Factors Affecting the Dynamics of Frosty Pod Rot in the Main Cocoa Areas of Santander State, Colombia

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Abstract

Frosty pod rot (FPR) caused by *Moniliophthora roreri* is the primary disease affecting cacao production in the major producing countries of the Americas and is one of the major threats to cacao worldwide. The incidence of FPR on clones with different levels of resistance was investigated in four localities of Santander State, Colombia, between July 2013 and May 2015. Dynamics of diseased pods were modeled using boosted regression trees, a machine learning technique that allows regressions to be performed without prior statistical assumptions. The results suggested that FPR epidemics varied according to plot location, clone, weeks of observation, and total pods produced. Dynamics in the phenology of pods had an effect on the epidemics, and this dynamic could

partially explain the difference in resistance among clones. Although not total, partial resistance of ICS 95 was confirmed. An important wilt effect was observed, particularly in the resistant clones; consequently, differences in harvested pods were not significant among clones. Pod stripping remains a good practice for the management of the disease and this practice could also have an effect on the pod dynamics and wilt phenomenon.

Keywords: boosted regression tree, disease development and spread, disease resistance, epidemiology, frosty pod rot, fruit production, *Moniliophthora roreri*, *Theobroma cacao*, trophic interaction.

Frosty pod rot (FPR) caused by *Moniliophthora roreri* can result in a considerable reduction in cacao bean yield and is one of the most devastating diseases of cacao in intertropical America, including Colombia (Evans et al. 2003; Jaimes and Aranzazu 2010; Phillips-Mora and Wilkinson 2007). Although FPR is a relatively minor disease in global terms because of its specific location, the potential danger presented by the disease is enormous (Evans et al. 2003; Phillips-Mora and Wilkinson 2007). The disease is twice as destructive as black pod (*Phytophthora* spp.) and more difficult to control than witches' broom (*M. pernicioso*) (Phillips-Mora and Wilkinson 2007). In Colombia, FPR is the most damaging disease for cacao production, with losses exceeding 40%, depending on crop management (Cubillos 2017; Jaimes and Aranzazu 2010).

M. roreri is a hemibiotroph and attacks only the fruit of species of the genera *Herrania* and *Theobroma*, causing internal and external pod damage that results in total pod loss (Phillips-Mora et al. 2007). FPR development can be lengthy, progressing over the 5 or 6 months required for pod development and ripening (Bailey et al. 2013). Under favorable conditions, the disease can occur 50 to 70 days after infection, beginning as expanding brown lesions on the pod surface, with the development of white mycelia and conidia 5 days later (Jaimes and Aranzazu 2010).

Current management strategies for FPR are primarily agronomic practices such as pod stripping (removal of infected pods) or pruning, combined with improved varieties and possibly biological

control (Ali et al. 2015). However, some of these measures have been effective only on an experimental scale (Phillips-Mora et al. 2007), and some tolerant clones show increases in disease losses as they age (Ali et al. 2015). However, efficient use of these management practices as part of an integrated management program requires prior knowledge of the host–parasite–environment interaction that governs disease development (Mouen Bedimo et al. 2007).

In Costa Rica, a conceptual model based on management and economics with field dynamics was developed (Leach et al. 2002). The aim of the model was to evaluate net returns of different management strategies for Central American farmers, and the efficiency of frequent pod stripping was demonstrated. This strategy is applied in Colombia. In Tabasco (Mexico), FPR epidemics are also associated with the number of fruit, suggesting that each epidemic must have independent integrated actions to manage and reduce FPR incidence (Torres de la Cruz et al. 2011). In that study, high levels of FPR damage were associated positively with low temperature (20 to 26°C) and relative humidity higher than 90% during the 49 days prior to the onset of symptoms (Torres de la Cruz et al. 2011). In Colombia, the FPR cycle can begin at any time because the source of primary inoculum is present throughout the year and is favored by the rainfall distribution throughout the year; the FPR cycle is positively correlated with rainfall that occurs 2 months before an epidemic (Merchán 1981).

