Research Article

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Tree spacing impacts the individual incidence of *Moniliophthora roreri* disease in cacao agroforests

Marie Ange Ngo Bieng,[a,b,c*] Laudine Alem,[a,b,c] Chloé Curtet[a,b,c] and Philippe Tixier[c,d]

Abstract

BACKGROUND: Using conventional pesticides in crop protection has raised serious environmental concerns and there is therefore a need for integrated pest management (IPM) methods. In this paper, we found that the spacing of trees can impact disease, which could result in a reduction in pesticide applications and may act as a potential IPM method. We studied Frosty Pod Rot (FPR) in 20 cacao agroforests in Costa Rica (Upala region).

RESULTS: Using a generalized linear mixed model, we analyzed the impact of the neighborhood composition and distance from a studied cacao individual on its individual FPR incidence. We found that the number of cacao tree neighbors in a radius of 3.7 m and the number of fruit trees in a radius of 4.3 m had a significant negative influence on the incidence of FPR on individual cacao trees. Moreover, cacao tree neighbors had the most significant local influence compared to the neighborhood of other taller categories such as fruit or forest trees.

CONCLUSION: The mechanisms involved are related to the barrier effect, due to the effectiveness of the cacao tree's architecture as an efficient barrier against FPR spore dispersal. This paper provides new insights into optimization of the spatial environment around each host as an original IPM method.

Supporting information may be found in the online version of this article.

Keywords: local environment; individual plant scale; frosty pod rot; *Theobroma cacao*; barrier effect; spore dispersal

1 INTRODUCTION

There is an increasing need for more sustainable management of pests and diseases in cropping systems. Indeed, the continuous use of pesticides has resulted in great harm to agriculture, the environment, and human health.[1–5] Consequently, several integrated pest management (IPM) methods have been developed in order to ensure, amongst other things, more ecological management of pests and diseases as a way of reducing the systematic use of pesticides. IPM methods include biological control (use of natural enemies, bio-pesticides and bio-stimulants), genetic control (use of resistant varieties), cultural control (trap crops, adjusting the timing of planting or harvesting, sanitary harvesting, clearing and thinning), and adding plant diversity in cropping systems (growing two or more cultivars or species, hosts and non-hosts of a particular pathogen).[6–9]

Only a few studies have investigated how the spatial organization of diverse plants (hosts and non-hosts combined) alters pest and disease incidence within agroforest plots.[10–12]

The effects of the spatial organization of plants on pest and disease regulation include the physical barrier effect. The physical barrier effect is the restriction of pathogen spread by the presence of a physical barrier.[4] This physical disruption of pathogen spread may be derived from the effects of the canopy architecture on dispersal processes; canopy porosity may be related to dispersal mechanisms. In our context, canopy porosity may be defined as the ability of a pathogen to pass through the canopy. Canopy porosity depends on the density and spatial arrangement of tree organs (branches, leaves, trunk).[5,13] Thus, the spatial arrangement of non-host organs may act as a barrier to the dispersal of a pathogen between host organs.[13] In such a case, the non-host organs of a host plant may also participate in the barrier effect, and allow host organs to escape infection.

In multi-species systems, the effects of the spatial organization of plants on pest and disease regulation may also include a reduction in the connectivity between host plants. The reduction in connectivity in multi-species systems does not take into account only the

* Correspondence to: MA Ngo Bieng, CATIE, UR F&S, 30501 Turrialba, Costa Rica. E-mail: marie-ange.ngo_bieng@cirad.fr.

a CIRAD, UMR SYSTEM, 30501 Turrialba, Costa Rica

b CIRAD, UMR SYSTEM, F-34398 Montpellier, France

c CATIE, 30501 Turrialba, Costa Rica

d CIRAD, UPR GECO, F-34398 Montpellier, France

physical disruption of pathogen spread, but also the host/non host status of the different plants.[4,14,15] In this case, pathogen spread is decreased following a decrease in host abundance, due to the interception of infectious spores by non-host plants.[4,16,17] Consequently, the reduction in connectivity between host plants may reduce disease incidence in accordance with the spatial organization of non-host individuals around host individuals.

