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Several enemies at the same time: interaction between two cocoa pod diseases and a cocoa pod borer and their impact in Peruvian agroforestry systems

Mónica Arias[1], Jhoner Alvarado Labajos[2], Marcos Ramos[3], Clémentine Allinne[4], Gerben Martijn Ten Hoopen[5], Leïla Bagny-Beilhe[6]

[1] PHIM Plant Health Institute, Univ. Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France, monica.arias@cirad.fr
[2] Fundación Amazonía Viva, Juanjui, Peru
[3] ABSys, Univ Montpellier, CIHEAM-IAMM, CIRAD, INRAE, Institut Agro, Montpellier, France, marcos-javier.ramos@cirad.fr
[4]ABSys, Univ Montpellier, CIHEAM-IAMM, CIRAD, INRAE, Institut Agro, Montpellier, France, clementine.allinne@cirad.fr
[5] PHIM Plant Health Institute, Univ. Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France, gerben-martijn.ten_hoopen@cirad.fr
[6] PHIM Plant Health Institute, Univ. Montpellier, CIRAD, INRAE, Institut Agro, IRD, Montpellier, France, leila.bagny@cirad.fr

ABSTRACT.

Farmers frequently need to deal with several pests simultaneously. Despite this co-occurrence, damages produced by pests and diseases (P&D) are often studied and treated separately as they can be caused by distant taxa including fungi, nematodes and insects. P&D can enhance or hamper the incidence of each other, making of these interactions important modulators of the real impact of P&D on the plant. Interactions between P&D, abiotic conditions and resource availability can affect the composition of the P&D community at the temporal and spatial scale. Therefore, comprehensive analyses including all these factors are needed to better understand the spatio-temporal effect of P&D on crop production and to help design sustainable management strategies. In this preliminary study we explored spatio-temporal patterns in P&D incidence affecting cocoa agroforestry systems, their possible correlation with pod production and climate (mean temperature and precipitation), and potential spatio-temporal patterns of co-infection at the tree level in the Peruvian Amazon region of San Martin. Over the course of one year we collected data on the incidence of three cocoa pests: black pod disease (BPD) – due to *Phytophtora palmivora*, frosty pod rot disease (FPRD) – due to *Moniliophtora roreri* and the emergent local pest American cocoa pod borer (APB) - *Carmenta foraseminis*. Damages produced by other agents such as birds and sunburns were also recorded. We found that P&D incidence was correlated to total cocoa pod production. No geographical pattern was detected on P&D incidence profile suggesting that P&D population species are not expanding or contracting in the area and that none of the plots has enhancers or hampers that favour differently P&D incidences. FPRD is the prevalent diseases in the area, thus managing practices such as removal and proper elimination of infected pods need to be the priority in the region. We found that warmer temperatures were correlated to higher incidences of BPD and BPD+APB and drier conditions to damages produced by “other” agents. Correlation between P&D incidences and microclimatic conditions still need to be explored to better understand the effect of climate on P&D incidence and to provide recommendations to counteract this trend through microclimate manipulation. Neither temporal nor spatial conditions favour coinfection at the tree level, suggesting that pods are not a limiting resource in the area and that pests avoid interactions. Whether results are similar at pod level needs to be explored.

Keywords: multipest, injury profile, pest interaction, spatio-temporal dynamics, pest emergence