A mixture of susceptible and resistant plants has the potential to reduce disease occurrence in natural and agriculture ecosystems (Severns et al. 2014). Although no cacao varieties have complete resistance, some clones such as ICS 95 or the newly selected varieties from Centro Agronómico Tropical de Investigación y Enseñanza demonstrate partial resistance against FPR (Ali et al. 2015; Phillips-Mora et al. 2005), which varies depending on pathogen aggressiveness (Phillips-Mora et al. 2005) and the environment (Jaimes et al. 2011). Moreover, varieties selected for disease tolerance in one location can be susceptible to the same disease in another location. Hence, studies of FPR epidemics that account for different environments and cacao varieties are required to understand their influence on the temporal and spatial dynamics of a

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disease outbreak. Therefore, the objectives of this research were to monitor temporal dynamics of FPR in several cacao clones with different levels of resistance at four localities in Santander State (Colombia) and to determine the relationship between FPR epidemics and environment.

Three primary scientific questions were addressed. (i) Which genotypic, environmental, and production factors are important in the development of FPR epidemics? (ii) Is clone behavior stable across

environments? (iii) What is the effect of yield on the disease expression?

Materials and Methods

Experimental locations and design. The study was conducted in four plots in four locations in Santander State (Colombia): three commercial plantations (Cimitarra, El Carmen, and San Vicente) and one research station in Rionegro (Fig. 1). The five cacao clones

Fig. 1. Experimental plots were located in four localities of Santander State, Colombia: La Suiza in Rionegro, El Trebol in Cimitarra, El Venado in El Carmen, and El Libano in San Vicente.

ICS 95, ICS 60, and ICS 39 (Imperial College Selection), CCN 51 (Colección Castro Naranjal), and SCC 61 (Selección Colombia Corpoica) with different levels of resistance were selected to analyze disease progress over time. ICS 95 is a resistant clone, CCN 51 and ICS 60 are moderately resistant clones, and SCC 61 and ICS 39 are susceptible clones (Jaimes et al. 2011). Clones CCN 51, ICS 95, and SCC 61 were planted in all four locations; ICS 39 was in El Carmen and San Vicente; and ICS 60 was in El Carmen and Rionegro. The plot in Rionegro was an experimental plot with four blocks.

Criteria for selection of the plots were (i) at least three of the five cacao varieties were planted in plantations located in each location, (ii) the presence of FPR, (iii) the same general crop agronomic management practices were used (i.e., pruning, fertilization after pruning according to the onset of rainfall, removal of diseased fruit every 2 weeks, weed control, and opportune harvest of fruit), and (iv) each location needed to be representative of a different environmental condition of Santander State. All localities have a tropical forest climate characterized by monthly rainfall above 60 mm throughout the year, annual rainfall exceeding evapotranspiration, and no monthly average temperature below 18°C (Table 1). Cimitarra and Rionegro have relatively high mean temperatures (27.6 and 26°C, respectively) but differ in annual rainfall, with Cimitarra being much wetter on average (annual rainfall = 2,766 mm). El Carmen and San Vicente have relatively low mean temperatures (24.5 and 25.3°C, respectively) but El Carmen is much wetter on average than San Vicente (2,156 and 1,820 mm, respectively).

Sampling plots were chosen in commercial plantings, except in Rionegro, which is the experimental farm of Agrosavia, formerly Corpoica (Table 1). Five randomly selected, productive adult plants of each clone were marked per sampling location, except in Rionegro, where five plants were marked in each of the four blocks.

Disease assessment. Cacao fruit were classified into cherelles (fruit less than 2 months old) and pods (fruit more than 2 months old). The age of the pods was estimated based on their size, based on the results of an earlier study (Jaimes et al. 2011). To analyze disease progress over time for each marked plant, we recorded pod status at intervals of 15 days between July 2013 and July 2015 (two production periods of 12 months). Three categories of pods were differentiated: pods between 2 and 4 months old, pods over 4 months old (large pods [LPo]), and diseased pods (DPo; pods with advanced symptoms of FPR). DPo were harvested after each counting. On the same dates, fruit with cherelle wilt (Wi; fruit less than 2 months old that died) were counted and removed. Mature pods were harvested but their number was recorded only in the four blocks of Rionegro; farmers collected the mature pods in their plots. At each sampling date, the variable “total pods” (TPo) was defined as the sum of pods on a cacao tree (pods between 2 and 4 months old, LPo, and DPo) and did not include cherelles.

