.77	0.95	0.90	1.09
Sca6	-0.07	0.05	-0.03	0.26	0.45	0.85	0.99	1.41	1.11	1.08	1.59	0.96	0.72

Progenitor	M		1	2	3	4	5	6	7
Be4	0,67	1							
Cepec89	0,90	2	ns						
CSul7	1,07	3	ns	ns					
EEG29	0,85	4	ns	ns	ns				
ICS98	0,87	5	ns	ns	ns	ns			
Oc67	1,84	6	**	**	**	**	**		
Rb39	1,09	7	*	**	ns	ns	*	*	
Sca6	0,72	8	ns	**	**	*	ns	**	**

ns, *, ** - not significant, significant at 5% and significant at 1% of probability of error.

Table 3: Proportion between the average of Vegetative Brooms of each progeny and the general average of all progenies, for each counting period *Moniliophthora perniciosa /Theobroma cacao* pathosystem, Ilhéus, Bahia

Progeny	2001	2002	2004	2005	2006	2006	2007	2008	2009	2009	2010	2010	Mean
Be4XCCN10	0.00	1.63	1.41	1.08	1.27	1.62	0.68	1.02	1.04	1.78	1.80	0.92	1.19
Be4XCepec86	2.35	0.64	1.04	1.02	0.74	0.98	0.39	1.00	0.68	0.16	0.74	0.04	0.81
Be4XICS9	0.00	0.76	0.38	0.26	0.51	0.38	2.08	0.62	3.77	0.92	0.65	2.48	1.07
Be4XIMC76	1.88	0.69	0.00	0.56	0.17	1.03	1.08	2.15	0.82	0.41	0.43	0.95	0.85
Be4XP4B	0.00	0.25	0.00	0.51	0.84	1.82	0.28	0.62	0.79	1.60	0.00	0.00	0.56
Be4XSGu54	2.00	1.22	1.31	1.40	1.51	1.24	1.10	1.22	1.48	1.16	1.59	1.85	1.42
Cepec89XCCN10	0.00	2.25	1.08	1.23	1.66	1.27	0.98	1.28	0.99	1.01	1.20	0.73	1.14
Cepec XCCN51	0.32	0.75	0.89	0.39	0.97	1.09	1.20	0.54	1.05	0.59	0.88	1.62	0.86
Cepec X Cepec 86	4.00	3.72	2.94	2.78	2.19	1.74	1.90	0.84	1.56	0.96	2.14	0.38	2.10
Cepec 89XICS9	0.00	0.23	0.00	0.51	2.24	3.67	1.04	3.59	2.63	3.03	1.95	1.35	1.69
Cepec 89XIMC76	0.68	0.14	0.24	0.22	0.66	1.67	0.49	0.43	0.62	0.30	0.68	0.63	0.56
Cepec 89XNa33	0.78	0.89	0.58	1.39	0.99	0.91	1.22	0.71	0.73	1.13	0.74	1.20	0.94
Cepec 89XP4B	0.29	0.41	0.48	0.17	0.31	0.50	0.76	0.49	0.83	0.95	0.58	0.54	0.53
Cepec 89XSGu54	0.88	1.26	0.34	0.64	0.89	1.49	1.42	0.90	1.73	2.43	2.38	1.72	1.34
CSul7XCCN10	2.30	1.00	1.04	0.83	0.53	1.01	0.83	1.78	0.69	0.75	0.52	3.55	1.24
CSul 7XCCN51	0.59	2.56	1.31	1.33	0.71	0.67	2.64	1.16	1.95	1.97	1.34	0.88	1.42
CSul 7XCepec86	1.10	1.00	1.08	0.57	1.43	0.31	0.62	0.78	0.44	0.40	0.65	0.31	0.73
CSul 7XICS9	0.34	0.53	0.33	0.18	0.82	0.95	0.65	1.74	1.35	0.34	1.24	0.87	0.78
CSul 7XIMC76	1.26	1.13	0.93	0.69	2.56	1.45	0.18	0.98	1.24	0.21	0.77	0.83	1.02
CSul 7XNa33	0.24	0.25	0.04	0.20	0.34	0.11	0.17	0.22	0.19	0.00	0.48	0.17	0.20
CSul 7XP4B	0.00	0.34	0.70	0.07	0.07	0.10	0.05	0.11	0.11	0.02	0.70	0.33	0.22
CSul 7XSGu54	0.06	0.34	1.63	0.49	0.71	0.50	1.03	1.01	0.55	0.15	0.50	0.63	0.63
EEG29XCCN10	0.00	0.64	0.42	0.56	0.88	0.85	1.50	1.53	1.28	2.13	1.69	2.53	1.17
EEG29XCepecC86	4.31	2.66	3.84	2.55	0.96	0.63	1.02	0.47	0.32	1.14	1.08	0.28	1.61
