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Shade tree *Chloroleucon eurycyclum* promotes coffee leaf rust by reducing uredospore wash-off by rain

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ABSTRACT

Shade trees stimulate many pathways that influence disease development in opposite directions, and that, in addition, may interact with environment. To better understand shade trees effects on coffee leaf rust (CLR), we studied three disease stages separately: sporulation, uredospore wash-off by rain, and uredospore deposition on leaves. The study was conducted during almost one year in the long-term trial on coffee-based agroforestry systems established by CATIE in 2000, in Turrialba, a low altitude area of Costa Rica. We only used the *Full Sun* and *Shade* provided by *Chloroleucon eurycyclum* treatments. For studying sporulation, we harvested diseased leaves every three weeks and collected the uredospores present on the lesions. For assessments of uredospore wash-off, we located containers at ground level below the coffee bushes and in the interval between rows of coffee bushes, and removed them after rainfall events (43 rainfall events studied) to count the number of uredospores collected. For uredospore deposition, we used varnish to capture deposited uredospores on apparently healthy coffee bush leaves (55 dates). We also studied the raindrop kinetic energy by using splashcups, on 19 rainy days. The number of uredospores produced and preserved was 2.22 times higher below shade trees than in full sun, whereas the number of uredospores lost by wash-off, below the coffee bush, was 1.62 times lower. Reduced wash-off was probably due to raindrop interception by shade trees and stemflow and to the increased kinetic energy of the raindrops in the understory of the *Shade* treatment (twice as high as that measured in full sun), which reduced the capacity of coffee leaves to intercept raindrops. In addition, we found 1.43 times more uredospores deposited on apparently healthy leaves below shade trees than in full sun, partly due to the higher number of uredospores produced and preserved below shade trees. Increasing throughfall and reducing raindrop kinetic energy below shade trees seem crucial to improved CLR regulation. This can be achieved by selecting specific shade tree functional traits and by implementing shade pruning during the rainy season.

1. Introduction

Shade trees in agroforestry systems provide pest and disease regulating services, particularly by providing refuges to a series of species such as birds, ants, spiders and fungi that help control pests and diseases (Avelino et al., 2011, 2018; Leakey, 2014; Schroth et al., 2000). Shade trees also have direct effects on pests and pathogens by modifying microclimate (Avelino et al., 2011, 2018; Schroth et al., 2000) as well as indirect effects through modifications of the physiological status of the host plant (Avelino et al., 2011; Schroth et al., 2000). As a result, shade trees have been reported to help manage several coffee diseases such as phoma blight, caused by *Phoma costarricencis*; brown eye spot caused by *Cercospora coffeicola*; and branch die back, a syndrome exacerbated by several *Colletotrichum* fungi (Avelino et al., 2011, 2018; Schroth et al., 2000). However, other pests and diseases have a higher abundance below shade trees, such as the American leaf spot disease, caused by *Mycena citricolor*; thread blight, caused by *Corticium koleroga*; pink disease caused by *C. salmonicolor*; and even coffee wilt disease, caused by *Fusarium xylarioides* teleomorph *Gibberella xylarioides*. Humid conditions in the understory probably favor these disease developments, but some

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shade trees are also alternate hosts for *M. citricolor*, *C. koleroga* and *C. salmonicolor* (Avelino et al., 2011, 2018; Schroth et al., 2000).

Shade trees effects on pests and diseases are normally more variable. For instance, controversies are found in the literature concerning coffee berry borer, caused by *Hypothenemus hampei*; coffee berry disease caused by *Colletotrichum kahawae*; and coffee leaf rust (CLR) caused by *Hemileia vastatrix*. Shade trees effects on these pests and diseases are variable, probably because shade trees stimulate many pathways that affect pest and disease development in opposite directions, and that, in addition, may interact with the environment (Avelino et al., 2011). The balance of these effects is thus uncertain. In the case of the coffee berry borer, the number of insects in coffee berries was found to be larger and the sexual ratio biased toward females in full sun, whereas the percentage of bored berries was larger below shade trees, suggesting that different antagonistic pathways were stimulated at the same time (Mariño et al., 2016). Opposite effects of shade trees on coffee berry disease have been reported recently (Motisi et al., 2019), with decreased symptom appearance along the branch, but also a delayed disease extinction, under a dense and permanent shade provided by a kola tree when compared with a moderate and non-permanent shade provided by banana plants. Microclimate modifications and their effect on the disease through different pathways have been suggested. Similarly, opposite effects of shade trees on CLR have been noted. Shade trees favor infection, i.e. uredospore germination and penetration into the leaf, when compared with coffee in full sun, due to better temperature and wetness conditions. At the same time, it reduces leaf receptivity, i.e. physiological resistance, by regulating fruit load (Lopez-Bravo et al., 2012). Additionally, shade trees interact with rain and wind for uredospore dispersal in the air (Boudrot et al., 2016). When raining, dispersal in the air is more abundant below shade trees than in full sun, probably because the kinetic energy of the raindrops that hit the coffee leaves and release the uredospores is increased (due to accumulation of rainwater on shade tree leaves and increased drop size in the understory). In dry periods, however, shade trees reduce uredospore dispersal in the air when compared with full sun, because they intercept wind, which is the main factor driving dispersal in the absence of rain (Boudrot et al., 2016). Interactions between shade trees and altitude for CLR have been recently highlighted. At altitudes >1400 masl, higher CLR incidence occurred in shaded plots, whereas at altitudes <1400 masl, CLR reached higher levels in open coffee systems (Liebig et al., 2019).