The spatial organization of the plant community also alters the microclimate (light, humidity, wind), which may influence the epidemiology of pests and diseases.[18] These processes are particularly important in agroforestry systems in which the spatial structure may be involved in pest and disease regulation.[10,11,18] Tropical agroforestry systems are often multi-species and multi-strata cropping systems, where the commercial crops are located in the understory, and associated with a diversity of companion trees presenting a set of spatial organizations.[12] The composition and the spatial organization of trees in the higher vertical stratum (associated shade trees) modify microclimatic variables and the quantity and the quality of transmitted light available in the lower stratum.[12,18,19] These effects of associated shade trees are known as 'shade effects', which are an important issue in pest and disease management in agroforest systems.

More generally, and not only related to the spatial organization of plants in a multi-species system, the introduction of plant diversity in a cropping system may also decrease host abundance and thereby decrease disease intensity following a decrease in the quantity of inoculum. This relationship between host abundance and inoculum quantity is known as the resource hypothesis, which predicts that fewer resources induce reduced pathogen incidence.[4,18,20]

In this paper we investigated the effect of the spatial organization of individuals as a potential IPM method in cacao agroforestry systems. The cacao tree (*Theobroma cacao* L.) is a native species of the understory of Central and Latin American forests, and a member of the Malvaceae family.[21–23] It is a tropical perennial cash crop of economic significance around the world.[24] The fruits, called pods, contain beans, which are the raw material for the chocolate industry.[25,26] The disease studied here was Frosty Pod Rot (FPR), a major threat to cocoa production in Latin America and, due to its invasive nature, a threat for world cocoa production.[21,27] FPR is probably the most severe cacao disease as it causes substantial losses, averaging 30% and up to 100% under favorable conditions.[21] FPR is a fungal disease caused by *Moniliophthora roreri*.[28–30] *M. roreri* is a basidiomycete of the family of the Marasmiaceae. The disease cycle is linked to the biological cycle of this hemibiotrophic fungus.[30–33] In the first biotrophic phase of germination and infection, *M. roreri* spores penetrate, germinate and invade the pod cells, causing pod malformations. Thus the cycle progresses to the second necrotrophic phase, where cell death leads to the mummification of the pods, followed by spore production on the pod surface (white mycelium similar to frost). Pods remaining suspended in the cacao tree crown can release a huge number of spores (44×10^6 cm^{-2}), which are viable for up to 9 months and can be detected 1 km from the nearest cocoa field.[34] *M. roreri* spores are readily wind-dispersed over large distances ranging from dozens of meters to more than a kilometer.[30,35] The biotrophic and necrotrophic phases are favored by low ventilation, free water, and high relative humidity. The dispersal of spores is greater under conditions of low air humidity and strong wind speeds, because dry spores are lighter.

Optimization of the spatial environment around each cacao tree may be an efficient way of altering microclimatic conditions and promoting the barrier effect against spore dispersal. In previous studies, we highlighted interactions between the spatial structure of forest trees (the highest vertical stratum) and FPR disease incidence in cacao agroforest systems in the Talamanca region of Costa Rica.[10,12] Indeed, greater pest and disease intensity was found in a plot with an aggregated spatial organization of forest shade trees, in comparison with plots with random or more regular spatial organization of forest trees. We made the assumption that different spatial organizations of shade trees define different local neighborhoods which impact the biological cycle of FPR through microclimatic variations propitious to the dynamics of FPR. In highly complex cacao plots, each tree has a unique local environment. It appeared interesting to go further, i.e. on an individual plant scale, to ascertain the effects of the neighborhood on individual disease incidence.

