

Behavior of introduced regional clones of *Theobroma cacao* toward the infection *Moniliophthora roreri* in three different regions of Colombia

Comportamiento de clones regionales e introducidos de *Theobroma cacao* ante la infección de *Moniliophthora roreri* en tres regiones diferentes de Colombia

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ABSTRACT

Moniliasis (frosty pod rot), caused by *Moniliophthora roreri*, is the most limiting disease of cacao bean production in Colombia. Disease control has focused primarily on the implementation of cultural methods, which are inefficient and increase costs of production. A promising alternative for the control of *M. roreri* is the genetic approach, involving the search for plants with known resistance in the field. The aim of this study was to evaluate the behavior of 13 regional clones of Corpoica Selection Colombia (SCC), ten regional Fedecacao clones (with the initial F), three clones Caucasia (CAU) and 13 introduced clones for resistance to *M. roreri*, by monitoring symptoms and signs of the pathogen in fruits of the cacao at three experimental sites located in Arauquita (Arauca), San Vicente and Rionegro (Santander). To estimate the degree of susceptibility or resistance of each clone, the pathogen was inoculated into fruits 75±5 days old. Eight weeks later, the variables Incidence, Internal Severity Index and External Severity Index were evaluated. In the three experimental sites, the ICS clone 95 behaved as a resistant plant with ISI ranging from 1.4 to 1.9. In the other clones tested, susceptibility varied according to altitude and environmental conditions. However, the ICS clone 95 showed partial resistance, varying the spread of the pathogen in the plant tissue.

Key words: moniliasis, corn, cacao, clones, resistance.

RESUMEN

La moniliasis, causada por *Moniliophthora roreri*, es la enfermedad más limitante de la producción de granos de cacao en Colombia. El control de la enfermedad se ha enfocado principalmente en la implementación de métodos culturales, que resultan ineficientes y elevan los costos de producción. Una alternativa promisoría para el control de *M. roreri* es el método genético, involucrando la búsqueda de materiales con reconocida resistencia a nivel de campo. El objetivo del presente trabajo fue evaluar el comportamiento de 13 clones regionales Selección Colombia Corpoica (SCC), diez clones regionales Fedecacao (con la inicial F), tres clones Caucasia (CAU) y 13 clones introducidos por resistencia a *M. roreri*, mediante el seguimiento de síntomas y signos del patógeno sobre frutos de cacao bajo condiciones de tres sitios experimentales ubicados en Arauquita (Arauca), San Vicente y Rionegro (Santander). Para estimar el grado de susceptibilidad o resistencia de cada clon, se inoculó el patógeno sobre frutos de 75 ± 5 días de edad. Ocho semanas después, se registraron las variables de Incidencia, Índice de Severidad Externa e Índice de Severidad Interna. En los tres sitios experimentales, el clon ICS 95 se comportó como un material resistente, con ISI que varió entre 1,4 a 1,9. En los demás materiales evaluados, la susceptibilidad varió de acuerdo con la altitud y las condiciones medio ambientales. Sin embargo, el clon ICS 95 presentó una resistencia parcial, variando el avance del patógeno en el tejido de la planta.

Palabras clave: moniliasis, mazorca, cacao, clones, resistencia.

Introduction

Cacao (*Theobroma cacao*) is one of the most important tropical crops: economically, socially and environmentally (Hebbar, 2007). For 2010, FAO reported world production of 4 million t of dry grain, of which over 60% was used in manufacturing in the chocolate industry (FAO, 2011). However, this crop is attacked by a large number of pathogens, which are responsible for up to 30% of global

production losses, including *Moniliophthora roreri* H.C. Evans, Stalpers, Samson & Benny; *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora, comb. nov. and *Phytophthora* sp. as the most important (Hebbar, 2007).

Moniliasis, the common name for the disease caused by *M. roreri*, is considered a highly destructive disease (Evans, 2007), with its introduction to the African continent considered a major threat to the cacao economy (Ploetz, 2007).