To better understand shade trees effects on coffee pests and diseases, particularly on CLR, and disentangle this complexity, we decided to focus our studies on each of the disease processes separately, as was already done for the study of uredospore dispersal in the air (Boudrot et al., 2016). Through this approach, we hope to (1) better understand shade trees effects on CLR, the sum of the individual effects of shade trees on the different disease processes (2) identify key effects on one or more of these processes and (3) eventually deduce traits of shade trees that could be selected in order to reduce the intensity of CLR epidemics.

Here, we studied three CLR stages particularly important for disease development: sporulation, uredospore wash-off by rain and deposition on leaves. Rayner (1961b) quantified almost 400 000 uredospores produced by a single lesion in three months, but McCain and Hennen (1984) mentioned a potential of 2 000 000 uredospores in an 18 mm diameter lesion. Though this production of uredospores is high, not all survive. As a biotrophic fungus, *H. vastatrix* cannot survive long outside the leaf (Deepak et al., 2012): any released uredospore that is not able to reach a coffee leaf in a short time will die (Waller, 1982). Rain-generated wash-off of the released uredospores to the ground is one of the main threats to uredospores survival (Savary et al., 2004). Another is the parasitism of uredospores by natural enemies, such as *Lecanicillium lecanii* (Vandermeer et al., 2009). Conversely, any factor that contributes to the conservation of uredospores will considerably increase the intensity of the CLR epidemic. Infection will be the result of the uredospores able to reach the lower surface of the leaves where the necessary stomata for fungus penetration into the leaf can be found (Rayner, 1961a).

2. Materials and methods

2.1. Study site and period

The study was conducted from 19 February 2016 to 1 December 2016 in Turrialba, Costa Rica, in the experimental station at CATIE (Centro Agronómico Tropical de Investigación y Enseñanza), specifically in the long-term trial on coffee-based agroforestry systems that was established in 2000 by CATIE (Haggar et al., 2011). This low-altitude site (600 masl) is under the influence of the Caribbean Sea. The area is wet (2683 mm on average in the 2010–2016 period) with no marked dry season. However, rain is less from February to April (123 mm per month, average of the 2010–2016 period). Temperatures are warm, with monthly minima of 14.1–18.2 °C and monthly maxima of 28.3–30.5 °C (2010–2016). These conditions are favorable for CLR development.

2.2. Experimental design and studied treatments

The long-term trial on coffee-based agroforestry systems established in Costa Rica covers 9.2 ha. It comprises 20 treatments that result from the partial combination of seven shade levels (three shade tree species, alone and combined two by two, plus full sun) with two management strategies (conventional, organic) and two input levels each. Each treatment is replicated three times (three blocks). The planted coffee cultivar is the dwarf Caturra of the *Coffea arabica* species, susceptible to most of the CLR races. The planting density is 5 000 coffee bushes ha^{-1} (2 m between rows and 1 m between coffee bushes within the row). Two coffee trees were planted in each hole (Haggar et al., 2011; Schnabel et al., 2018).

Our study was conducted in only two of these 20 treatments: *Full Sun* and *Shade* provided by *Choroleucon eurycyclum*, the cashá tree, with 71 trees ha^{-1}. The cashá is a tall tree (about 15 m in height) with a wide canopy. This species, which is mostly used in cacao-based agroforestry systems in Central America (Somarriba et al., 2014), is promising for coffee, particularly in organic systems, due to its N-fixing property and valuable timber (Schnabel et al., 2018). Shade cover at ground level was 70% on average, which can be considered as dense. In both treatments, coffee was conventionally managed. Nutritional management consisted of 500 kg ha^{-1} of an N–P–K–Mg–B fertilizer (18-5-15-6-2) plus 180 kg ha^{-1} of urea in three applications during the year. Weeds were managed chemically within the coffee bush rows and mechanically between rows. A systemic fungicide (cyproconazole, 10% WG) was also sprayed on two opportunities (March and June) to control coffee diseases, particularly CLR. Despite being managed equally, in 2016, yield in full sun reached 2853 kg ha^{-1}of green coffee; below shade trees, only 618 kg ha^{-1}.