1. Introduction

Attacks by pests and diseases (P&D) are often studied individually. However, it is well known that organisms are often confronted with several P&D simultaneously. These P&D can interact among each other and produce differences in the abundance and the characteristics of the different P&D composing the local community. Three main kinds of interactions between co-infecting pathogens have been described in the literature: *via* the resource, the host or by interference (Dutt et al., 2022). Resource-mediated interactions occur when a given pathogen is affected by the remaining amount of resource left after the exploitation by another pathogen. As an example of its outcome, resource-mediated interactions can increase pathogen virulence, in order to exploit more rapidly the available resource (Dutt et al., 2022). Pathogens can also interact *via* their host, as infections turn-on host defence mechanisms that can either facilitate or hamper subsequent infections. For instance, *Pseudomonas syringae* activates the salicylic acid defence pathway, rendering plants more susceptible to the fungus *Alternaria brassicola* given that the jasmonic acid pathway is suppressed (Abdullah et al., 2017; Spoel et al., 2007). Pathogens can also interact *via* interference, that is when a pathogen can modulate chemically or mechanically the colonization, reproduction or transmission of another pathogen (Mideo, 2009). For example, proteins produced by two tomato viruses interact and favour colonization of both viruses within the same host (Tollenaere et al., 2012; Tripathi et al., 2015). Given that these interactions can shape the structure of the P&D compendium community at a given time and place and even the virulence and the subsequent success of the different agents, it is necessary to study the interactions between agents to design appropriate management strategies for different localities.

Farmers growing cacao in South America must face a number of P&D acting simultaneously on their cocoa pods. For example, unpublished data from the San Martin region in Peru show that in certain localities mostly planted with CCN51, only around 22% of the cocoa pods produced are healthy when harvesting. From the remaining 80%, up to 58% are infected by one or several of the members of the local pest and diseases community. The main agents of this community are: *Moniliophtora roreri*, a fungal pathogen that produces frosty pod rot disease (FPRD), *Phytophtora palmivora*, an oomycete responsible for black pod disease (BPD), and the American pod borer (APB), *Carmenta foraseminis*, a lepidopteran pest from the Sesiidae family and emergent in the region. Damages produced by rodents, birds or the sun can attain up to 20% of the pods. Interactions between some of these agents have been proposed and explored such as the facilitation of BPD colonisation upon the infection by APB (Alomía et al., 2021).

To better understand the possible interactions between pathogens and pest within a cocoa agroforest system, we explored the spatiotemporal dynamics of the P&D community in the San Martin region, Peru. This is the principal cocoa production region in the country. We monitored trees during 12 months on 8 different plots located along the upper Huallaga river.

We explored whether 1) there was a prevalent disease in the region and in each of the localities, 2) whether infections produced by a combination of diseases followed a geographical pattern (whether neighbouring plots show more similar infection profiles than plots further apart), 3) whether attacks by the pest and diseases show any pattern over time, that could be related to resource availability and/or climate oscillations. Finally, we explored 4) whether coinfection events at the tree level were promoted by season and/or by spatial differences.

2. Materials and Methods

2.1. Study area and monitoring period

To explore the spatiotemporal dynamics of the pest and diseases we monitored eight plots located in 5 localities along the upper Huallaga river (upper Huayabamba) in the San Martin Region, Peru (-7.32°, -76.78°): the localities were Huicungo, Santa Ines, Santa Rosa (1 and 2), Dos de Mayo (1 and 2), Marisol (1 and 2) (Figure 1). CCN51 is the prevalent cocoa genotype in the region. A total of 40 trees per plot were followed over a period of 12 months, from August 2020 until September 2021. Monitoring consisted on inspection of the fruits and registering of healthy and damaged fruits per tree each 15 days. For damaged/infected pods the responsible organisms were assigned and these pods were subsequently removed from the tree. Although Witches' broom, Certaocystis, Rosellinia, parasitic plants and mirids can also be present in this region we only focused on the diseases that affected the largest number of

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pods: frosty rot pod diseases, black pod diseases, American cacao pod borer and damages produced by "other" causes including birds, squirrels and sunburns. The number of fruits, either healthy or affected by the different pest/diseases, was calculated either by pooling all data, by plot or by date, depending on the question.