EEG29XICS9	0.29	0.13	0.38	0.26	2.78	1.82	3.61	1.64	1.53	0.74	0.54	0.46	1.18
EEG29XIMC76	2.10	1.54	2.22	2.99	1.86	0.80	0.79	1.18	0.50	0.54	0.62	1.26	1.37
EEG29XNa33	0.29	0.86	1.49	1.18	1.05	0.87	1.41	0.96	0.93	0.94	0.89	0.47	0.95
EEG29XP4B	0.07	1.69	0.79	0.57	1.05	0.34	0.61	0.14	0.26	0.07	0.19	0.30	0.51
EEG29XSGu54	0.94	2.56	4.54	6.74	2.60	1.24	2.44	1.25	1.74	1.12	1.08	1.08	2.28
ICS98XCCN10	0.88	0.40	0.86	0.89	0.63	1.15	0.87	0.27	0.55	0.77	1.41	0.80	0.79
ICS98XCCN51	2.20	1.58	1.06	0.77	0.30	0.98	1.18	0.59	0.61	1.06	2.65	0.69	1.14
ICS98XCepec86	2.12	2.71	2.65	1.89	4.72	3.89	3.30	2.47	3.30	3.39	1.73	1.98	2.85
ICS98XICS9	0.59	0.68	0.41	0.99	1.18	1.08	0.99	1.43	1.18	2.26	1.20	1.53	1.13
ICS98XIMC76	2.88	1.14	2.11	1.66	0.91	0.76	0.90	0.70	1.30	0.77	1.25	0.91	1.28
ICS98XP4B	1.24	1.45	1.54	1.62	1.76	0.76	2.68	0.97	1.25	1.08	0.72	0.39	1.29
ICS98XSGu54	1.23	1.17	0.85	0.28	1.06	1.88	1.29	3.28	1.37	1.87	1.36	1.78	1.45
OC67XCCN51	0.22	1.18	1.90	0.42	1.54	0.90	0.24	0.20	0.13	0.21	0.35	1.01	0.69
OC67XCepecC86	0.59	0.88	2.02	1.60	1.31	2.87	0.97	0.86	0.44	1.95	0.97	0.62	1.26
OC67XIMC76	7.13	2.70	1.90	2.36	1.24	1.24	1.39	1.58	1.41	1.40	0.54	1.32	2.02
OC67XNa33	2.94	1.21	0.54	0.56	1.05	0.70	1.22	0.34	0.96	1.19	0.65	1.16	1.04
OC67XSGu54	0.00	2.15	0.00	1.94	0.91	1.26	0.97	0.44	0.54	1.58	0.65	2.57	1.08
RB39XCCN10	1.32	1.26	2.07	1.21	0.53	0.37	0.78	0.57	0.56	0.80	0.73	0.81	0.92
RB39XCCN51	1.18	0.67	0.67	0.89	0.17	0.72	0.21	1.64	0.96	3.32	0.54	0.46	0.95
RB39XCepecC86	2.00	1.70	1.77	1.12	0.64	0.20	0.58	0.31	0.19	0.11	0.22	0.44	0.77
RB39XICS9	0.00	0.59	0.58	0.34	0.79	1.01	0.65	1.25	0.88	0.69	1.08	0.67	0.71
RB39XIMC76	1.76	1.78	2.42	1.99	0.88	1.07	0.06	0.09	0.63	0.34	1.25	1.10	1.12
RB39XNa33	0.24	0.28	0.65	0.41	0.12	0.02	0.04	0.14	0.30	0.00	0.37	0.17	0.23
RB39XP4B	0.13	0.29	0.53	0.31	0.09	0.12	0.19	0.29	0.19	0.38	0.70	0.54	0.31
RB39XSGu54	1.18	0.59	0.58	2.81	1.01	0.95	2.43	3.28	3.29	0.74	0.65	3.49	1.75
Sca6XCCN10	0.07	0.17	0.13	0.20	0.47	0.09	0.57	0.95	1.36	2.37	3.17	0.72	0.86
Sca 6XCCN51	0.00	0.11	0.19	0.51	1.39	1.44	1.08	0.86	0.50	1.09	0.59	0.55	0.69
Sca 6XCepecC86	0.00	0.05	0.00	0.11	0.22	0.22	0.20	0.45	0.07	0.06	0.58	0.52	0.21
Sca 6XICS9	0.00	0.03	0.06	0.60	0.45	0.92	0.81	1.33	0.42	0.38	2.41	1.48	0.74
Sca 6XIMC76	0.05	0.03	0.00	0.00	0.05	0.36	0.34	0.54	0.60	0.67	0.55	0.92	0.34
Sca 6XNa33	0.00	0.05	0.00	0.03	0.08	0.35	0.35	0.74	0.65	0.82	1.08	0.52	0.39
Sca 6XP4B	0.00	0.05	0.04	0.00	0.13	0.17	0.22	0.32	0.46	0.24	0.50	0.92	0.26
Sca 6XSGu54	0.00	0.04	0.02	0.10	0.04	0.47	0.30	0.72	1.05	0.49	1.05	0.60	0.41