2.3. Sporulation measurements

Every three weeks on average, we collected all the diseased leaves on six randomly selected branches on six coffee bushes (one per coffee bush) in the *Full Sun* and *Shade* treatments of the three blocks. Branches were distributed within the coffee bushes so that two branches came from the upper level, two from the middle and two from the low level. Leaves were gathered per branch. For each group of diseased leaves, the uredospores were rasped from the CLR lesions (with no *L. lecanii*) and then placed into micro tubes with 1 ml of sterilized water and 2.5% Twin. The samples were then homogenized during 5 min at 30 °C in an ultrasonifier. Finally, the number of uredospores in each suspension was counted three different times, using a Neubaeur® counting chamber (HBG, Germany) with the 10 X power objective lens, and then averaged. The inoculum size, i.e. the number of uredospores per branch, at each date and in each treatment, was obtained by averaging the counts of the six branches.

2.4. Measurements of uredospore washed off by rain

Rain wash-off of uredospores was measured in 43 daily rain events during the study period. The sampling devices consisted of 15 containers placed at ground level below three coffee bushes (five per coffee bush) and 10 placed in the interval (between coffee bush rows) in the full sun and shade plots of one specific block. The containers were placed in the morning (rain usually starts in the afternoon) and were removed the morning following the rain. Due to the heavy workload involved in this activity, we only sampled one block at a time. However, the sampling devices were moved from time to time, from one block to another, to take advantage of the replicates of the trial. A total of 13 evaluations were done in one block; 15 in each of the other two blocks.

The suspensions of uredospores were processed in the laboratory as follows: (1) the volume of collected water in each container was measured with a measuring cylinder; (2) the container and the measuring cylinder were washed using 10 ml of water at 2.5% of Tween; (3) all the water was recovered and filtered in a 50 μm mesh to eliminate large particles; (4) the sample was homogenized in an ultrasonifier for 5 min and agitated for 1 min; (5) the sample was then centrifuged for 3 min at 2 000 rpm; (6) the supernatant was removed and only the remaining 5 ml were used; (7) the centrifuge tube was placed in an ultrasonifier for 5 min and then agitated for 30 s; (8) 12 countings were done per sample by using a Neubaeur® counting chamber (HBG, Germany) with the 10 X power objective lens, and the data were averaged.

Method calibration trials with different known suspensions of uredospores revealed that the relationship between the number of uredospores ml^{-1} in the final 5 ml used for countings in the laboratory (U_l) was well-related ($r^2 = 0.90$) to the number of uredospores ml^{-1} of the initial suspension in the containers (U_c): $U_c = 0.64U_l$. We used this relationship to deduce the real number of uredospores into the containers.

2.5. Uredospore deposition on leaves measurements

Uredospore deposition assessments were conducted on 55 dates: 38 on mornings following rainy days, and 17 following dry days. As for the trial on wash-off of uredospores by rain, sampling was performed in one specific block on each date. At the end, one block was used 16 times, another 18 times and the last 21 times.

On each sampling date, we selected three coffee bushes and three branches per coffee bush (one per coffee stratum) in the *Full Sun* and *Shade* treatments. Two apparently healthy leaves per branch among the young leaves of the branches were chosen (on the second to the fourth node from the tip). A varnish was applied on a 3 cm^2 surface on both sides of these leaves, on the troughs of the leaf border undulations where water accumulates. After a while, the dry varnish was removed and the uredospores caught were counted with the 10 X power objective lens.

2.6. Rainfall and kinetic energy measurements

Rainfall was monitored by using a Campbell Scientific© rain gauge (TE525MM-L, 0.1 mm precision) placed on the agroforestry trial in full sun, above coffee bushes.

In addition, the rain kinetic energy was compared below shade trees and in full sun by using splashcups almost identical to the ones developed by Scholten et al. (2011). The only difference was the size of the sand used for the measurements (150–250 instead of 125–200 μm). The principle of this device is to derive the kinetic energy of the raindrops from the loss of a known quantity of sand encased into a cup and exposed to rain. The higher the loss after the rain, the higher the kinetic energy: 1g m^{-2} of sand lost is equivalent to a kinetic energy of 0.1455 J m^{-2}. These measurements were decided on after considering that uredospore wash-off is dependent on water interception by coffee leaves, and water interception on drop size (Calder, 1996) which in turn influences the kinetic energy of the raindrops (Vis, 1986).

We studied the kinetic energy of raindrops on 19 rainy days simultaneously in the three blocks. Splashcups were placed above the coffee bushes at 2.5 m from the ground. We used three splashcups in each full sun plot and eight below shade trees. Four of the splashcups below shade trees were placed at half distance from a shade tree trunk and the tip of the longest branch in four directions and four below the edge of these longest branches. The cups with the weighted sand were placed in the morning and removed the day following the rain.