However, final counts were calculated by tallying each fruit produced by a cacao tree and categorizing them as either (i) diseased and removed (SDPo = sum of DPo), (ii) mature and harvested (SHPo = sum of harvested pods), or (iii) wilted and removed (SWi). Because harvested pods were recorded only in Rionegro, we could define the fate of each fruit produced only at this location. For all sites, the sum of all the produced fruit (i.e., TPo + Wi) corresponded to the potential production of each cacao tree during 1 year of production (i.e., one production period, from July to June). The relationship among all the variables defined is shown in Figure 2.

Statistical analyses. To investigate the clonal effect (four clones) on produced fruit (STPFR) and SHPo, we analyzed temporal variations in production among clones, focusing on one location (Rionegro). Four negative binomial generalized linear models (GLM) (Zuur et al. 2013) were constructed to assess the effects of production period and clone on each type of fruit produced by a cacao tree in each of the two production periods in the four blocks of Rionegro (SDPo, SHPo, SWi, and STPFR). The significance of the clone effects was tested by likelihood ratio tests of the models with and without the clone variable, using a 5% risk threshold ($P \leq 0.05$). Post hoc tests based on the Tukey’s honestly significant difference were used to compare estimated marginal means.

To quantify the contributions of environmental (location), genetic (clone), temporal (week), and total production (TPo) variables on the rate of epidemic development, we modeled the dynamics of DPo in all locations using boosted regression trees (BRT) (Friedman 2001). A BRT is a machine learning algorithm based on regression trees (Bhatt et al. 2013) that is increasingly used in ecological modeling (Bhatt et al. 2013; Elith et al. 2008). The flexibility of regression trees allows complex ecological responses to be modeled; for example, when multiple interactions occur. Boosting (iterative addition of regression trees) reduces the variance of predictions, whereas the cross-validation procedure introduces stochasticity to reduce overfitting.

First, we used a BRT to quantify the contributions of environment (location), genetics (clone), time (week), TPo, LPo, and number of Wi (model 1). In a second step, we removed the clone variable from the model to determine whether other variables compensated for the clone effect (model 2).

The first regression tree was constructed to maximize the homogeneity of the response variable within partitioned groups. Then, at each step of the iterative BRT procedure, a proportion of the residual data (defined by the “bag” setting) was randomly selected to construct a new regression tree. The boosting algorithm added the new regression tree to the linear combination of all regression trees. To evaluate the optimal number of BRT, a 10-fold cross-validation was used. The optimal number of regression trees was that which minimized the average hold-out residual deviance (Hijmans et al. 2017).

Because the response variable was a count, the number of DPo per cacao tree for every 14 days was modeled using a Poisson distribution. Only cacao trees with at least four pods were selected because we were interested in relating disease to multiple variables rather than in demonstrating the effect of a small number of pods on disease. We chose BRT model settings from the range recommended by Elith et al. (2008). The bag fraction, set to 0.75, determined the proportion of data to be selected at each step. The learning rate, set at 0.08 for both models, determined the contribution of each regression tree to

Fig. 2. Relationship among the variables used in the study of the used variables.

Table 1. Characterization of monitored plots for frosty pod rot dynamics; location in Santander State and average climatic conditions

Property	Location	Latitude (N)	Longitude (W)	Altitude (m)	Temp (°C) ^x	Rain (mm) ^y	RH (%) ^z
El Trebol	Cimitarra	06° 15.10'	73° 54.46'	390	27.6	2766	85
El Venado	El Carmen	06° 43.713'	73° 33.38'	315	24.5	2156	85
El Libano	San Vicente	06° 49.723'	73° 27.37'	757	25.3	1820	85
La Suiza	Rionegro	7° 22.200'	73° 10.65'	500	28	1700	87

^x Annual average temperature.

^y Annual rainfall.