Table 4: Proportion between the average of Cushion Brooms of each progeny and the general average of all progenies, for each counting period *Moniliophthora perniciosa /Theobroma cacao* pathosystem, Ilhéus, Bahia

Progeny	2001	2002	2004	2005	2006	2006	2007	2008	2009	2009	2010	2010	Mean
Be4XCCN10	0.00	0.34	0.42	1.05	0.61	1.53	0.79	0.68	0.98	1.03	1.07	0.87	0.78
Be4XCepec86	0.00	0.00	0.19	0.21	0.68	0.56	0.20	0.32	0.85	0.08	0.23	0.00	0.28
Be4XIMC76	0.00	0.00	0.42	0.31	1.80	0.04	1.00	1.56	0.81	1.94	1.04	0.68	0.80
Be4XSGU54	0.00	0.00	0.09	0.96	1.09	2.38	0.92	1.06	1.14	0.63	1.33	1.00	0.88
Cepec89XCCN10	0.00	4.37	0.28	1.76	2.63	2.93	0.76	0.74	0.88	0.66	0.41	2.16	1.47
Cepec89XCCN51	0.00	0.62	0.60	0.84	1.46	1.46	0.76	0.44	0.81	0.37	0.75	1.03	0.76
Cepec89XCepec86	0.00	3.55	0.39	1.38	2.02	1.03	0.60	0.16	0.90	0.92	1.06	0.79	1.07
Cepec9XICS9	0.00	0.00	0.00	0.67	0.86	4.16	2.37	2.68	2.46	3.00	1.73	1.01	1.58
Cepec89XIMC76	0.00	0.09	0.34	0.41	0.26	0.51	0.21	0.26	0.26	0.38	0.20	0.30	0.27
Cepec89XNa33	0.00	0.23	0.00	0.25	0.75	0.78	0.64	0.86	0.53	0.85	0.97	1.35	0.60
Cepec89XP4B	0.00	0.00	0.00	0.10	0.39	0.53	0.22	0.24	0.43	0.30	0.46	0.79	0.29
Cepec89XSGu54	0.00	0.29	0.00	1.39	1.99	2.51	0.52	0.39	2.11	4.19	3.17	3.38	1.66
CSul7XCCN10	3.16	1.86	1.92	1.72	1.49	0.97	0.74	1.59	0.85	0.95	0.43	2.73	1.54
CSul7XCCN51	0.22	8.27	1.72	1.64	1.46	2.88	3.26	1.33	2.35	2.27	2.30	1.23	2.41
CSul7XCepec86	0.00	2.28	1.25	0.79	2.53	0.46	1.23	0.75	1.25	0.57	0.61	0.53	1.02
CSul7XICS9	0.00	0.05	0.42	1.77	1.15	2.48	2.39	1.10	1.59	0.73	1.84	1.49	1.25
CSul7XIMC76	0.30	1.42	0.28	0.54	0.59	0.36	0.41	0.71	0.33	0.14	0.84	1.49	0.62
CSul7XNa33	0.00	0.00	1.60	0.46	0.31	0.06	0.22	0.00	0.14	0.27	0.00	0.11	0.27
CSul7XP4B	0.00	0.00	0.24	0.09	0.08	0.05	0.33	0.18	0.22	0.07	0.84	0.19	0.19
CSu7XSGu54	0.00	0.11	0.40	1.03	0.74	0.40	1.10	0.36	0.51	0.05	0.36	0.55	0.47
EEG29XCCN10	0.00	0.00	0.00	0.34	0.78	0.37	0.53	0.32	0.46	1.90	2.38	2.18	0.77
EEG29XICS9	0.00	0.20	0.00	0.10	0.23	0.18	0.00	0.00	0.35	0.24	0.00	0.90	0.18
EEG29XIMC76	0.00	0.91	0.37	1.64	0.00	0.27	0.33	0.78	0.65	2.22	0.54	0.23	0.66
EEG29XNa33	0.00	0.73	1.63	1.55	1.03	0.24	0.93	0.56	0.89	0.50	0.43	1.03	0.79
EEG29XP4B	0.00	0.59	0.56	0.26	1.72	0.37	0.52	0.39	0.46	0.15	0.17	0.45	0.47
EEG29XSGu54	0.00	0.07	0.74	4.17	1.56	0.98	0.78	1.82	0.67	0.26	0.35	0.75	1.01