In this paper, we analyzed the effect on FPR incidence of the plant composition and structure (spatial neighborhood and tree) within a cacao agroforest. To do this, we measured the species composition and the spatial organization of 20 plots of cacao agroforestry systems in Costa Rica, and we assessed the incidence of FPR on 894 cacao trees. We distinguished between the effects of forest trees, fruit trees, banana plants, and cacao trees (from the highest to lowest stratum, respectively). The analysis was based on two steps: (1) determination of the range of distances at which the number of trees of a given stratum explained most of the FPR incidence on each cacao tree, and (2) testing of the significance of those effects using a generalized linear mixed model. We discuss our findings in terms of potential mechanisms involved in disease regulation. Lastly, we address the prospects for improving our knowledge of the mechanisms involved in order to take into account the optimization of spatial organization as a lever in IPM.

2 MATERIAL AND METHOD

2.1 The study plots: cacao agroforests in the Upala region of Costa Rica

We studied 20 plots of cacao-based agroforests in Costa Rica, in the region of Upala (Fig. 1, see all coordinates in Table S1 in the Supporting Information). Upala has a tropical humid climate with a 3-month dry season (February, March and April), and a 9-month rainy season. The mean annual precipitation is 2323 mm (131.4 mm mean for the 3-month dry season and 2192.4 mm for the 9-month rainy season) and the mean daily temperature is 26.5 °C. In this region, as in the Talamanca region in Costa Rica, FPR has led to the decline of cacao yields and the production area since the 1970s.[36]

In each plot, we studied a 40 m × 40 m area. The studied areas (Table S1) were located as much as possible in the centre of the agroforest plot to minimize border effects. Following a survey conducted with local farmers in May 2015, we selected the 20 plots under the same environmental conditions and with the following characteristics: managed (i.e. not abandoned), and under different rates of sanitary harvesting (removal of diseased pods, from every 3 days to twice a month), planted with cacao varieties not resistant to FPR and comprising local susceptible varieties, and organic, i.e. with no chemical inputs (fertilizers, herbicides, pesticides or fungicides).

2.2 Measurements of the plant community structure

In June and July 2015, we mapped each plot and characterized the composition of its vegetation. The Cartesian coordinates of

Figure 1. Study site. The plots were located in the Upala region of Guanacaste, north-western Costa Rica. The GPS coordinates of the 20 plots set up in that region are provided as Supporting information (Table S1).

Table 1. Number of individuals in each category of plants in the 20 study plots.

Plant category	Number of individuals
Assessed cacao trees	894
Total number of cacao trees	1722
Forest trees	499
Fruit trees	90
Banana plants	184

each plant (all cacao individuals and, for other species, individuals of 2 m tall or over) were recorded in each plot using a theodolite (Leica Builder 409; Leica Geosystems, Heerbrugg, Switzerland). Theodolites are distance measurement instruments with very high precision, evaluated at 2 mm + 2 ppm (2 mm every km). We assumed that our distance measurements were precise to within a millimeter as we worked with small distances, shorter than a kilometer. More details can be found in the user manual (https://geog.sfsu.edu/sites/default/files/leica-builder-user-manual.pdf).

Plants were identified up to either the species or family level. Cacao trees were the first category. The plants associated with the cacao trees were then classified in three other categories: forest trees, fruit trees, and banana (see Table 1, with the number of individuals per category). As in our earlier work in cacao agroforests in Costa Rica[12] (see specifically figure P331 of the cited reference illustrating the vegetation layers), we wanted to take into account the different layers of these multi-strata systems, along with the different architectural features and level of shade intensity. The native forest trees were the tallest, forming the top vertical stratum and provided the highest level of shade. The intermediate vegetation layers were formed of fruit trees, and banana, which provided a more localized, closer and deeper shade. Cacao trees formed the layer of the understory.