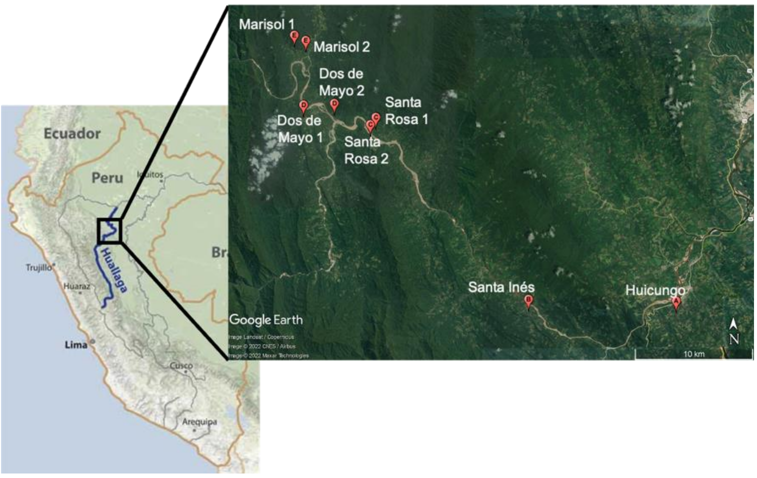

Figure 1. Map of the study area in the upper Huallaga river basin, San Martin, Peru

2.2. Statistical analyses

All analyses were performed using R (R Foundation for Statistical Computing, 2014). To test whether there was a geographical pattern on the amount of pods affected by the different diseases, we performed a Cluster analysis using the amount of healthy and infected pods produced per plot. Infected pods were classified in five categories: pods affected by FPRD, BPD, APB, BPD+APB or other agents.

To test for temporal infection patterns of different infection categories (the five previously mentioned), we calculated Spearman correlations for each of the different categories and a) resource availability (total amount of pods produced per tree per date), and b) two climatic variables: mean precipitation and mean temperature. Climatic variables were obtained from the CRU TS 4.06 dataset (Harris et al., 2020). Both variables were obtained for the region that was monitored (half-degree resolution) for August 2020 to September 2021 (Figure 2). Data was accessed using Google Earth. Cross correlations between variables including lags between them were also tested (for example, whether the amount of infected pods by a given disease was correlated to precipitation or temperatures some days/weeks before).

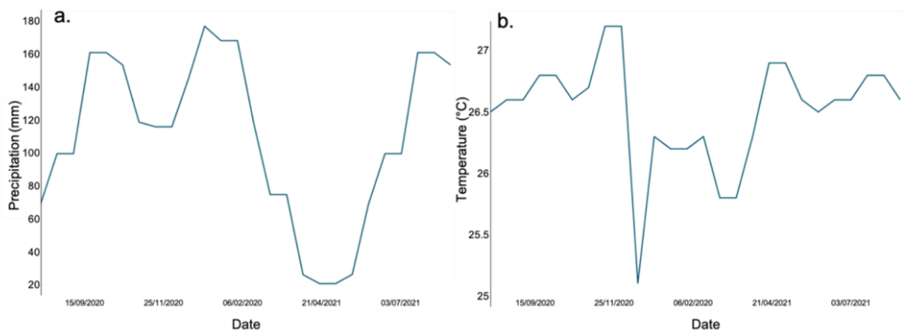

Figure 2. Temporal variations in a) precipitation (mm) and b) temperature (°C) in the region during the monitoring for this study.

To evaluate the likelihood of coinfection events at the tree level (i.e. at least two diseases at the same time infecting the same pod) for different dates and at the different plots, we compared the real number of coinfection events to a simulated dataset. Simulations were based on the number of infection events for each of the three main pests (FRPD, BPF, APB) per tree and per date. The real number of infections was simulated 1000 times and it produced a simulated dataset of coinfections at the tree level. The possible coinfection combinations are four: FRPD+BPF, FRDP+APB, BPF+APB and FRDP+BPF+APB. Real and simulated coinfection events were compared and a p-value was calculated for each disease combination per date and for each disease combination per plot. To calculate the p-value, we first calculated the mean of real coinfections event either per date or per plot for each of the possible disease combinations. Second, we calculated how many simulated data were equal or lower than the real mean (*t*). Third, we divided *t* by the number of simulated points either per date or per plot.