ICS98XCCN10	0.00	0.00	0.00	0.00	0.00	0.29	0.03	0.00	0.12	0.12	0.77	0.08	0.12
ICS98XCCN51	0.00	6.44	0.28	0.51	3.99	3.89	0.75	0.29	0.46	2.24	1.27	0.23	1.70
ICS98XCepec86	0.00	0.00	0.19	1.64	1.35	1.10	7.25	2.40	4.31	1.01	1.46	1.50	1.85
ICS98XICS9	0.00	0.00	0.28	0.46	1.92	2.59	2.07	3.61	2.17	0.97	0.63	2.70	1.45
ICS98XIMC76	0.00	0.22	1.15	0.62	0.42	0.46	0.14	0.00	0.17	0.05	0.39	0.26	0.32
ICS98XP4B	0.00	1.30	2.13	1.16	3.39	0.78	1.46	0.84	1.50	0.93	0.92	0.00	1.20
ICS98XSGU54	0.00	0.17	0.00	0.39	0.38	0.70	0.85	1.32	1.18	1.01	0.74	1.13	0.66
Oc67XCCN51	0.00	0.07	1.25	0.14	0.36	0.26	0.17	0.16	0.19	0.14	0.31	0.00	0.25
Oc67XCepecC86	0.00	0.00	0.76	0.46	0.43	1.47	0.57	1.07	1.44	1.06	1.38	1.91	0.88
Oc67XIMC76	11.82	3.25	5.99	3.97	1.89	1.83	1.03	1.52	0.81	2.32	0.20	0.83	2.95
Oc67XNA33	27.40	3.90	0.65	0.48	0.10	0.00	0.40	0.78	0.81	0.73	0.31	0.83	3.03
Oc67XP4B	0.00	0.00	0.28	0.41	0.39	0.07	0.95	0.88	1.79	0.97	0.69	1.24	0.64
Oc67XSGu54	0.00	3.03	3.68	3.39	0.55	1.71	0.27	0.73	0.75	4.25	0.63	2.36	1.78
RB39XCCN10	2.95	3.69	0.71	0.82	0.92	0.93	0.30	0.24	0.42	0.55	0.56	1.15	1.10
RB39XCCN51	0.00	0.39	0.06	0.49	1.78	0.62	1.32	2.42	1.87	2.35	0.28	1.04	1.05
RB39XCepec86	7.25	0.33	0.69	1.33	0.42	0.65	0.10	0.55	0.27	0.00	0.38	0.00	1.00
RB39XICS9	0.00	0.00	4.59	0.72	1.72	0.44	2.64	0.97	0.81	1.09	0.46	0.79	1.19
RB39XIMC76	0.00	2.24	5.28	4.26	1.17	0.55	0.80	0.44	0.55	0.49	1.50	0.90	1.51
RB39XNa33	0.00	2.28	0.93	0.44	0.25	0.26	0.46	0.10	0.52	0.04	0.46	0.28	0.50
RB39XP4B	0.69	0.39	2.94	1.32	0.25	0.13	0.51	0.78	0.51	0.97	2.27	1.48	1.02
RB39XSGu54	1.21	1.17	9.17	4.10	1.09	0.59	2.54	1.07	1.56	0.30	0.23	1.91	2.08
Sca6XCCN10	0.00	0.10	0.00	0.41	0.29	1.79	1.42	2.39	2.83	3.75	4.00	3.52	1.71
Sca 6XCCN51	0.00	0.00	0.00	1.13	1.67	2.71	2.94	1.33	1.50	0.87	1.46	0.86	1.21
Sca 6XCepec86	0.00	0.00	0.03	0.07	0.26	0.28	0.14	0.78	0.10	0.09	0.31	0.23	0.19
Sca 6XICS9	0.00	0.00	0.00	0.68	1.04	1.03	2.76	8.97	3.27	2.43	6.99	1.20	2.36
Sca 6XIMC76	0.00	0.00	0.00	0.00	0.07	0.10	0.27	0.28	0.34	0.35	0.58	0.45	0.20
Sca 6XNA33	0.00	0.00	0.00	0.05	0.23	0.18	0.16	0.49	0.45	0.94	1.01	0.51	0.34
Sca 6XP4B	0.00	0.00	0.04	0.05	0.13	0.11	0.44	0.29	0.45	0.15	0.68	0.15	0.21
Sca 6XSGU54	0.00	0.07	0.09	0.07	0.31	1.00	0.48	1.01	0.92	0.12	0.61	0.30	0.42