2.3 Measurements of FPR disease incidence

Disease incidence was assessed over the same period, in June and July 2015, around the first pod production peak. Production peaks are periods when the number of mature cacao pods on cacao plants is expected to be optimum. We therefore expected to have the maximum resource and inoculum intensity within each selected plot. We counted the total number of pods and the number of pods showing FPR symptoms for a total of 894 assessed cacao trees, out of a total of 1722 cacao trees in the 20 plots. Thus, around 50% of the cacao trees, randomly chosen, were assessed within the study plots. The FPR symptoms included premature ripening or pod deformation, visible irregular dark brown lesions, up to the whole pod being mummified and covered with a white mycelium (Fig. 2). We defined FPR incidence as the percentage of pods showing FPR symptoms out of the total number of pods (%podFPR).

2.4 Statistical analyses

In this study, our aim was to determine the effect of neighboring plants on FPR incidence on individual cacao trees. We used a generalized linear mixed effect model (GLMM[37,38]) to explain FPR incidence as a function of the number of plants in a given radius around each of the assessed cacao trees. The number of plants was evaluated taking into account the four defined categories: cacao trees, forest trees, fruit trees, and banana. We used a binomial GLMM since initial FPR incidence, the percentage of damaged pods, typically follows a binomial distribution. We included two random factors on the intercept of the model: the plot and the total number of pods of each observed cacao tree. Having the plot as a random factor enabled us to take into account variability due to the variability of each plot: soil, landscape context, management applied, in particular sanitary harvesting. Having the total number of pods as a random factor enabled us to take into account the resource effect, i.e the positive correlation between the total number of pods and disease incidence. We carried out the analysis in two steps. First, we explored at what distance (radius in which plants were counted) each category of plants better predicted the FPR incidence of each cacao tree. We tested radii ranging from 0 to 10 m around each assessed cacao tree in 0.1 m steps. The difference in AIC[39] between the model with the number of plants in a given radius as a predictor and the null model was used as an estimator of the goodness of fit (we called this difference in AIC from the null model ΔAIC). The null model represented the variation in individual FPR incidence within 800 assessed cacao trees (excluding cacao trees close to the border, i.e. < 3 m), only taking into account the two random factors. For each category of plants, we selected the radius with the greatest ΔAIC, highlighting a major effect of the neighborhood in the selected category and of distance on individual disease incidence. Here, we used AIC as an indicator to identify the 'signal' of the response of disease incidence to the number of plants in a given radius. In the second step, we tested the significance of the effect of the four predictors (number of plants of each category of plants counted in the radius determined in the first step of the analysis) inside a complete model. All models were fitted with the 'glmer' function in the 'lme4' package,[40] in which the maximum likelihood of parameters is approximated by the Laplace method.[38] We verified that there was no over-dispersion in the model. The optimum model was obtained by using a backward model selection process (Drop1 function) to eliminate less significant variables.[41] All statistical analyses were performed with R 3.3.0.[42]

3 RESULTS

The number of cacao trees in a radius of 3.7 m (nca3.7) and the number of fruit trees in a radius of 4.3 m (nfu4.3) were the strongest

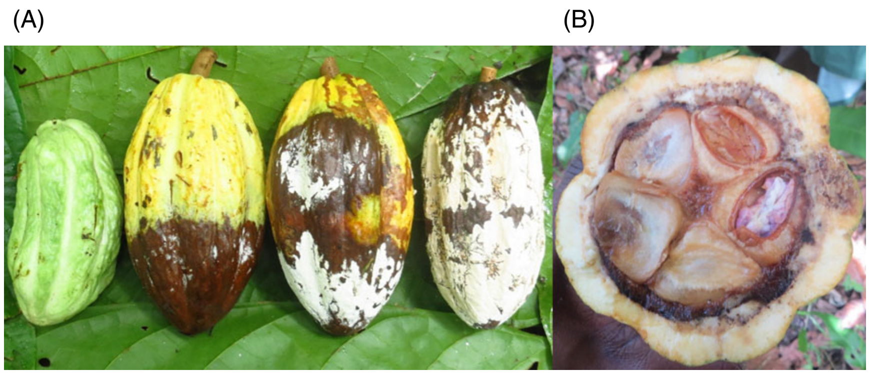

Figure 2. FPR symptoms. FPR symptoms include (A) pod deformation, premature ripening, visible irregular dark brown lesions, whole pod mummified and covered with a white mycelium. (B) Mycelium germination inside the pod.