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This pathogen is limited to Spanish-speaking countries (Evans, 2007). Farmers recognize *M. roreri* primarily by external symptoms on the fruits of cacao, especially by the appearance of signs of the pathogen, such as white mycelium, ashen in appearance, or the complete sporulation of the pathogen on the affected fruit tissue (Phillips-Mora and Wilkinson, 2007). The origin of the disease comes from the northeastern Colombian region where the domestication of cacao involved the association of *T. cocoa* with other related species such as *Theobroma gireli* for thousands of years. Hence, at present, the genus *Theobroma*, like *Herrania*, is host to *M. roreri* (Evans, 2007).

In Colombia, moniliasis can cause losses from 40 to 100% of the dry grain, depending on the farmers' practices (Castellanos *et al.*, 2007). Control of *M. roreri* is mainly based on cultural practices, but this must be supplemented with other control methods because the pathogen inoculum level and aggressiveness, especially in the origin region (Aime and Phillips-Mora *et al.*, 2005), mean that these practices are inefficient. Other studied control methods, rare in Colombia, are biological and chemical control. However, the adoption of these practices or methods is difficult due to the low price of dry grain nationwide. As an alternative to this problem and complementary to other methods, genetic control is promising for the management of the disease (Phillips-Mora *et al.*, 2005), considering that the selection of resistant plants could provide a control method with a longer duration and lower costs, which together with the implementation of other methods would result in the successful control of the disease.

The nature of disease resistance of any plant is present under the cultivation conditions. This resistance can occur as a survival response of the plants to parasitic organisms, involving a wide range of defense mechanisms that stop or prevent the attack of the pathogen. The selection of resistant materials involves measuring

the degree of susceptibility to the pathogen; this requires evaluating the direct and indirect effects of the pathogen on the host plant (Ribeiro *et al.*, 2001). According to the above, the objective of this study was to evaluate the behavior of some local plants as well as introduced ones against infection by *M. roreri*, by monitoring symptoms and signs of the pathogen on fruits of each cacao clone under field conditions.

Materials and methods

Location of experimental sites

Two experimental sites were selected on the Mountain Santandereana and a place in the Llanos Foothills in the department of Arauca. These sites were: Experimental Station La Suiza in Rionegro (Santander, Colombia) Corpoica located at 7° 22' N; 78° 10' W, at an altitude of 500 m, average temperature of 28°C, average annual precipitation of 1,800 mm and average relative humidity of 85%; Villa Monica Farm Fedecacao in San Vicente de Chucuri (Santander, Colombia) located at an altitude of 850 m, with an average temperature of 24°C, average annual precipitation of 1,800 mm and average relative humidity of 82% and Santa Elena Farm Fedecacao in Arauquita (Arauca, Colombia) located at 7° 60' N; 71° 61' W, at an altitude of 162 m, average temperature of 28°C, average annual precipitation of 2,500 mm and average relative humidity of 75%.

Plant material

Regional and introduced materials were selected taking into account those most widely used commercially and their availability in each of the three clonal gardens selected as the experimental sites (Tab. 1).

The fruits were obtained by hand pollination of flowers of each material (clone mother) and with clone IMC 67 (clone father). The flowers of each material were covered with

TABLE 1. Cacao materials evaluated for monilia resistance at La Suiza (Corpoica), the farms Villa Mónica and Santa Elena (Fedecacao) between the years 2006 to 2007.

Corpoica		Fedecacao	
Regional	Introduced	Regional	Introduced
SCC 41, 45	ICS 1, 40, 60, 95	FLE 2, 3	ICS 1, 6, 39, 95
SCC 46, 52	TSH 565, 812	F 302	TSH 565
SCC 56, 64	EET 8	FSA 11, 12 y 13	EET 8
SCC 70, 76	IMC 67	FTA 1 y 2	IMC 67
SCC 77, 79	CCN 51	FEAR 5 y 12	UF 613
SCC 80, 91	CAP 34	CAU 37, 39 y 43	CCN 51
SCC 61		SCC 61	