2.7. Analyzed variables and statistical analyses

To model *H. vastatrix* sporulation, uredospore wash-off by rain, uredospore deposition on leaves, rainwater collected in the containers and kinetic energy of the raindrops, we used generalized additive mixed-effects models (GAMM) for their flexibility in considering non-linear relationships.

For *H. vastatrix* sporulation, the model included, as fixed effects, the shade factor (*Full Sun* and *Shade*), coffee branch stratum (upper, middle and low parts of the plant), their interaction, and sampling date as a covariate. As random effects, the model considered the randomization restrictions for the measurement of this variable (block and shade (block)). Since the data presented overdispersion, we used the negative binomial distribution with the log link function.

The number of uredospores lost by wash-off and deposited on leaves necessarily depend on the available uredospores. However, in our study, sporulation assessments only occurred every three weeks on average and did not always coincide with the dates of uredospore wash-off by rain and deposition assessments. We therefore estimated the inoculum size per branch on these dates by calculating the time-weighted mean of the sporulation values obtained in the two evaluations closest to each uredospore wash-off and deposition assessment date. This calculation provided estimates for each full sun and shade plots individually.

For the wash-off of uredospores by rain, the model comprised, as fixed effects, the shade factor, container location (below coffee bushes, between coffee bush rows), and their interaction. As a covariate, we used rainfall in a first model. In a second one, we included another covariate, the inoculum size per branch, which was estimated as explained just before, to examine how this variable affected the eventual significant effects highlighted with the previous model. Randomization restrictions for the measurement of this variable were considered in the models as random effects (block and shade (block)). For these models, we also used the negative binomial distribution with the log link function.

The model for uredospore deposition on leaves included, as fixed effects, the shade factor, leaf side (upper and under surfaces), coffee branch stratum and their interaction. Rainfall was included as a covariate in a first model. As for wash-off of uredospores by rain, we included the inoculum size per branch in a second model as an additional covariate. Randomization restrictions for the measurement of this variable led to include in the models these random effects: (block, shade (block) and branch (shade (block))). In this case, we used the normal distribution with the log link function.

Throughfall, i.e. rainwater collected in the containers and kinetic energy of the raindrops were modeled as a function of shade, the position of the devices (containers and splashcups, respectively), rainfall and interactions. As random effects, the model considered the randomization restrictions for the measurement of this variable (block, shade (block) and device position (shade (block))). We used the normal distribution with the identity function.

In all models, the term thin-plate spline (tp) was used for covariates. The REML estimation method was used in all adjusted models, using the gam function of the mgcv library (Wood, 2003, 2011, 2017) with the R 3.5.1 software (R Core Team, 2019). For model validation, we used graphic diagnostics of the residuals by means of the gam.check function and the check function of the mgcViz library (Fasiolo et al., 2019). For treatment comparisons, we used the emmeans function of the package of the same name (Lenth, 2019). To assess the importance of interactions of factors with covariates, we performed a likelihood ratio test comparing a model without interaction with a model that considers the interaction. For that purpose, we run an anova test with a F-statistic in R.

3. Results

3.1. Sporulation

The sporulation model fitted well the data with $r^2 = 0.85$ between observed and fitted data. We found an interaction shade x coffee branch stratum for sporulation ($\chi^2 = 80.9$, $P < 0.0001$). Until mid-June, i.e. until the rainy season was well-established, the number of uredospores per branch remained low, but this number increased considerably after this period (Fig. 1). Higher quantities of uredospores were then quantified below shade trees than in full sun (Figs. 1 and 2). On average, we estimated 39 539 uredospores per branch below shade trees and only 17 824 in full sun (adjusted means), 2.22 times more below shade trees than in full sun (Fig. 2). The highest production of uredospores was found in the middle stratum of the coffee bush below shade trees, with 52 226 uredospores per branch on average (adjusted mean). No differences were found between the low and upper branches. In full sun, the low and middle parts of the coffee bushes showed more uredospores than the highest one (Figs. 1 and 2). The highest quantity was, on average, 22 296 uredospores per branch (adjusted mean) in the middle coffee bush branches.