3. Results

3.1. Prevalent disease in the study area

When pooling all data we found that frosty pod rot disease is the prevalent diseases in the area, affecting more than 3816 fruits out of the 13419 monitored (28.4%), even more than healthy pods (2946 pods, 22.0% of total fruits). Other damages reached 2612 fruits (19.5% of total fruits). Black pod disease affected 2014 fruits (15.0% of total fruits), while American pod borer affected 975 fruits (7.3% of total fruits). Combination between APB and BPD was found in 1055 pods (7.9%). When visualizing data per locality FPRD was prevalent in 5 out of the 8 studied plots (Figure 3). Santa Rosa-MS and Santa Ines-MS produced more healthy than sick pods (Santa Rosa: 312 out of 1860 fruits produced; Santa Ines: 312 of 1735 fruits produced). In Huicungo, pods showing "other" damages represented the more frequent fruit status (355 out of 1300 fruits produced).

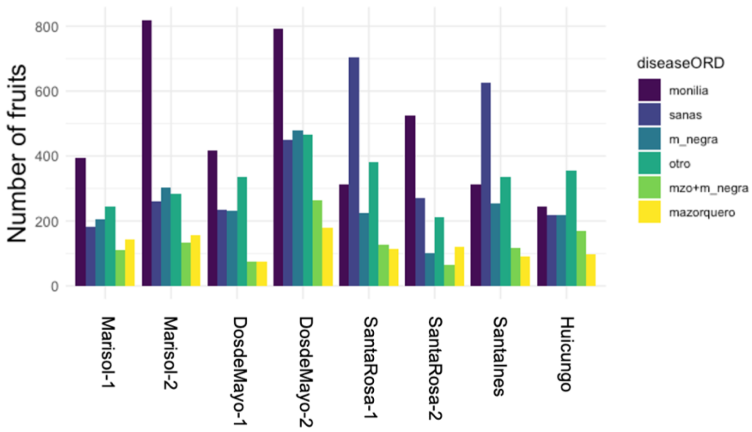

Figure 3. Disease prevalence per locality expressed on number of fruits affected by the different diseases. FPRD: Frosty pod rot diseases, BPD: black pod diseases, APB: American pod borer.

3.2. Geographical pattern on disease infection

To explore whether a spatial pattern was present in our data we applied a Cluster analysis. We obtained two clusters based on the amount of healthy and infected pods per tree and per plot. When visualizing the representation of the clusters for the different localities we detected no spatial pattern on infection profiles. As shown in Figure 4 there is only the "Dos de Mayo-2" plot, located towards the centre of the study area, contributes mostly to the cluster blue and is the only on that shows this pattern.

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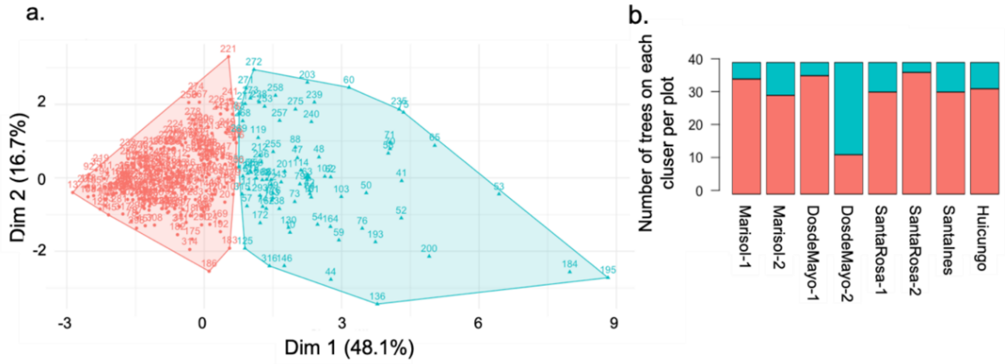

Figure 4. Results of cluster analysis performed on the total amount of healthy and infected pods produced per tree. Colors correspond to the two clusters that were obtained (a). Their representation in the different plots is shown in b.