CAU = Caucasia (Colombia); CAP = Comercial Agrícola Pechical (Ecuador); CCN = Colección Castro Naranjal (Ecuador); EET = Estación Experimental Tropical (Ecuador); F = Florida (USA); FEAR = Fedecacao Arauquita (Colombia); FLE = Fedecacao Lebrija (Colombia); FSA = Fedecacao Saravena (Colombia); FTA = Fedecacao Tame (Colombia); ICS = Imperial College Selection (Trinidad); IMC = Iquitos Marañon Collection de (Perú); SCA = Scavina (Perú); SCC = Selección Colombia Corpoica (Colombia); TSH = Trinidad Seleted Hybrid (Trinidad); UF = Unit fruit (Trinidad).

high-density polyethylene bags to protect the environment from the pathogen (Phillips-Mora *et al.*, 2005).

Obtaining pathogen inoculum

The pathogen inoculum was obtained from a collection of diseased fruits with symptomatic stains. These were subjected to disinfection with sodium hypochlorite 1%, with subsequent washing with distilled water. The fruits were placed in a humid chamber with environmental conditions equal to those of the test sites for 15 d. After this period, the pathogen spores were obtained by scraping the sporulated fruit.

Evaluation of cacao clones resistance

Resistance assessments were made on fruits of the cacao at 75±5 d old. For inoculation of the pathogen, dry spores were taken with the tip of an entomological pin, taking approximately 3.4·10⁴ spores/cm² (Merchán and Restrepo, 1980). The dry spores were dispersed over an area of 4 cm² on the fruit, moistened with sterile distilled water. The inoculated fruits were covered with high-density perforated bags and moistened absorbent paper for 5 d to create favorable conditions for the onset of the infection process.

After 8 weeks, the progress of the disease was assessed. For this, the variables recorded were: incidence (I) (% of diseased fruits) external severity index (ESI), using the modified severity scale of five degrees of Sánchez and González (1989) (Tab. 2), and internal severity index (ISI) (Tab. 3). The classification ISI of the cacao material was based on the scale proposed by Phillips-Mora *et al.* (2005) (Tab. 4).

The materials selected at La Suiza in Corpoica were evaluated twice, once in 2006 and again in 2007. The selected materials in Fedecacao were evaluated three times in each location, once per semester in 2006 and the second half

TABLE 2. External severity index (ESI) based on symptoms and signs observed on inoculated fruit.

Index	Symptom
0	Absence of any symptoms
1	Points, oily and / or deformed
2	Points, concentric Stains
3	Area of the stain equal to or less than the marked area
3.4	Area of the stain greater than the marked area
3.9	Area of the stain more than 50% of the fruit
	Signs
4	Emergence of mycelium
4.3	Appearance of spores
4.6	Sporulation greater than 50% of fruit
4.9	Sporulation equal to the stain

TABLE 3. Internal severity index (ISI) according to the symptoms and signs observed on inoculated fruit.

Index	Symptom (% of damage)
0	0
1	1 – 20
2	21 – 40
3	41 – 60
4	61 – 80
5	81 – 100

TABLE 4. Classification of *T. cacao* materials, according to their resistance to *M. royeri* according to Phillips-Mora *et al.*, 2005.

Classification (SI)	Range
Resistant (R)	0 – 1.25
Moderately resistant (MR)	1.26 – 2.50
Moderately susceptible (MS)	2.51 – 3.75
Susceptible (S)	3.76 – 5.0

of 2007. We used a completely randomized design, where each clone had a treatment. In each test, we used a sample of 10-15 fruits; each fruit was considered an experimental unit. Each test was considered a repetition over time.

Statistical Analysis

For the statistical analysis, the ISI and ESI variables were analyzed for each region separately, submitting the data for each site for analysis of variance and comparison of means by the Tukey method ($P \leq 0.05$). The data analysis was performed using SAS software version 8 (SAS Institute, Cary, NC).

Results

Behavior of materials in the Llanos Foothills

At the Santa Elena Farm tested clones had an incidence higher than 76% (Tab. 5), with the exception of clones Caucasia, CAU 51 and 43, which had an incidence of 45 and 53% respectively.