predictors of FPR incidence (Fig. 3A and B), with a ΔAIC of 13.73 and of 3.4, respectively. The numbers of forest trees in a radius of 5.2 m (nfo5.2) and of banana plants in a radius of 5.1 m (nba5.1) were weaker predictors of FPR incidence (Fig. 3C and D), with a ΔAIC of 1.8 and of 0.13, respectively. In the case of cacao trees, we encountered two peaks of low ΔAIC (3.7 m and 7.6 m). As we wanted to mitigate the border effect, and because that distance was about twice the value of the first peak (3.7 m), we decided to choose the first peak of 3.7 m to characterize the distance effect of the neighborhood of cacao trees.

The significance of the four predictors selected in the first step (nca3.7, nfu4.3, nfo5.2, nba5.1) for FPR incidence was tested in a model. After backward selection, only nca3.7 and nfu4.3 were found to be significant (Table 2). Interestingly, these two significant variables had a negative effect on FPR incidence (Fig. 4). The model with nca3.7 and nfu4.3 as predictors led to a RMSE equal to 0.105 (about 10% of prediction error). Moreover, as we can see in the model prediction (Fig. 4), the prediction of FPR incidence ranged between 16% for nca3.7 = 1 and 2.5% for nca3.7 = 8, and between 8% for nfu4.3 = 0 and 1% for nfu4.3 = 3. Nca3.7 explained about 15% and nfu4.3 explained about 7% of the variation in individual disease incidence among the simulated percentages of infected pods.

4 DISCUSSION

In this study, we found that the number of cacao tree neighbors in a radius of 3.7 m had a significant negative influence on individual FPR incidence. Moreover, cacao tree neighbors had the most significant influence compared to the vicinity of the other three categories: fruit trees, forest trees and banana plants.

We suggest that this effect may be attributed to a barrier effect related to cacao canopy architecture. Canopy architecture has been demonstrated to have a major effect in limiting spreading distance through a barrier effect.[5,13] Cacao architectural traits, such as LAI, canopy height, canopy roughness, shoot lengths, shoot and leaf positions may reduce the canopy porosity of cacao trees, resulting in shorter dispersal distances. Moreover, the position of cacao branches from near the soil up to the top of the tree may certainly influence cacao canopy porosity, which has an effective impact on inoculum interception by other potential cacao hosts. These architectural traits are relevant in passive spore dispersal, which is the case for the main fungal diseases.[5,13] The non-host organs of cacao trees may form an efficient barrier to the dispersal of FPR spore between pods, the host organs.[18,43] Therefore, the cacao trees around each individual cacao tree may certainly serve as an effective barrier to pathogen dispersal. An optimum distribution of non-host organs around each individual cacao tree results in a reduction in disease dispersion: a large proportion of spores is retained on the 'parent' host, or in the close neighborhood, leading to inefficient dispersal. The position in the understory and the density of cacao trees supported the suggestion that cacao trees may be an effective barrier to pathogen dispersal. Other trees (fruit and forest trees), in higher vertical stratum and less dense in the stand, were probably a poorer barrier between two cacao trees, with only their trunk acting as a barrier.