3.3. Temporal pattern on disease infection

The frequency of each of the diseases fluctuated along the year and show a similar pattern to the temporal oscillation of the total fruit production (Figure 5, Spearman correlation results for total fruit production and FPRD ($P<0.001$), BPD ($P=0.003$), APB ($P<0.001$), BPD+APB ($P=0.001$), other agents ($P<0.001$)). Production peaked twice during the monitoring period: around September 2020 (beginning of the monitoring period analysed, thus after the COVID-related lockdown) and around the beginning of August 2021 (towards the end of the monitoring period). FPRD, APB, the combination BPD+APB, as well as damages by "other" agents also show two peaks that match those of total fruit production (Figure 5). Although BPD also has high incidence values around September 2020 and August 2021, its peak of incidence was in April 2021. Climate was correlated to BPD, BPD+APB and "other" agents damages: warmer temperatures were positively associated to BPD (Spearman correlation $P=0.001$) and APB+BPD (Spearman correlation $P=0.03$). Damages caused by other agents were negatively correlated to precipitation (Spearman correlation $P=0.006$). No correlation between the different variables including lags of different spans were found.

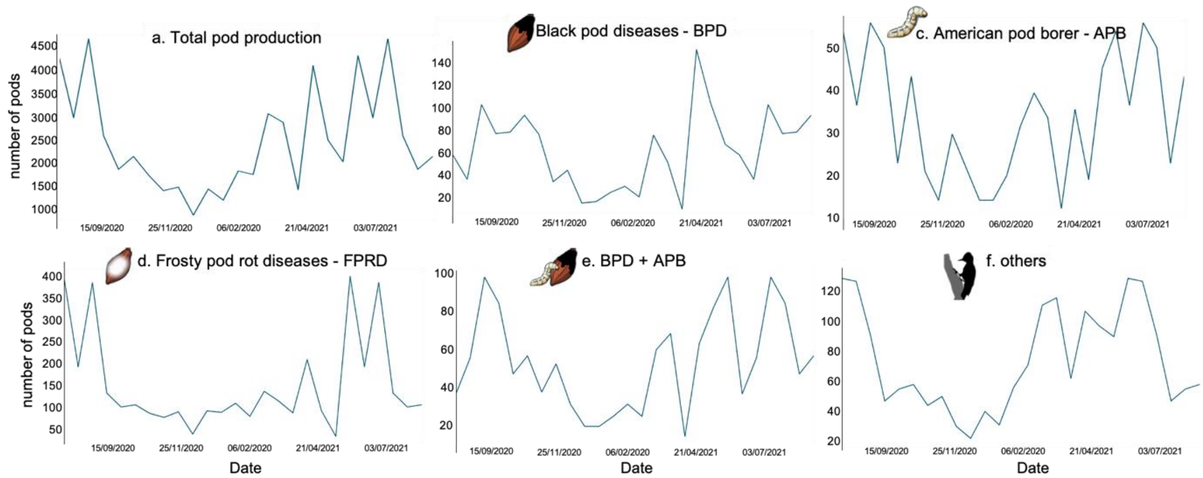

Figure 5. Temporal dynamics of the number of pods produced (total and infected) between August 2020 and September 2021. In a) total pod production (healthy and infected pods), from b to f number of pods affected by b) black pod diseases, c) American pod borer, d) Frosty pod rot diseases, e) the combination of black pod diseases and American pod borer and f) other agents such as bird, rodents or sun.

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3.4. Spatio-temporal patterns in co-infection events at the tree level

Real data fell below the median of 1000 simulations results for all the possible disease combinations. P-values ranged from 0.02 to 0.6. At the tree level, coinfections between BPD + FPRD and APB+FPRD occurred less often than expected by random in some cases (values below 0.05 are indicated by * and values below 0.1 are indicated by ~ in Figures 6 and 7).