In assessing the severity of the disease internally, ICS materials 95, CAU 39, CAU 43, 51 and CAU 37 behaved as resistant (R) (Tab. 5). The Clone of note, the ICS 95 (clone introduced) had the lowest ISI, which was 0.25±0.13, with an average damage of 25%. As for the clones classified as MR, only clone FSA 12 (regional material) presented an ISI with no significant difference with respect to materials R, showing an average internal damage of 29.5%. The other materials showed internal damage exceeding 40%. In assessing the severity of the disease outside, it was observed that clone CCN 51 had the lowest ESI average value, this being 0.78 with the first symptoms of the disease. (Tab. 5),

the disease in the introduced clones, as at the Villa Monica farm, only clone ICS 95 behaved as a resistant material, with an average ISI of 1.4 (Tab. 7), indicating an average internal damage of 16%. The other introduced materials behaved from moderately susceptible to susceptible, with internal damage above 58% (Tab. 7). In the external assessment of severity, only clone ICS 95 presented an ESI mean of 1.4, equivalent to the onset of initial symptoms (Tab. 7) (Phillips-Mora *et al.*, 2005).

In the evaluation of the regional clones, the ranking ranged from moderately susceptible (MS) to susceptible, presenting an ISI above 2.5, i.e. internal damage exceeding 50% (Tab. 8), including the clone SSC 61, which presented an average ISI of 4.8, i.e. an internal damage average of 96%. In most of the clones, a higher than average ESI, 2.5 (Tab. 8), indicating that most of the materials developed stain symptoms, especially in clones SCC 45 and 70, which completed the disease cycle, i.e. until sporulation (Tab. 8).

Discussion

In this study, only clone ICS 95 behaved as a resistant material with respect to the other clones evaluated, with internal damage below 20% (Fig. 1), coinciding with that reported by other authors (Tab. 9) (Aránzazu, 2003; Phillips-Mora *et al.*, 2005; Rodríguez and Medina, 2005). The same results found by Phillips-Mora *et al.* (2005) evaluated this material against seven strains of *M. roreri*, noting that the material retained its behavior as resistant material. Other materials that showed partial resistance under environmental conditions at the Santa Elena and Villa Monica were the clones, CCN 51 (Fig. 1), CAU 37, CAU39, CAU 43 and IMC 67 (Fig. 1) (Tab. 9). These materials had atypical symptoms, such as subsidence, green ripening, wilting in the affected area and the formation of green islands on senescent plant material (Fig. 2A). In the specific case of the green islands, this is because of the pathogens hemibiotrophics such as *M. roreri* that invade living cells, reverse the metabolism

TABLE 7. Incidence, internal and external severity of *M. roreri*, and classification of *T. cacao* clones introduced in Rionegro (Santander) for 8 weeks, following the internal severity index (ISI) proposed by monilia (Phillips-Mora *et al.*, 2005).

Clone	n	Incidence (%)	Mean ESI±ES	Mean ISI±ES	Classification
ICS 95	29	100	1.4±0.57 a	0.8±0.45 a	Resistant
CCN 51	30	100	2.9±0.34 bc	3.2±0.45 b	Resistant susceptible
TSH 812	27	100	3.5±0.44 bc	3.6±0.43 b	
ICS 60	27	100	3.4±0.38 bc	3.6±0.38 b	
EET 8	25	100	3.8±0.54 cd	4.1±0.37 bc	
UF 613	30	100	3.8±0.38 d	4.2±0.47 bcd	Susceptible
IMC 67	30	100	2.9±0.41 bc	4.4±0.38 cd	
CAP 34	27	100	4.5±0.43 d	4.6±0.44 d	
ICS 40	26	100	4.4±0.44 d	4.6±0.38 d	
TSH 565	29	100	4.6±0.51 d	4.8±0.41 d	
ICS 1	27	100	4.7±0.37 d	4.9±0.37 d	

ESI, external severity index; ISI, internal P.

Means followed by same letter do not differ statistically by the method of Tukey ($P \leq 0.05$), n = experimental units. ES, standard error.