3.2. Wash-off of uredospores by rain

The value of the determination coefficient of the regression between observed and fitted data, $r^2 = 0.84$, provides evidence of the correct goodness-of-fit of the model to data. The number of uredospores collected in the containers after rainfall events tended to increase with daily rainfall ($\chi^2 = 460.4$, $P < 0.0001$; Fig. 3), with high quantities observed when daily rainfall >10 mm. In addition, we highlighted an interaction between shade and container location ($\chi^2 = 16.0$, $P = 0.00296$; Fig. 4), which was still significant ($\chi^2 = 82.6$, $P < 0.0001$) when including in the model the positive relationship between the inoculum size per branch and the number of uredospores transported to the ground by rainwater ($\chi^2 = 39.9$, $P < 0.0001$). The highest quantity of uredospores was observed in the containers in full sun below the coffee bush, with an average of 51.7 uredospores lost cm^{-2} of ground after a rainy day (adjusted mean). The number of uredospores recovered in the other studied conditions were ranked as follows (adjusted means): full sun in the interval between coffee bush rows (41.7 uredospores cm^{-2} of

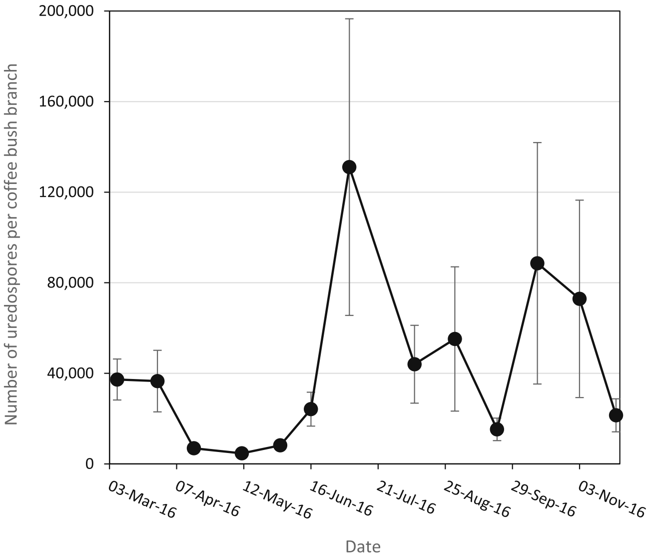

Fig. 1. Evolution of the number of uredospores produced per coffee bush branch (means and standard errors).

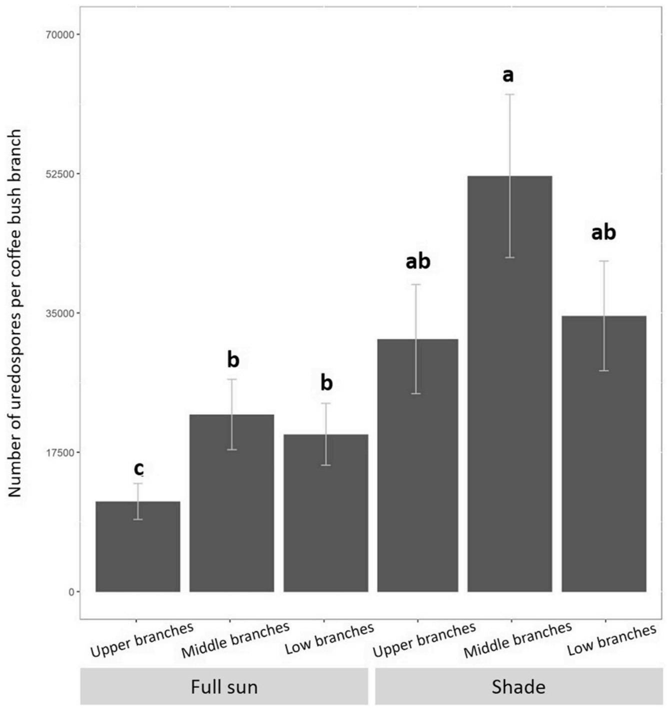

Fig. 2. Adjusted means of the number of uredospores produced per coffee bush branch as a function of branch stratum and shade condition. Predicted values deduced from the generalized additive model with mixed effects (GAMM) on sporulation. Bars represent standard errors.

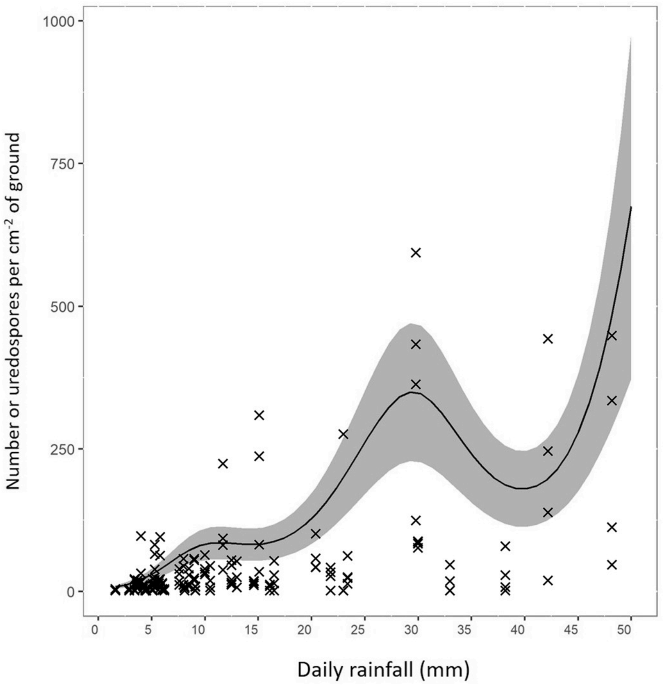

Fig. 3. Relationship between daily rainfall and wash-off of uredospores. Predicted values deduced from the generalized additive model with mixed effects (GAMM) on uredospore wash-off by rain. Area represents standard error. Dots represent means of 15 data below the coffee bushes and 10 data in the interval between coffee bush rows.