The good performance for CB and bad for VB of EEG 29, which is a selection within traditional varieties cultivated in Bahia, portrays this type of varieties (Pires, 2003).

For Group 2 of progenitors, the best performance for VB was that of P 4B, which did not differ significantly only from Na 33, the second best performance (Table 5). For CB, the P 4B, also with the best numerical values for the 12 averages, did not differ from Na 33, IMC 76 and Cepec 86, all with general averages well below 1.00 (Table 6). All of these genotypes did not show an increase in incidence in the last seven evaluations, which indicates that they also have different resistance factors than Scavina.

The best combinations with P 4B for VB were with CSul 7, Sca 6 and RB 39, (Table 3) being the four Upper Amazon clones. And only the progeny with the Trinitarian ICS 98 showed high averages, notably in the first evaluations – significantly different from the ones of the crosses with CSul 7, Sca 6 and RB 31 (Wilks' Lambda, $p < 0.05$ - not shown). For Na 33, the best combinations were with CSul 7 and RB 39 and the worst with OC 67 (Table 3), the latter being very close to the overall average of the experiment. And there were no significant differences among the progenies.

For CB, the best combinations of P 4B were with CSul 7, Sca 6 and Cepec 89 (Hybrid of unknown origin, possibly descendant of Scavina) (Table 4). Again, the crossing with ICS 98 showed the worst set of means, but there was not enough experimental precision to achieve significance in the differences among progenies. For Na 33, the best combinations were with CSul 7 and Sca 6, all three Upper Amazon clones, and the worst with the Criollo OC 67, the latter being significantly different from all others that included Na 33 (Wilks' Lambda, $p > 0.05$ - not shown).