TABLE 8. Incidence, internal and external severity of *M. roreri* and classification of regional *T. cacao* clones in Rionegro (Santander) for 8 weeks, following the internal severity index (ISI) proposed by monilia (Phillips-Mora *et al.*, 2005).

Clone	N	Incidence (%)	Mean ESI±ES	Mean ISI±ES	Classification
SCC 56	28	100	2.5±0.43 a	2.5±0.45 a	Moderately susceptible
SCC 64	26	100	2.7±0.39 ab	2.9±0.43 ab	
SCC 52	30	100	3.4±0.41 b	3.2±0.39 ab	
SCC 91	29	100	3.4±0.38 bc	3.3±0.41 ab	
SCC 76	27	100	3.8±0.40 bc	3.6±0.34 b	
SCC 41	27	100	3.6±0.42 bc	3.8±0.39 bc	
SCC 79	26	100	3.8±0.45 bc	3.9±0.46 bc	
SCC 77	29	100	4.5±0.46 c	4.3±0.37 c	
SCC 46	29	100	3.8±0.36 bc	4.3±0.45 c	
SCC 61	26	100	4.6±0.40 c	4.6±0.34 c	Susceptible
SCC 80	26	100	4.0±0.47 bc	4.8±0.33 cd	
SCC 45	28	100	4.7±0.30 c	5.0±0.08 d	
SCC 70	30	100	4.3±0.35 bc	5.0±0.10 d	

ESI, external severity index; ISI, internal P.

Means followed by same letter do not differ statistically by the method of Tukey ($P \leq 0.05$), n = experimental units. ES, standard error.



FIGURE 1. Behavior of the clones ICS 95, IMC 51 and CCN 67 under three different environmental conditions.

of the host to favor their growth and reproduction. That is when the green islands form on the senescent plant material, in order to ensure their survival (Hammond-Kosack and Jones, 1997).

In the specific case of a limited area or minor external damage to the fruit skin, reduction of the amount of inoculum in the environment is guaranteed. However, estimating the amount of affected tissue is generally a good estimate of the amount of the pathogen. But the amount of the pathogen is not just dependent on the level of cultivar resistance, since there are other factors such as interference between plots, the relationship between symptoms of the disease, the number and density of inoculum of the pathogen, and precocity and habitat of the host (Ribeiro *et al.*, 2001). This could elucidate the ICS 95 clone, where this material under the conditions of the E.E. La suiza showed a small affected area, but with full internal tissue damage (Fig. 2),

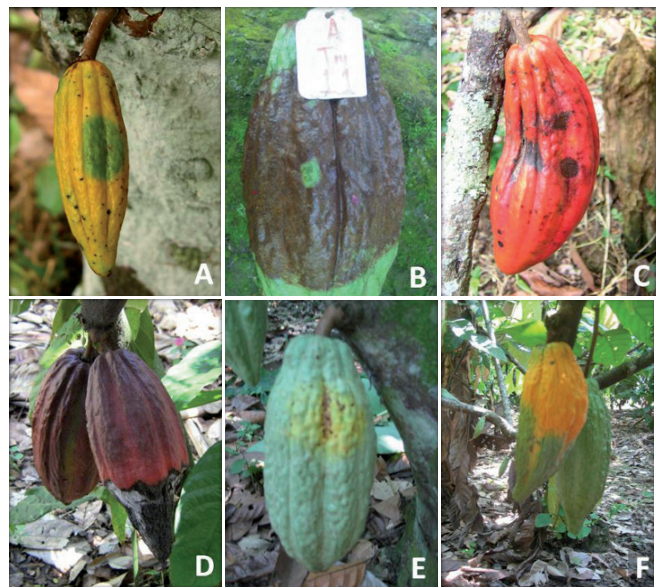


FIGURE 2. Atypical symptoms of moniliasis of cacao. A, green island; B, fruit affected by *Phytophthora* sp. (chocolate spot) and *M. roreri* (green island); C, subsidence; D, wilted area affected by the pathogen; E, green ripening; F, green ripening.

in contrast to that observed at the Villa Monica farm and Santa Elena (Fig. 2).