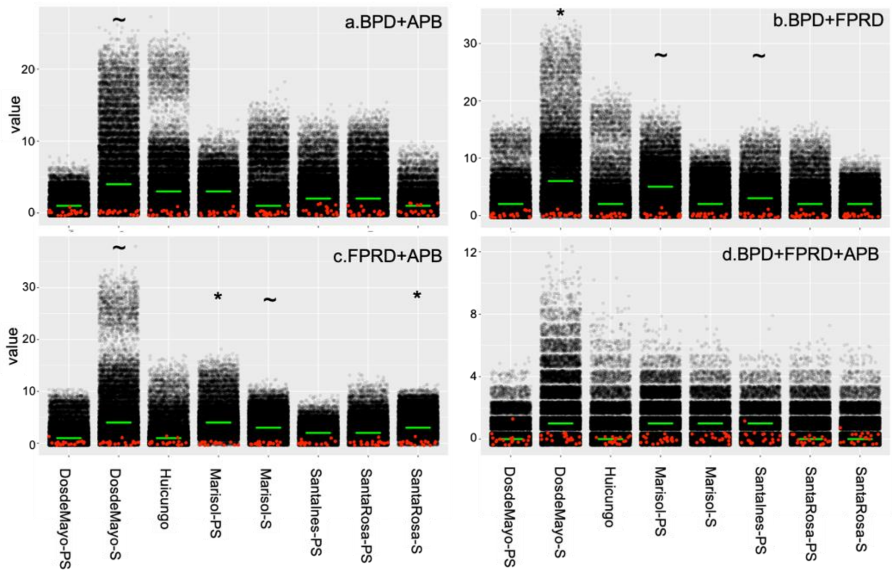

Figure 6. Comparison between simulated and real data by plot for the coinfection by a) black pod disease and American pod borer, b) black pod borer and frosty pod rot disease, c) frosty pod rot disease and American pod borer and d) all the three agents. In black are results of simulations, in green their median and in red the observed data. * states for p values < 0.05 and ~ for p values < 0.1.

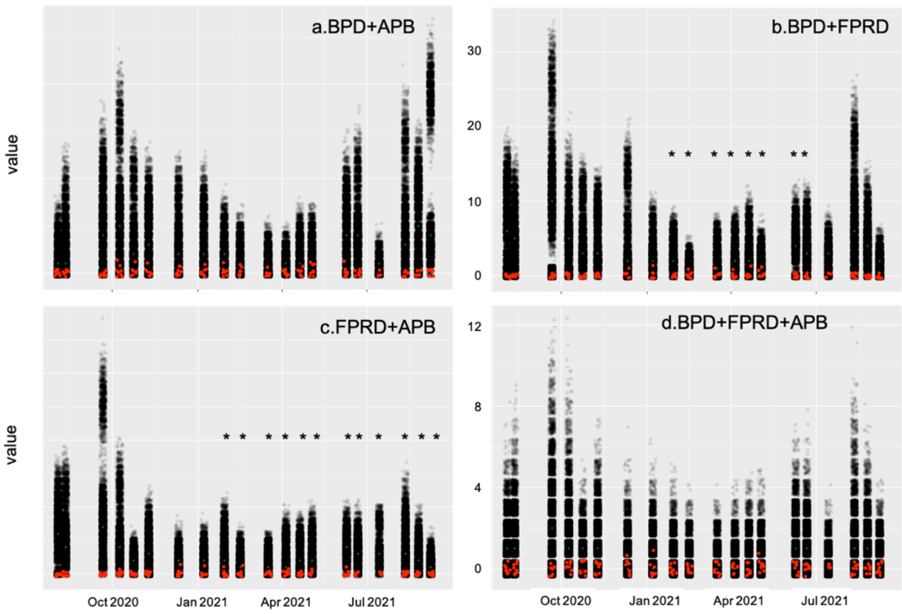

Figure 7. Comparison between simulated and real data by date for the coinfection by a) black pod disease and American pod borer, b) black pod borer and frosty pod rot disease, c) frosty pod rot disease and American pod borer and d) all the three agents. In black are results of simulations, in green their median and in red the observed data. * states for p values < 0.05.

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4. Discussion

We analysed the spatio-temporal dynamics of pod damages produced by FPB, BPD, APB and other agents on 8 plots in 5 localities in the upper Huallaga river at the San Martin Region (Peru). We found that the most important disease in the region is frosty pod rot disease. Its prevalence has been documented by several other authors in regions dominated by the clone CCN51 (Carvajal-Rivera et al., 2022). Other diseases and pests include black pod disease and American cocoa pod borer. APB is a new pest in the area and whether it is increasing cocoa losses or sharing losses produced by another agent from the local P&D community remains undefined. Comparisons between agroforestry plots with similar tree composition, similar managing practices and similar climate conditions but differing in the presence of the APB will be useful to better understand the real effect of the emergent pest on cocoa production.