^z Annual average relative humidity (RH).

the expanding model (Buston and Elith 2011). The “complexity parameter” of the regression tree, fixed at 2, determined the number of nodes in a regression tree, indicating that we fitted a model with up to two-way interactions.

The relative importance of each predictor and the contribution of interactions are provided for each model. The relative importance was based on the number of times a variable was selected for splitting, weighted by the squared improvement of the model following each splitting, and averaged over all regression trees (Friedman 2001; Williams et al. 2010). The partial dependence function shows the marginal effect of each variate on the count response after averaging the effects of all other variates (Bhatt et al. 2013).

The fitting of all models was performed using R 3.4.3 (R Core Team 2018). The MASS package (Venables and Ripley 2002) was used for the negative binomial GLM, the emmeans package (Lenth et al. 2019) and multcompView package (Graves et al. 2015) for post hoc tests, and the gbm (Greenwell et al. 2019) and dismo (Hijmans et al. 2017) packages for the BRT analysis. Graphs were done using ggplot2 (Wickham 2016).

Results

Visualization of the dynamics (all locations). FPR (DPo) was recorded on the different sampling dates at each of the four locations. DPo was low (mean of 0.7) and varied from 0 to 8 depending on location, clone, and production period (Fig. 3). The pattern of FPR symptoms varied as a function of location; for example, the first disease peak occurred approximately on the same date for the susceptible and moderately resistant clones (SCC 61, CCN 51, and ICS 60) in Rionegro but the dynamics of the disease were different in the other locations. The higher peak occurred earlier in El Carmen or occurred in the second part of the production period, depending on clone and production period.

Similarly, peak TPo occurred approximately in the same weeks for the different clones in any given location but these peaks differed among locations (Fig. 4). Nevertheless, between peaks, the cacao trees always bore pods. The distributions of Wi were approximately similar to those TPo, although the peaks in Wi were often between 1 and 2 weeks later. The proportions of Wi and TPo varied between clones. LPo was an indicator of pods near maturity, and the peaks for number of pods (TPo and LPo) were shifted in time according to the location.

Clonal differences in the fate of fruit (Rionegro). The fate of each fruit produced during a production period could be categorized as wilted (SWi), diseased (SDPo), or harvested (SHPo). The average number of fruit per category showed large differences among clones: CCN 51 and ICS 95 produced many fruit, of which most wilted (80 and 83%, respectively), whereas SCC 61 and ICS 60 produced few fruit, of which a smaller proportion wilted (35 and 44%, respectively) (Fig. 5). Negative binomial models demonstrated a significant effect of clone on the number of fruit in the three categories and on the total number of fruit produced (Table 2). Despite these effects, post hoc tests did not differentiate the clones for the quantities harvested (SHPo) (Table 3). The SDPo per cacao tree was significantly lower for ICS 95 than for ICS 60 and SCC 61; CCN 51 was intermediate. The SWi per cacao tree was significantly lower for SCC 61 than for ICS 60 and lower for ICS 60 than for ICS 95 and CCN 51. Overall, CCN 51 and ICS 95 produced more fruit than ICS 60 and SCC 61 (Table 3).

Modeling of DPo (all locations). Modeling of DPo was based on the data collected every 2 weeks in all locations. Model 1 included environment (location), genetics (clone), time (week), and the different variables Wi, LPo, and TPo. The final BRT model combined 1,800 regression trees and had a good predictive performance, with

Fig. 3. Mean of diseased pods (DPo) per cacao tree every 2 weeks, shown by clone (CCN 51, ICS 39, ICS 95, SCC 61, and ICS 60), location (Rionegro, Cimitarra, El Carmen, and San Vicente), week, and production period. Line type represents different production periods (A and B). Clones ICS 39 and ICS 60 were only recorded in two locations and not over the entire production period for ICS 60. Arrows indicate the maximum value of DPo during a production period.

a cross-validation correlation of 0.64. The mean residual deviance was 50% of the initial deviance (Table 4).

The two variables that most influenced the dynamics of FPR were TPo and clone, with the same relative influence of 29% (Table 4). Week, location, LPo, and Wi had less of an influence (between 6 and 15%). Production period had almost no relative influence (4%) but was involved in an interaction in combination with week (Table 5).