According to the above, one can conclude that clone ICS 95 has partial resistance, where the expression of resistance genes can be modified by the action of other genes (epitaxis), the state of development of plant tissue or the environment. In the atmosphere, the temperature plays an important role, which has been reported by several authors, where the expression of certain genes depends on the temperature at which they are exposed. This is the case of genes L1, L3, L7, L8, L10 and L11 in flax for the rust *Melampsora lini* (Islam and Mayo, 1990), Sr6 in oats for stem rust, LR6 and Lr17 in oats for leaf rust (Browder, 1985), among many other reported genes (Ribeiro *et al.*, 2001).

Significantly, the behavior of clone CCN 51, which is the product of crossing ICS 95 X BMI 67, can show partial resistance to *M. roreri* as an inherited characteristic. In the case of clone ICS 95, Johnson *et al.* (2009) highlighted in their study the behavior of these materials, and their source of resistance displayed on TSH materials. This shows that resistance is an inherited trait associated with resistance.

Although partial resistance was observed in clone ICS 95 at the three experimental sites (Tab. 9), and to a lesser extent in CCN51 and CAU materials under the conditions of the Villa Monica farm and Santa Elena (Tab. 9), the imple-

TABLE 9. Classification of the reaction of different universal cacao materials to *M. royeri* at the three experiment sites and in other areas as reported in the literature.

Clones	Behavior			Classification according to the literature
	Santa Elena farm (300 m a.s.l.)	La Suiza Station (400 m a.s.l.)	Villa Monica farm (900 m a.s.l.)	
ICS 95	R	R	R	MR*
CCN 51	R	MR	MR	MS*;MR**
ICS 39	MR	S	MR	S*;S**
TSH 565	MS	S	S	S*,S**,S***
IMC 67	MR	S	S	S*
EET 8	-	S	MS	S*
UF 613	-	S	MR	-
ICS 1	-	S	MS	S*,S**,S***
CAP 34	-	S	-	S*
ICS 60	-	S	-	S*
ICS 40	-	S	-	S*
TSH 812	-	MS	-	-
ICS 6	-	-	MS	-

R, Resistant; MR, Moderately resistant; MS, Moderately susceptible; S, Susceptible.

* Rodríguez and Medina, 2005; ** Aránzazu, 2003; Phillips-Mora *et al.*, 2005 .

mentation of a process of improvement by conventional methods is a slow process. Furthermore, although there is much genetic variability of clonal material, the majority of improvement projects have used a reduced genetic base (Motamayor *et al.*, 2002; Kuhn *et al.*, 2003). This is why the selection of new materials with resistance to monilia with current methodologies is a challenge. This is because field trials are expensive and take more than 5 years to find results (Kuhn *et al.*, 2003).

In the specific case of clone ICS 95, as a self-compatible material, it could be integrated into a genetic enhancement program by selecting filial generation 2, i.e. F2 the population, allowing the purification of the genes involved in the resistance of the materials. However, keep in mind that the disease resistance of *T. cocoa* is multigenic, and the associated phenotypic resistance is currently unknown, it is necessary to integrate molecular tools that reduce the cycle of selection in clonal materials with complete resistance to *M. royeri* (Kuhn *et al.*, 2003).

According to the above, it is important to focus research on the generation of materials resistant to *M. royeri* in three aspects: (1) Identification of genes or alleles responsible for partial resistance of clone ICS 95 and (2) Searching for new sources of resistance genes in hybrids that would expand the genetic base for resistance sources to moniliasis.

This, given the current trend to expand planting areas, it is a goal both globally and for Colombia as a control measure using genetic improvement programs for the control

of disease in cacao producing countries. Incorporation of resistant materials into production systems will result in an environmentally friendly and cost effective alternative, thus allowing the reduction of the spread of *M. royeri* (Johnson *et al.*, 2009), which is important because the disease is currently invasively and rapidly spreading throughout Mesoamerica.

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