No geographical pattern in the incidence of the different pest and diseases was found after comparing our eight plots. This suggests that the local community of pests and diseases is already well stablished in the area and that no expansions or contractions of species are occurring in the area. This also suggest that the general conditions along the study area are rather similar in terms of enhancers and hampers for the different diseases, as no particularly different profiles were found on the different zones of the study. Whether a spatial pattern can be found at smaller geographical scale in this region remains to be explored. The presence of trees established between crop plants decreases temperature and wind speed and increases humidity (Sileshi et al., 2008). These microclimatic conditions have already been identified as modifiers of the dispersion and incidence of several P&D in different agroforestry systems (Dupont et al., 2022; Koech & Whitbread, 2000; Schroth et al., 1995). Moreover, differences in the proximity to potential sources of pest, diseases and their natural enemies can also produce spatial differences in the presence and incidence of different P&D in a given plot. For instance, proximity to forest patches affects the composition of invertebrate natural enemies of pest insects (i.e. spiders) in Indonesian agroforestry systems of cocoa, which perhaps affects also pest community (Stenchly et al., 2012). This important yet unexplored within-plot variation in our system needs to be studied to propose coherent P&D managing strategies adapted to the local conditions.

We found strong temporal patterns between total pod production and the number of pods affected by the different diseases. Pod production, and the number of pods infected by the different P&D, peak on September 2020, drop strongly around December and January and peak again on August 2021. Our results suggest that as expected, pathogen cycles are mediated by the resource and that FRPD, BPD and APB are well adapted to the local pod production cycle as their populations can rise after strong population reductions. Climate variations were also associated to the oscillation in the number of damaged pods. Damages by BPD, BPD+APB were positively associated to warmer temperatures while damages by “other” agents were higher at drier conditions. We found no association between precipitation and BPD incidence, even including considering lags of different time spans between the variables, contrasting what has been previously reported for BPD in Cameroon (Ndoumbè-Nkeng et al., 2009). Further analyses including more precise information on climatic conditions as well as microclimatic variations are required to confirm and better understand these patterns. However, our preliminary results suggest that the current climate change with warmer temperatures and longer and stronger drier seasons will increase the damages caused by BPD, BPD+APB and “other” agents in the region. If this pattern is confirmed, managing practices that can mitigate warmer and drier climatic conditions by influencing the microclimate, such as an increase in shading (Lasco et al., 2014) should be further explored to be properly tuned to be efficient and subsequently encouraged in the region.

Regarding the general spatial and temporal patterns of coinfection at the tree level we found that coinfection events are rather rare, especially for BPD + FPRD and APB + FPRD coinfections. This suggests that there is enough resource for P&D to frequently infect healthy pods and that P&D avoid already-infected trees. Although results for the coinfection between BPD+APB were not significantly rare, they were still below the median value of coinfections expected by chance. Some authors have reported a facilitation dynamic at the pod level between them as the BPD could infect more easily the pod taking advantage of the hole produced by the APB (Alomía et al., 2021). Further analyses at the pod level might confirm the already reported pattern.

5. Recommendations

The highest incidence of most pest and diseases occurs at the beginning of the studied monitoring period. This is just after three months of COVID crisis that prevented the sanitary harvesting for three months. This suggests that not managing pests and diseases is a losing strategy for farmers.

The main problem in the San Martin area continues to be FPRD. Therefore, managing practices that are efficient against FPRD dispersion, such as infected pod removal and their subsequent efficient elimination needs to be encouraged. However further research is needed to understand the role of the emergent pest APB in the ecosystem and its potential to become a major cocoa pest in the continent.

Our preliminary results suggest that managing practices that help reducing temperature and water loss might reduce the damage produced by three of the five pest and diseases status explored in our study. However, our results require further exploration.