The relationship between the number of TPo and DPo was not linear. Indeed, TPo had a strong influence on DPo when few pods were on a cacao tree (less than 30) and then that influence reached a plateau (Fig. 6). The ICS 95 clone had the strongest negative influence on DPo, that of CCN 51 was intermediate, and ICS 39, SCC 61, and ICS 60 had a positive influence on DPo. The dynamics of FPR symptoms began with rapid growth in the first 10 weeks, followed by a plateau and a slow decrease. DPo differed among location, with San Vicente having the fewest and Rionegro having the most.

The interaction effects estimated in the BRT model showed that the largest interaction was between clone and TPo (Fig. 7). The number of DPo of CCN 51 and ICS 95 increased with the TPo until a total of 20 pods were on a cacao tree, whereas the other clones had a positive relationship between DPo and TPo until approximately 70 pods were on a cacao tree.

Modeling of DPo counts without the clone variable (all locations). Model 2 included environmental (location), temporal (week), and production (Wi, LPo, and TPo) variables. Dropping the clone variable led us to construct a model combining 1,500 regression trees. As a result, average residual deviance represented 57% of the initial deviance (Table 4). The percentage of deviance explained by model 1 and model 2 was 51 and 43%, respectively. Prediction performance was higher for model 1 than for model 2,

with a cross-validation correlation between predicted values and real values of 0.64 versus 0.51 for model 2.

The variables that most influenced the dynamics of FPR were TPo (33%) and the week (31%) (Table 4). The influence of the week

Fig. 5. Clonal differences in the fate of cacao fruit in Rionegro. Average number of fruit in each fate category—diseased, wilted, or harvested—per cacao tree per production period. One production period corresponds to 1 year of cacao production (from July to June).

Fig. 4. Mean of total pods (TPo) (bold), large pods (LPo) (light), and wilted pods (Wi) (dot) per cacao tree every 2 weeks shown by clone (CCN 51, ICS 39, ICS 95, SCC 61, and ICS 60), location (Rionegro, Cimitarra, El Carmen, and San Vicente), and week. Dotted vertical lines separate production periods.

effect was higher in model 2 than in model 1. All other variables had a relative influence in model 2 that was similar to that in model 1. LPo and Wi had a relatively small influence (10 and 7%, respectively). Maturation (based on LPo) had a positive, although slight, effect on DPo (not shown). Wi had a light but opposite influence depending on the model (Fig. 8). In model 1, when the clonal effect was included, Wi was associated with a high level of disease. In model 2, when the clonal effect was not included, a high number of Wi was

Table 2. Clonal differences in the fate of cacao fruit in Rionegro^x

Response variable, model ^y	Df	2× Log	Test	df LR	Stat	Pr(χ) ^z
SDPo						
1) Period	158	-1,153	1 versus 2	3	10.7	0.013*
2) Period + clone	155	-1,143	...	...	...	...
SWi						
1) Period	158	-1,546	1 versus 2	3	123.7	0.000*
2) Period + clone	155	-1,422	...	...	...	...
SHPo						
1) Period	158	-966	1 versus 2	3	7.9	0.048*
2) Period + clone	155	-958	...	...	...	...
STPFR						
1) Period	158	-1,647	1 versus 2	3	74.3	0.000*
2) Period + clone	155	-1,572	...	...	...	...

^x Likelihood ratio tests comparing negative binomial models with and without the clone effect for each of the three fruit fate categories and for the number of fruit produced (sum of the three categories). One production period corresponds to 1 year of cacao production (from July to June). Abbreviations: df = degree of freedom, 2× Log = logarithm of likelihood (×2), Test = comparison of the two models (χ² test), df LR = degree of freedom of likelihood ratio test, Stat = value of the χ² test, and Pr(χ) = probability associated to the χ² test.

^y SDPo = sum of the diseased pods, SWi = sum of the wilted cherelles, SHPo = sum of the harvested pods, STPFR = sum of the total fruit, and Period = production period of 12 months (from July to June).

^z Significance code: * if *P* < 0.05.