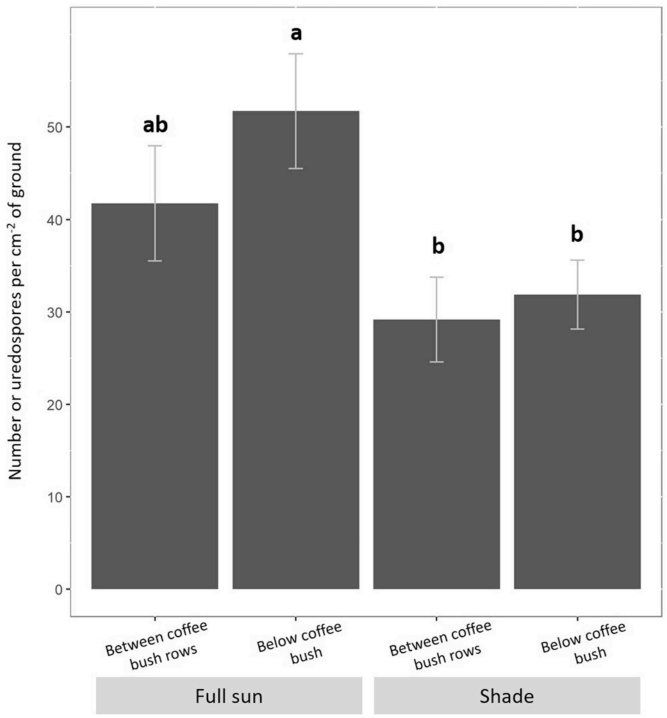

Fig. 4. Adjusted means of the number of uredospores lost by wash-off as a function of shade condition and container location. Predicted values deduced from the generalized additive model with mixed effects (GAMM) on wash-off. Bars represent standard errors.

ground), below shade trees and below the coffee bush (31.9 uredospores cm^{-2} of ground) and below shade trees in the interval between coffee bush rows (29.2 uredospores cm^{-2} of ground). On average, we obtained 1.62 times more uredospores in full sun than below shade trees in the containers located below the coffee bush. When including the inoculum size per branch as a covariate, this multiplicative factor was increased to 2.49.

3.3. *Uredospore deposition*

Our model fitted well the data with $r^2 = 0.97$ between observed and fitted data. The number of uredospores recovered after their deposition on apparently healthy leaves was very low, 0.79 cm^{-2} of leaf on each side (adjusted mean). However, we found an interaction between shade and coffee branch stratum ($F = 54.4$, $P < 0.0001$). Uredospore deposition differed in *Shade* and *Full Sun* treatments (Fig. 5), with 0.92 uredospore cm^{-2} of leaf on each side, deposited below shade trees on average, and only 0.65 in full sun (adjusted means), 1.43 times more below shade trees than in full sun. In addition, uredospore distribution within the coffee bush was homogenous below shade trees, whereas in full sun, fewer deposited uredospores were quantified in the upper branches (0.52 cm^{-2} of leaf on each side, adjusted mean) compared with the low and middle strata (0.70 cm^{-2} of leaf on each side, adjusted mean) (Fig. 5). When including the positive relationship between the inoculum size per branch and the number of deposited uredospores on apparently healthy coffee leaves in the model ($F = 55.1$, $P < 0.0001$), we still found an interaction between shade and coffee branch stratum ($F = 18.2$, $P < 0.0001$). However, this effect was slightly weakened: we only found 1.21 (instead of 1.43) more uredospores deposited below shade trees compared with full sun. The number of deposited uredospores did not differ significantly according to the side of the leaf and we did not find any effect of daily rainfall on deposited uredospores.