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References

Abdullah, A. S., Moffat, C. S., Lopez-Ruiz, F. J., Gibberd, M. R., Hamblin, J., & Zerihun, A. (2017). Host–multi-pathogen warfare: Pathogen interactions in co-infected plants. *Frontiers in Plant Science*, *8*, 1806.

Alomía, J., Alomía, C., & Vega, B. (2021). Carmenta foraseminis Eichlin y Phytophthora palmivora en frutos de Theobroma cacao L. en Satipo, Perú. *Manglar*, *18*(3), 283–288.

Carvajal-Rivera, A. S., Jaimes-Suárez, Y. Y., Guzmán-Plazola, R. A., Ortiz-García, C. F., & Sandoval-Islas, J. S. (2022). Temporal dynamics of witches' broom disease (Moniliophthora perniciosa) in six cocoa clones with and without shading. *Journal of Plant Pathology*, *104*(1), 37–48.

Dupont, S., Irvine, M. R., Motisi, N., Allinne, C., Avelino, J., & Beilhe, L. B. (2022). Wind-flow dynamics and spore-like particle dispersal over agroforestry systems: Impact of the tree density distribution. *Agricultural and Forest Meteorology*, *327*, 109214.

Dutt, A., Andrivon, D., & Le May, C. (2022). Multi-infections, competitive interactions, and pathogen coexistence. *Plant Pathology*, *71*(1), 5–22.

Harris, I., Osborn, T. J., Jones, P., & Lister, D. (2020). Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Scientific Data*, *7*(1), 1–18.

Koech, E., & Whitbread, R. (2000). Disease incidence and severity on beans in alleys between Leucaena hedgerows in Kenya. *Agroforestry Systems*, *49*(1), 85–101.

Lasco, R. D., Delfino, R. J. P., & Espaldon, M. L. O. (2014). Agroforestry systems: Helping smallholders adapt to climate risks while mitigating climate change. *Wiley Interdisciplinary Reviews: Climate Change*, *5*(6), 825–833.

Mideo, N. (2009). Parasite adaptations to within-host competition. *Trends in Parasitology*, *25*(6), 261–268.

Ndoumbè-Nkeng, M., Efombagn, M. I. B., Nyassé, S., Nyemb, E., Sache, I., & Cilas, C. (2009). Relationships between cocoa Phytophthora pod rot disease and climatic variables in Cameroon. *Canadian Journal of Plant Pathology*, *31*(3), 309–320.

DOI: 10.5281/zenodo.10209817

R Foundation for Statistical Computing, R. C. (2014). *R: A language and environment for statistical computing.*

Schroth, G., Balle, P., & Peltier, R. (1995). Alley cropping groundnut with Gliricidia sepium in Cote d'Ivoire: Effects on yields, microclimate and crop diseases. *Agroforestry Systems*, *29*(2), 147–163.

Sileshi, G., Schroth, G., Rao, M. R., & Girma, H. (2008). Weeds, diseases, insect pests and tri-trophic interactions in tropical agroforestry. *Ecological Basis of Agroforestry. CRC Press, Boca Raton*, 73–94.

Spoel, S. H., Johnson, J. S., & Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences*, *104*(47), 18842–18847.

Stenchly, K., Clough, Y., & Tscharntke, T. (2012). Spider species richness in cocoa agroforestry systems, comparing vertical strata, local management and distance to forest. *Agriculture, Ecosystems & Environment*, *149*, 189–194.

Tollenaere, C., Susi, H., Nokso-Koivisto, J., Koskinen, P., Tack, A., Auvinen, P., Paulin, L., Frilander, M. J., Lehtonen, R., & Laine, A.-L. (2012). SNP design from 454 sequencing of Podosphaera plantaginis transcriptome reveals a genetically diverse pathogen metapopulation with high levels of mixed-genotype infection. *PLoS One*, *7*(12), e52492.

Tripathi, D., Raikhy, G., & Pappu, H. R. (2015). Movement and nucleocapsid proteins coded by two tospovirus species interact through multiple binding regions in mixed infections. *Virology*, *478*, 137–147.