Table 3. Clonal differences in the fate of cacao fruit in Rionegro^y

Response variable, clone ^z	Response	SE	Asymp LCL	Asymp UCL	Group
SDPo					
CCN 51	13.6	1.8	10.4	17.6	AB
SCC 61	15.0	2.0	11.6	19.5	B
ICS 95	8.9	1.2	6.8	11.6	A
ICS 60	16.2	2.1	12.5	21.0	B
SWi					
CCN 51	81.8	9.4	65.3	102.5	C
SCC 61	11.7	1.4	9.2	14.9	A
ICS 95	70.7	8.2	56.4	88.7	C
ICS 60	18.4	2.2	14.5	23.2	B
SHPo					
CCN 51	5.6	0.8	4.2	7.5	A
SCC 61	5.6	0.8	4.2	7.6	A
ICS 95	8.4	1.2	6.4	11.2	A
ICS 60	8.7	1.3	6.5	11.5	A
STPFR					
CCN 51	103.1	9.6	85.9	123.7	B
SCC 61	34.8	3.3	28.8	42.0	A
ICS 95	94.2	8.8	78.5	113.2	B
ICS 60	44.8	4.2	37.1	53.9	A

^y Estimation of clonal effects using estimated marginal means from negative binomial models for each of the three fruit fate categories and for the number of fruit produced. Least-squares means with the same letter, for the same response variable, are not significantly different (*P* > 0.05). Response = estimated marginal means; SE = standard error; Asymp LCL and UCL = asymptotic lower and upper control limits, respectively; and Group = clones with the same letter were not significantly different (Tukey's test, 5%).

^z SDPo = sum of the diseased pods, SWi = sum of the wilted cherelles, SHPo = sum of the harvested pods, and STPFR = sum of the total fruit.

associated with a low level of disease, and the largest interaction detected concerned location and week (Table 5).

Discussion

Location was a major factor in the difference in dynamics of fruit production and in the dynamics of the disease (Figs. 3 and 4). Availability of fruit and humid climatic conditions are required to initiate an epidemic (Phillips-Mora et al. 2007). In the studied locations, pods were present all year long, although the quantity was variable (Fig. 4), which contrasts with the observations of Maddison et al. (1995) in Ecuador, where there were no pods for several months in the year. As reported by Merchan (1981), the FPR cycle can begin

Table 4. Boosted regression tree modeling of diseased pod (DPo) counts in all locations^y

Variables ^z	Relative influence of variables (%)	
	Model 1	Model 2
TPo	29	33
Clone	29	...
Week	15	31
Location	10	14
Wi	7	8
LPo	6	10
Period	4	4
Mean deviance	2.11	2.11
Residual deviance: mean	1.04 (49)	1.20 (57)
Mean cross-validation deviance	1.26 (60)	1.45 (69)

^y Relative influence of variables on DPo in the location-clone model (model 1) and in the location-production model (model 2). The relative importance was based on the number of times a variable was selected for splitting, weighted by the squared improvement of the model following each splitting, and averaged over all regression trees. The relative influence of variables is an estimate of the relative contributions of each variable to the model. It is an average of the number of times a variable is selected for splitting, weighted by the improvement to the model resulting of each split. These are presented as percentage. A higher percentage means a greater contribution of the variable to the model.

^z TPo = total pods, Wi = wilted cherelles, LPo = large pods, and Period = production period of 12 months (from July to June).

Table 5. Boosted regression tree (BRT) modeling of diseased pod counts in all locations^z

Variables	Location	Clone	Period	Week	TPo	Wi	LPo
Model 1							
Location	0	3	32	168	1	9	1
Clone	...	0	1	13	267	30	29
Period	...	...	0	146	0	3	0
Week	...	...	...	0	27	4	31
TPo	...	...	...	...	0	26	96
Wi	...	...	...	...	...	0	11
LPo	...	...	...	...	...	...	0
Model 2							
Location	0	...	36	228	9	2	5
Period	...	...	0	48	0	2	0
Week	...	...	...	0	75	3	51
TPo	...	...	...	...	0	68	105
Wi	...	...	...	...	...	0	27
LPo	...	...	...	...	...	...	0