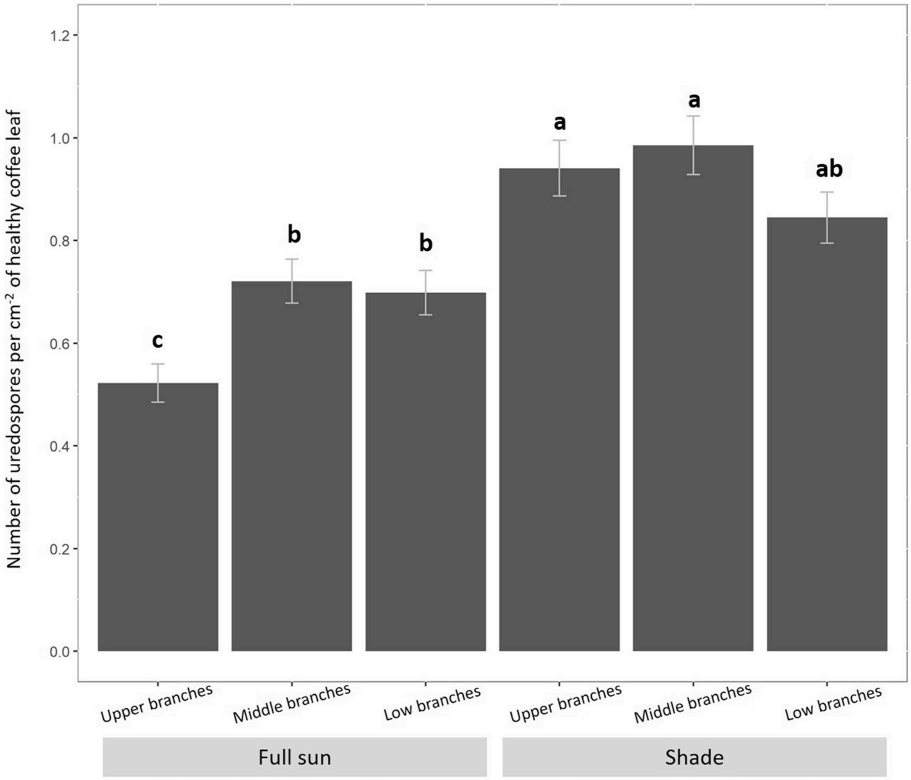

Fig. 5. Adjusted means of the number of uredospores deposited on apparently healthy coffee leaves as a function of shade condition and coffee bush branch stratum. Predicted values deduced from the generalized additive model with mixed effects (GAMM) on deposition. Bars represent standard errors.

3.4. Throughfall and kinetic energy of the raindrops

The goodness-of-fit of the throughfall model was also good with $r^2 = 0.93$ between observed and fitted data. We found an interaction between daily rainfall, shade and the position of the containers located at ground level (LRT = 41.31, $P < 0.0001$). The quantity of rainwater collected increased almost linearly with daily rainfall (except in full sun between coffee bush rows, with daily rainfall >37 mm, Fig. 6), but the slope of the relationship depended on the shade condition and the position of the container. In full sun, 9.8 mm were collected and only 7.7 mm below shade trees (adjusted means), for an average incident rainfall of 13.1 mm. However, the rainfall collected at ground level in the interval, between coffee bushes was very similar: 12.6 mm in full sun and 13.1 mm below shade trees (adjusted means).

The raindrop kinetic energy model fitted adequately the data with $r^2 = 0.68$ between observed and fitted data. We also found an interaction between rainfall and shade (LRT = 9.51, $P = 0.0002$). The measured kinetic energy was positively dependent on the quantity of rainfall accumulated during the 24 h periods when splashcups were exposed to rain, because this kinetic energy is a sum of kinetic energies of rain events that occurred during this period (Fig. 7). There were no differences between *Shade* and *Full Sun* treatments when daily rainfall was less than 5 mm. Above this limit, raindrop kinetic energy was larger below shade trees than in full sun, with no significant differences according to the location of the splashcups below shade trees (Fig. 7). On average, the kinetic energy below shade trees was twice as high as that measured in full sun: 269.3 J m^{-2} versus 133.6 J m^{-2} (adjusted means).