The number of cacao tree neighbors on an individual plant scale has a significant negative influence on individual FPR incidence. When we characterized this number as a local density, we found that this local density around each cacao trees was negatively correlated to individual FPR disease incidence. This result is consistent with observations from a study in Talamanca, Costa Rica, which showed, on a plot scale, that cacao tree density was negatively correlated to FPR intensity on adult pods.[10] The barrier effect seems to hold across very contrasting cacao agroforestry systems; indeed, the Upala systems are more intensively managed (less shade, more sanitary removal of infected pods) than those in Talamanca.[10,44]

The number of fruit tree neighbors in a radius of 4.3 m also had a significant negative influence on individual FPR incidence, but the effect was weaker. The number of forest trees or the number of banana plants in the neighborhood of cacao trees had no significant effect on the incidence of FPR. In the case of banana trees, this result may be linked to the small number of banana plants in the study plots. More interestingly, when comparing the number of forest trees to the number of fruit trees, forest trees were more numerous than fruit trees, but the effect of fruit trees was more significant (see in Table 1, the number of individuals in each category). This difference may have been attributable to the position of those trees in the vertical vegetation stratum which defines different shade intensity,[10] and the scale on which the two plant populations had an impact on the disease. Forest trees are the highest vertical vegetation stratum.[10−12] Moreover, they have a larger and more porous canopy than fruit trees, thus providing a poorer barrier at cacao level and more uniform shade than fruit trees. Fruit trees form an intermediate vertical vegetation stratum between forest trees and cacao trees. Fruit trees are smaller in size than forest trees and provide a more localized, closer and deeper shade for cacao trees.[10] This more localized shade may reduce the pod production of cacao individuals with fruit trees in their vicinity. Shade is already known to be a factor that reduces cacao production.[23] The effect of fruit trees may thus be related to the

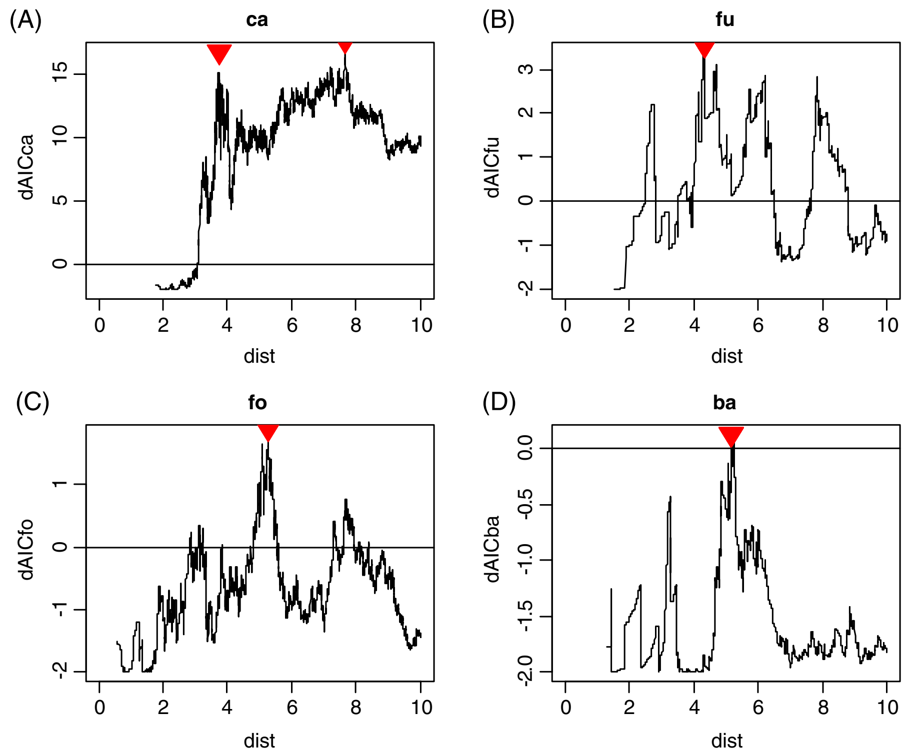

Figure 3. Selection of the distances at which each category of neighboring plants most affected FPR incidence. The distances correspond to the highest difference in AIC from the null model (ΔAIC) and are indicated by red triangles. The null model, the horizontal line in each figure, represented the variation in individual FPR incidence within the assessed cacao trees only taking into account the two random factors: the plot and the total number of pods. (A) shows the optimum distance for neighboring cacao trees (ca); (B) for neighboring fruit trees (fu); (C) for neighboring forest trees (fo); (D) for neighboring banana plants (ba).