^z Size effects of interactions between environmental (location), genetic (clone), temporal (week), and production (number of total pods [TPo], cherrille wilt [Wi], and large pods [LPo] on a tree) variables in model 1 and, in model 2, between environmental (location), temporal (week), and production variables, including TPo on a cacao tree, number of LPo (over 4 months), and number of Wi. Period = production period of 12 months (from July to June). A large size effect indicates a high interaction. Numbers represent a residual variance by pair of predictors (Elith et al. 2008); each number indicates the relative strength of interaction fitted by BRT; a residual variance of zero indicates that no interaction effects are fitted.

at any time in Colombia; however, the critical periods were associated with the periods of greatest fruit formation (Figs. 3 and 4), (Jaimes and Aranzazu 2010; Torres de la Cruz et al. 2011). Different environments could have been the major factor leading to the different dates of peak infection among locations. The final level of infected fruit did not reach 100%, as occurs in several countries such as Costa Rica (Leandro 2017). This could be due to specific climatic conditions, or perhaps pod stripping limited dispersion of secondary inoculum. Pod stripping is an agronomic practice often used by farmers to control the disease (Cubillos 2017) but was performed in our study to facilitate the counting of the pods in the different categories.

Clonal resistance was characterized in Rionegro by GLM and in all locations by the BRT model (Table 3; Fig. 6). Combined results showed that ICS 95 was resistant, CCN 51 was moderately resistant, and ICS 60 and SCC 61 were susceptible to FPR. According to Phillips-Mora et al. (2005), resistant clones such as ICS 95 have partial resistance, also called horizontal resistance. This type of quantitative resistance is characterized as nonrace-specific (Keane 2012), and this type of resistance trait in cacao can be environmentally sensitive (Young 1996). However, BRT modeling demonstrated both a clone and location effect (Table 4) but no interaction between these two variables (Table 5). The resistance level was the same regardless of the location for a clone. The clone effect interacted with the number of TPo (Table 5; Fig. 7) but the level of resistance apparently depended on fruit availability.

The study in Rionegro looking at the fate of the fruit showed differences among clones. The two clones classified as resistant or moderately resistant in our study (ICS 95 and CCN 51, respectively) produced many more fruit (Fig. 5) than the two susceptible clones (ICS 60 and SCC 61) (Table 3). Many of these pods died within 2 months (wilt). The more susceptible clones produced fewer fruit but many continued to grow, with some becoming diseased. In the

end, the number of pods harvested was relatively similar for the four clones (Table 3). Thus, in the conditions in Rionegro, the harvested production did not vary among clones, even with pod stripping. More studies of the wilt would be useful to better understand this

Fig. 7. Boosted regression tree modeling of diseased pod (DPo) counts with the clone variable in all locations. Predicted values of DPo are plotted depending on the two predictors with high interaction effect (clone and total number of pods on a cacao tree). Effects of all other predictors were set at their mean. Fitted functions correspond to prediction (effects of the other variables fixed to their mean) centered by subtracting the global mean.

Fig. 6. Boosted regression tree modeling of diseased pod (DPo) counts with the clone variable in all locations. Partial dependence functions of model 1 showing the relationships between the predicted number of DPo and the four most influential variables (total pods, clone, week and location). Fitted functions correspond to prediction (effects of the other variables fixed to their mean) centered by subtracting the global mean. The dotted line corresponds to the same function smoothed.

phenomenon (Valle et al. 1990). Because wilt affected a large proportion of young cacao fruit (Fig. 5), reducing wilt to increase yield is of interest.

Other studies used different models to describe FPR epidemics but location or clone effects were usually not considered (Bailey et al. 2013; Leach et al. 2002). The first study (Bailey et al. 2013) was conducted at the molecular level. The second study (Leach et al. 2002) was designed to evaluate net returns of various management strategies to farmers. The purpose of our study was to better understand the role of environment and genetics on FPR epidemics. We observed that the clone and location effects were of similar importance on FPR epidemics but did not interact (Tables 4 and 5; model 1), indicating that clones performed consistently in the different localities. Although all plots used similar management practices, FPR levels decreased from Rionegro to El Carmen to Cimitarra, and to San Vicente (Fig. 6). As stated above, these localities share a tropical forest climate with slight variations in their mean temperature and rainfall (Table 1). Because climate did not explain all of the differences in disease progress between the locations, other factors such as landscape context (for instance, what other crops are in the vicinity of cocoa plantations or landscape relief) could explain these differences (Holdenrieder et al. 2004).