The worst parents for VB were Cepec 86, which differed from all the others, and clones SGu 54 and CCN 10, not distinct from each other (Table 5). ICS 9 and IMC 76 had very close general averages, but differed significantly with, with the first having its averages with a tendency for growth and the second with a trend for reduction. For CB, the worst progenitors were ICS 9, different from all the others, CCN 10, only not different from SGu 54 and CCN 51, only not different from Cepec 86 (Table 6). Cepec 86, collected in a very old cultivation area in the Jequitinhonha River valley, in Bahia, repeats the performance of the other clone selected from local varieties, EEG 29, with poor performance for VB and good for CB.

ICS 9 and SGu 54 were expected to be the worst progenitors for resistance, and the performance of CCN 51, a variety resistant to witches' broom widely used in many countries, and CCN 10, also resistant, and largely cultivated in Bahia, were below expectations (Pires, 2003; Pires *et al.*, 2012). On the other hand, these last two clones had their best combinations, with very good results, with another Trinitarian or with a Criollo: ICS 98 x CCN 10 and OC 67 x CCN 51, as for VB as CB (Tables 3 and 4), which may suggest a different resistance mechanism in relation to Amazonian clones.

Table 5: Proportion between the average of Vegetative Brooms of each parent in group 2 and the general average of the eight parents, for each counting period, and significance for the differences between means, by the Wilks' Lambda test *Moniliophthora perniciosa /Theobroma cacao* pathosystem, Ilhéus, Bahia

Progenitor	**2001**	**2002**	**2004**	**2005**	**2006**	**2006**	**2007**	**2008**	**2009**	**2009**	**2010**	**2010**	**Mean**
CCN10	0.93	1.28	1.11	1.06	0.98	1.03	0.99	1.22	1.03	1.56	1.43	1.56	1.18
CCN51	0.45	0.99	1.16	0.71	0.94	0.87	1.16	0.69	0.91	0.95	0.96	1.03	0.90
Cepec86	2.02	1.72	1.79	1.57	1.57	1.30	1.23	0.92	0.98	0.98	1.04	0.56	1.31
ICS9	0.44	0.56	0.38	0.72	1.20	1.50	1.12	1.76	1.45	1.35	1.41	1.23	1.09
IMC76	2.00	0.98	1.11	1.22	0.95	1.04	0.66	0.89	0.94	0.59	0.70	0.94	1.00
Na33	0.78	0.59	0.57	0.65	0.67	0.63	0.86	0.67	0.78	0.84	0.77	0.64	0.71
P4B	0.52	0.78	0.68	0.55	0.62	0.50	0.75	0.38	0.56	0.55	0.56	0.56	0.58
SGu54	0.86	1.10	1.20	1.51	1.06	1.14	1.24	1.45	1.34	1.17	1.13	1.49	1.22

Progenitor	**M**		**1**	**2**	**3**	**4**	**5**	**6**	**7**
CCN10	1,18	1							
CCN51	0,90	2	.						
Cepec86	1,31	3	**	**					
ICS9	1,09	4	**	**	**				
IMC76	1,00	5	**	**	**	**			
Na33	0,71	6	**	.	**	**	*		
P4B	0,58	7	**	*	**	**	**	.	
SGu54	1,22	8	.	.	**	.	**	*	**

ns, *, ** - not significant, significant at 5% and significant at 1% of probability of error.

Table 6: Proportion between the average of Cushion Brooms of each parent in group 2 and the general average of the eight parents, for each counting period, and significance for the differences between means, by the Wilks' Lambda test *Moniliophthora perniciosa / Theobroma cacao* pathosystem, Ilhéus, Bahia

mãe/grupo 2	2001	2002	2004	2005	2006	2006	2007	2008	2009	2009	2010	2010	Mean
CCN10	1.91	1.70	0.99	1.17	1.13	1.23	0.70	1.11	1.09	1.61	1.26	2.2	1.34
CCN51	-0.19	2.48	0.82	0.92	1.56	1.66	1.81	1.06	1.38	1.26	1.17	0.7	1.22
Cepec86	1.09	1.26	0.65	0.97	1.28	0.64	1.05	0.72	1.00	0.52	0.66	0.6	0.87
ICS9	0.65	-0.20	0.90	1.08	1.19	2.10	2.14	2.80	1.91	1.63	2.08	1.5	1.48
IMC76	1.62	1.00	1.85	1.36	0.75	0.52	0.48	0.54	0.41	0.75	0.51	0.6	0.86
Na33	2.06	1.11	0.66	0.38	0.54	0.40	0.58	0.41	0.65	0.54	0.56	0.6	0.71
P4B	0.49	0.11	0.90	0.45	0.63	0.29	0.46	0.35	0.53	0.42	0.82	0.4	0.49
SGu54	0.39	0.55	1.23	1.66	0.92	1.16	0.78	1.00	1.02	1.27	0.94	1.3	1.02