Table 2. Result of the backward model parameter selection process.

Parameter	*df*	AIC	Pr(Chi)	ΔAIC	Estimate
Mc	–	374.94	–	–	–
Mwnca$_{3,75}$	1	379.56	0.01005*	4.62	−0.3730
Mwnfu$_{4,32}$	1	378.73	0.01614*	3.4	−0.9027
Mwnfo$_{5,27}$	1	373.73	0.37239	−1.94	NS
Mwnba$_{5,13}$	1	374.92	0.15873	−0.02	NS

Estimates are provided for significant variables.
The AIC of the complete model is equal to 374.94. Eliminating the fixed effect of cacao neighbors in a radius of 3.7 m and of fruit tree neighbors in a radius of 5.2 m led to a significant increase in the AIC. Eliminating the fixed effect of forest tree neighbors in a radius of 4.3 m and of banana plant neighbors in a radius of 5.1 m led to a non-significant reduction in the AIC. Nca3,7 and nfu5,2 were selected for the final model.
NS, not significant.

resource hypothesis[4,18] which predicts that fewer resources (pods) due to more localized shade participate in reducing pathogen incidence. In our analysis, we removed the resource effect using the total number of pods as a random factor. Moreover, in our study, the resource hypothesis did not include the rate of sanitary harvesting. On a plot scale, sanitary harvesting had a negative influence on plot FPR incidence, but was not correlated to cacao density (data available as Supporting Information, Table S2).

However, the reduction in production with an increasing number of cacao and fruit tree neighbors (Fig. 4) suggests that farmers are facing a trade-off between a barrier effect and the shading effect. Interestingly, while we observed a main effect of fruit trees with a radius of 4.3 m, the analysis of the variation in ΔAIC depending on the radius showed two other peaks at 6 and 8 m. These secondary peaks suggest that farmers probably have some flexibility in organizing the structure of their field, i.e. a radius of 8 m allows more diverse spatial patterns than a radius of 4.3 m.

In Upala, the absence of a forest tree effect on individual FPR incidence may be linked to their relatively lower density, but more especially to their architecture (high trees, large and porous canopy), which suggests a greater (plot) scale influence. These results, compared to those from Gidoin *et al.*,[10] suggest that forest trees in an adequate density and spatial distribution do not enhance individual FPR incidence, which is interesting as forest trees are related to other ecosystem services in cropping systems.[45] and more specifically in the Talamanca region, to the regulation of disease incidence on a plot scale.[10]

It is also known that the effectiveness of a particular barrier is variable and depends, among other factors, on inoculum intensity, and in our case on the spore density in the air.[43,46] Therefore, factors favoring the development of the disease, such as low ventilation, free water, and high relative humidity favoring the germination phase may counter-balance the barrier effect.[43,46] In Upala, the more effective sanitary harvest probably helped to increase the weight of the barrier effect (and perhaps also reduce wind speed). Future studies may address the issue of the inoculum in the air in relation to the spatial structure and the composition of the plant community. Moreover, associated trees may also influence abiotic variables in the system, such as light, humidity, wind. In particular, a reduction in wind speed may enhance the barrier effect.[18]