The relationship between the number of DPo and TPo was not linear (Fig. 6). A threshold occurred after which the increase in FPR was slow; this threshold depended on the clone (Fig. 7). The rate of FPR development increased when there were more pods. This suggests that, when there are just a few pods, the density of the spores is too low in the environment for an outbreak to start. After a threshold, the rate of increase in FPR slows because there are too many pods and the spores cannot infect all of the pods in the field.

The effect of week on FPR development was relatively low according to model 1 (Table 4). However, the observed variations of week on FPR development appeared important based on Figure 3; however, these variations were most likely due to the effect of fruit dynamics (TPo). This variable (TPo) was, with clone, the primary source of variation in FPR. However, 50% of the deviance was unexplained in model 1 (Table 4), highlighting the importance of the variation among trees and the possible existence of explanatory variables not included in the study. No strong link was detected between Wi dynamics and DPo dynamics (Table 4). Wi is primarily due to competition between leaf flushing and pod production (Melnick 2016).

To determine whether the clone effect was linked to environmental, production, or temporal effects, we compared BRT models with and without the clone effect (model 1 and model 2, respectively). Model 2 was slightly less predictive than model 1 (Table 4) but a part of the clone effect was explained by the variables TPo and weeks. The part of the clone effect not compensated for by the environment or production in time could be due to intrinsic resistance.

Another part of the clone effect was primarily explained by the week effect. This suggests that the availability of pods could explain a part of the genetic effect. In other words, there is a genetic effect on the pod dynamics (quantity of pods in time) on trees and this pod

dynamics could explain a part of the clone effect on FPR such as for FPR in Ecuador (Maddison et al. 1995) or *Phytophthora* pod rot in Cameroon (Efombagn et al. 2004). Peaks of pod production depended on the clone (Fig. 4) and could induce differences in disease dynamics.

The number of Wi did not have a strong relationship with the number of DPo in either model (Table 4) but the relationship between Wi and FPR was not the same for the two models (Fig. 8). Without the clone effect, trees with a high level of Wi had fewer DPo. This result confirmed dynamically that clones with increased resistance to FPR were clones with increased numbers of Wi (Fig. 5). This relationship should be confirmed with additional clones, and the mechanisms that could explain this phenomenon should be investigated.

Although currently recommended management practices were applied in this study, epidemics continued to occur and more DPo were recorded than harvested pods (Fig. 5). For this reason, improved integrated disease management approaches involving better agronomic practices, combined with improved planting materials, are needed (Ali et al. 2015).

Additional studies are required to evaluate the potential benefits of zoning cacao material according to its locality-dependent behavior (both the productivity and resistance to FPR) and tree spatial structure (i.e., the positioning of trees in plots) (Gidoïn et al. 2014). In this study, FPR development was lower in San Vicente and it could be possible to plant mainly productive clones in this area but not necessarily resistant clones. In the other locations, it is important to plant only resistant clones. Additionally, research defining the quantitative genetic control of FPR disease resistance, including quantitative trait loci mapping of resistance, is required to better understand the mechanisms involved. To determine the architecture of resistance at the gene level, it is also important to take into account the genetic diversity of *M. royeri*, because it was shown that *M. royeri* is genetically very diverse in Colombia (Jaimes et al. 2016). Once better defined, it should be possible to determine how resistance is affected by different traits, particularly those traits linked to fruiting periods and duration and wilt behavior (Efombagn et al. 2004).

Acknowledgments

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Fig. 8. Boosted regression tree modeling of diseased pod counts (DPo) in all locations. Dependence plots showing the relationships between the predicted number of DPo and cherelle wilt for model 1 and model 2 (effects of all other predictors were set at their mean). Predictions were centered. Dotted lines correspond to the same function smoothed.

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