mãe/grupo 2			1	2	3	4	5	6	7
CCN10	1,34	1							
CCN51	1,22	2	**						
Cepec86	0,87	3	**	.					
ICS9	1,48	4	**	**	**				
IMC76	0,86	5	**	**	.	**			
Na33	0,71	6	*	*	.	**	.		
P4B	0,49	7	*	**	.	**	.	.	
SGu54	1,02	8	.	**	.	**	.	.	.

ns, *, ** - not significant, significant at 5% and significant at 1% of probability of error.

Overall, there was a higher proportional frequency of crosses between Amazonian clones for those with the best results, and crossings including a Trinitarian or Criollo for those with the worst. Among the 20 best general averages for VB, only five combinations included a Criollo or Trinitarian, while among the 20 worst averages, 17 were crosses with at least one of these types. For CB, six of the 20 best averages, and 19 of the 20 worst were generated by progenies with at least one Criollo or Trinitarian parent. This is an inconvenience for the improvement of the species because some important characteristics are more frequent in these types (Pires, 2003).

For both types of broom, together, the progenies with the best performances were: Cepec 89 x IMC 76, Cepec 89 x D 4B, CSul 7 x Na 33, CSul 7 x P 4B, CSul 7 x SGu 54, EEG 29 x P 4B, ICS 98 x CCN 10, Oc 67 x CCN 51, RB 39 x Na 33, Sca 6 x Cepec 86, Sca 6 x IMC 67, Sca 6 x Na 33, Sca 6 x D 4B and Sca 6 x Sgu 58.

From this trial, new progenitors were selected for a second generation of recurrent selection, with many confirming the good performance for resistance (Benjamin *et al*, 2016; Pires *et al.*, 2021; Rodrigues *et al.*, 2020), and from this second generation to a third. Selected clones from the first and second generation of recurrent selection are being evaluated in regional trials to define new varieties for commercial planting. Obviously, for the selection of these new progenitors or clones, other factors not addressed in this work, such as resistance to other diseases, productivity, fruit characteristics, etc., were considered - the selection is based on progenitors averages (general combining ability), progenies averages (specific combining ability) and plants averages, and the selected plants are, then, tested as progenitors in new cycles of recurrent selection and/or as clones, in regional clonal trials.

Other characteristics of the progenitors tested here can be found in International Cocoa Germplasm Database.

CONCLUSIONS

For the two groups of progenitors evaluated: 1- Be 4, Cepec 89, CSul 7, EEG 29, ICS 98, Oc 67, RB 39 and Sca 6, e 2 - CCN 10, CCN 51, Cepec 86, ICS 9, IMC 76, Na 33, P4b, SGu 54, there were different additive effects in the inheritance for vegetative (VB) and cushion (CB) brooms, or differences among clones in terms of the general combining ability; and different non-additive effects, or differences in the specific combining ability. Differences among progenitors and among progenies, for both types of broom, were also found for the tendencies in the evolution of the number of brooms over the evaluation period.

It was confirmed the already reported evolution of populations of the pathogen, that occurred concurrently with the intensification of planting, in Bahia, of the first varieties indicated as resistant, all descendants of the clone Scavina 6. After a period, the Scavina's progenies, in this study were divided into

two groups: those that continue whit good performance and those that lose their prominence, and the effects on sustaining resistance by inheritances from other sources show the importance of processes of recurrent selection for the improvement of the level and durability of this character.

Overall, there was a higher proportional frequency of crosses between Amazonian clones for those with the best results and crossings including a Trinitarian or Criollo clone for those with the worst.

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