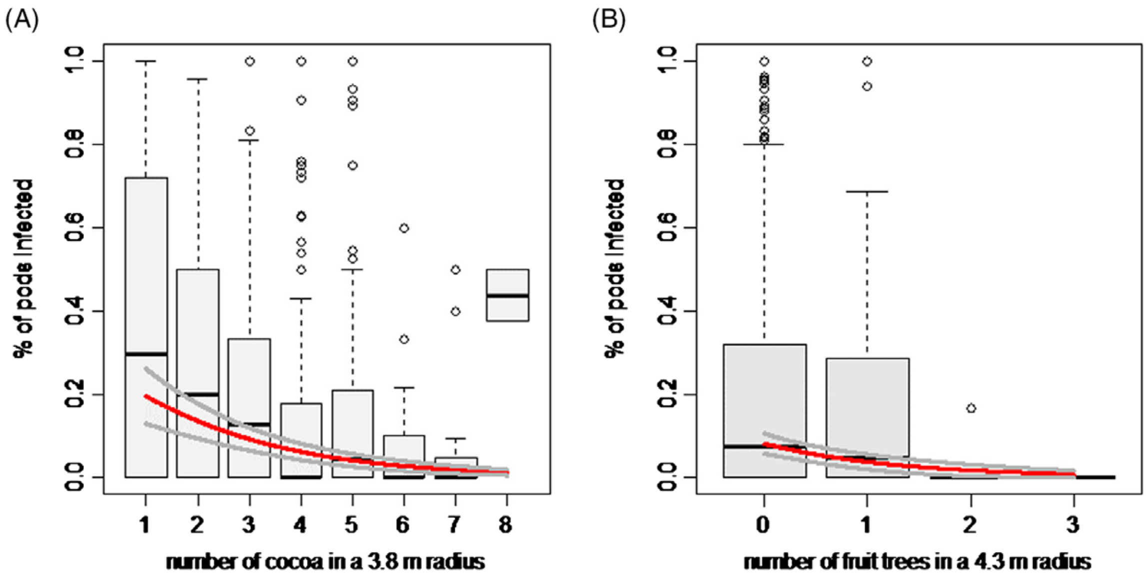

Figure 4. Variation in individual FPR incidence, according to nca3.7 (A) and nfu4.3 (B). Measurements (boxplot) and predictions of FPR incidence according to the number of cacao trees in a 3.7 m radius (nca3.7) (A) and of fruit trees in a 4.3 m radius (nfu4.3) (B). The red and gray lines show the mean responses and standard errors predicted by the GLMM (complete model including the two significant predictors), respectively. The predicted response of FPR incidence to each predictor (nca3.7 or nfu4.3) across its range of variation was achieved with the other predictor set at its mean value. The prediction of FPR incidence ranged between 16% for nca3.7 = 1 and 2.5% for nca3.7 = 8, and between 8% for nfu4.3 = 0 and 1% for nfu4.3 = 3. The two explanatory variables explained the individual disease incidence in the same direction: the more a cacao tree had neighboring cacao trees in a radius of 3.7 m, or neighboring fruit trees in a radius of 4.3 m, the less was its FPR incidence.

Our study was statistically powerful because it was based on a relatively large quantity of field data and was carried out on an individual plant scale. Furthermore, the choice of the distance effect of each type of plants, without any a priori assumptions, provided new information as to the scale on which the plant population may be organized to improve disease control. A temporal replication of this study, to ensure that the results were not unique to the microclimate in that season or the unique disease cycle of that year, may enhance the strength of our conclusions. Such an analysis could easily be applied to other pathosystems, especially those with poor existing knowledge and those in spatially heterogeneous environments.

5 CONCLUSION

In this study we found an interaction between the neighborhood and disease incidence on an individual plant scale. The number of cacao tree neighbors in a radius of 3.7 m and the number of fruit trees in a radius of 4.3 m had a significant negative influence on the individual incidence of FPR. These findings open up prospects for neighborhood management, in order to control pest and disease incidence. Indeed, the position of cacao and fruit trees can be more easily managed than the position of forest trees, as cacao and fruit trees are introduced during planting. Interactions between spatial structure and pest and disease incidence in cacao agroforests may lead to optimization of the neighborhood to reduce pests and diseases, which could be a lever for IPM. Optimizing the spatial structure of cacao and fruit tree individuals seems to be a good lever for the regulation of FPR development in cacao agroforests.

One prospect should be to measure microclimatic conditions on an individual plant scale (temperature, free water, relative humidity, wind speed) in different neighborhoods, along with FPR incidence, in order to identify the mechanisms involved in the impact on FPR of varying the number of neighbors.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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