4. Discussion

Despite having smaller coffee yields below shade trees, meaning a reduced receptivity to CLR (Lopez-Bravo et al., 2012), we found that sporulation was more abundant below shade trees than in full sun. This is consistent with the higher incidence and severity found below shade trees in this trial in previous years (Lopez-Bravo et al., 2012). Recently, however, it has been demonstrated in controlled conditions that the number of uredospores produced was larger under intense light than under low light, as that found below shade trees (Toniutti et al., 2017). Our results indicate that shade trees promote CLR by protecting uredospores from wash-off by rain, whereas in full sun, the large quantity of uredospores produced is more easily depleted during rainfall events. This effect, which has never been reported before, is possibly the main propitious effect of shade trees on CLR. To have a better idea of its magnitude, we estimated the average number of uredospores present on leaves at a coffee bush level, considering a five-year-old coffee bush with 100 branches: 3 953 900 uredospores below shade trees and only 1 782 400 in full sun. Now, considering that the largest branch is 0.6 m long for a dwarf variety, the coffee bush canopy cover is therefore about 11 304 cm^2, and the average number of uredospores lost below the coffee bush due to wash-off by rain in full sun is 11 304 × 51.7 = 584 417 uredospores, whereas this number is 11 304 × 31.9 = 360 598 below shade trees. That means that, after a rainfall event, in full sun, on average, 100 * 584 417/(584 417 + 1 782 400) = 25% of the inoculum produced is lost by rain wash-off below the coffee bush, compared with 100 * 360 598/(360 598 + 3 953 900) = 8% only below shade trees. The percentages are even higher if we take into account the number of uredospores recovered in the interval between coffee bush rows, but slightly diminished if we take into account the number of uredospores deposited on leaves. However, both numbers are not easy to evaluate. Despite being very approximate, these figures illustrate the importance of uredospore wash-off by rain for CLR epidemic regulation, as already proposed for the *Puccinia* spp., causal agents of wheat rust diseases (Sache, 2000) and quantified for *P. arachidis* (Savary and Janeau, 1986). This was also previously suggested for CLR (Savary et al., 2004). Our findings reinforce the view that the low rainfall regime observed in 2012, in the second half of the year, when CLR incidence usually increases, helps explain the high intensity CLR epidemics that the region experienced that year (Avelino et al., 2015). In general, any factor affecting uredospores washed off by rain will have heavy consequences on CLR development. Shade is one of these factors.

The higher quantity of uredospores deposited on apparently healthy coffee leaves below shade trees compared with in full sun, is partially explained by the higher inoculum amount found below shade trees. When including the inoculum size per branch in the model, shade trees effects were weakened; however, they did not totally vanish. We hypothesize that this increased deposition below shade trees is due to the improved conservation of uredospores (as explained before), and to a more efficient dispersal (see just below).

In full sun, the lowest parts of the coffee bush showed more uredospores produced and deposited. This trend is probably the consequence of the uredospore wash-off. Part of the washed-off uredospores will be deposited during their transport towards the ground. These will accumulate in the lowest parts of the coffee bush, causing more infections and lesions and therefore producing more uredospores, on those parts. These results help explain gradients of incidence and severity that have been reported in Colombia and Mexico in full sun or slightly shaded coffee plots (Avelino et al., 1991; Villegas-García and Baeza-Aragón, 1990). However, under dense shade, we found large quantities of uredospores produced and deposited in the upper branches, explained by the reduced wash-off of uredospores by rain and the increased uredospore dispersal in the air (Boudrot et al., 2016). In our study, we verified that the kinetic energy of the raindrops was increased below shade trees. These raindrops will heavily impact the coffee leaves and facilitate the release of the uredospores in the air and their spread toward different strata of the coffee bush, contributing to homogenize the distribution of CLR at coffee bush level.

Shade trees probably reduce wash-off of uredospores by rain because

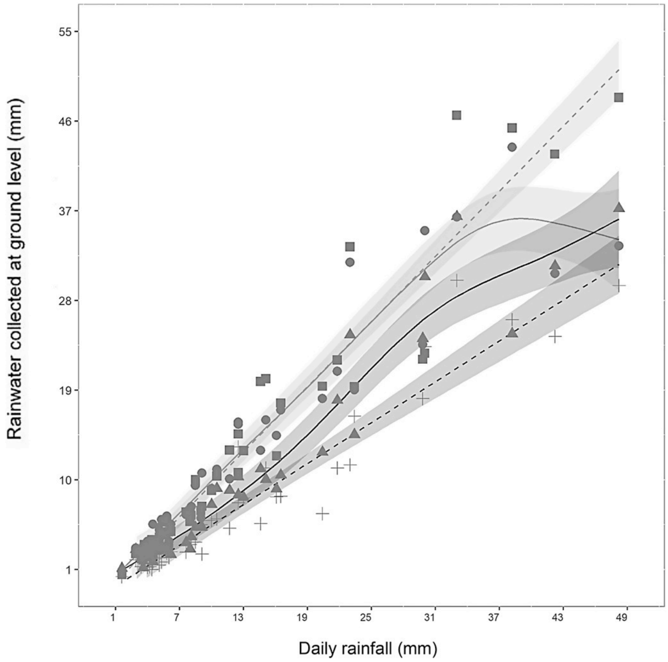

Fig. 6. Relationship between daily rainfall and rainwater collected at ground level as a function of shade condition and container location. Predicted values deduced from the generalized additive model with mixed effects (GAMM) on throughfall. Dotted line, light gray and squares: Below shade trees, in the interval between coffee bush rows; Solid line, light gray and solid circles: Full sun, in the interval between coffee bush rows; Dark gray, solid line and triangles: Full sun, below coffee bushes; Dotted line, dark gray and crosses: Below shade trees, below coffee bushes. Areas represent standard errors. Dots represent means of 15 data below the coffee bushes and 10 data in the interval between coffee bush rows.

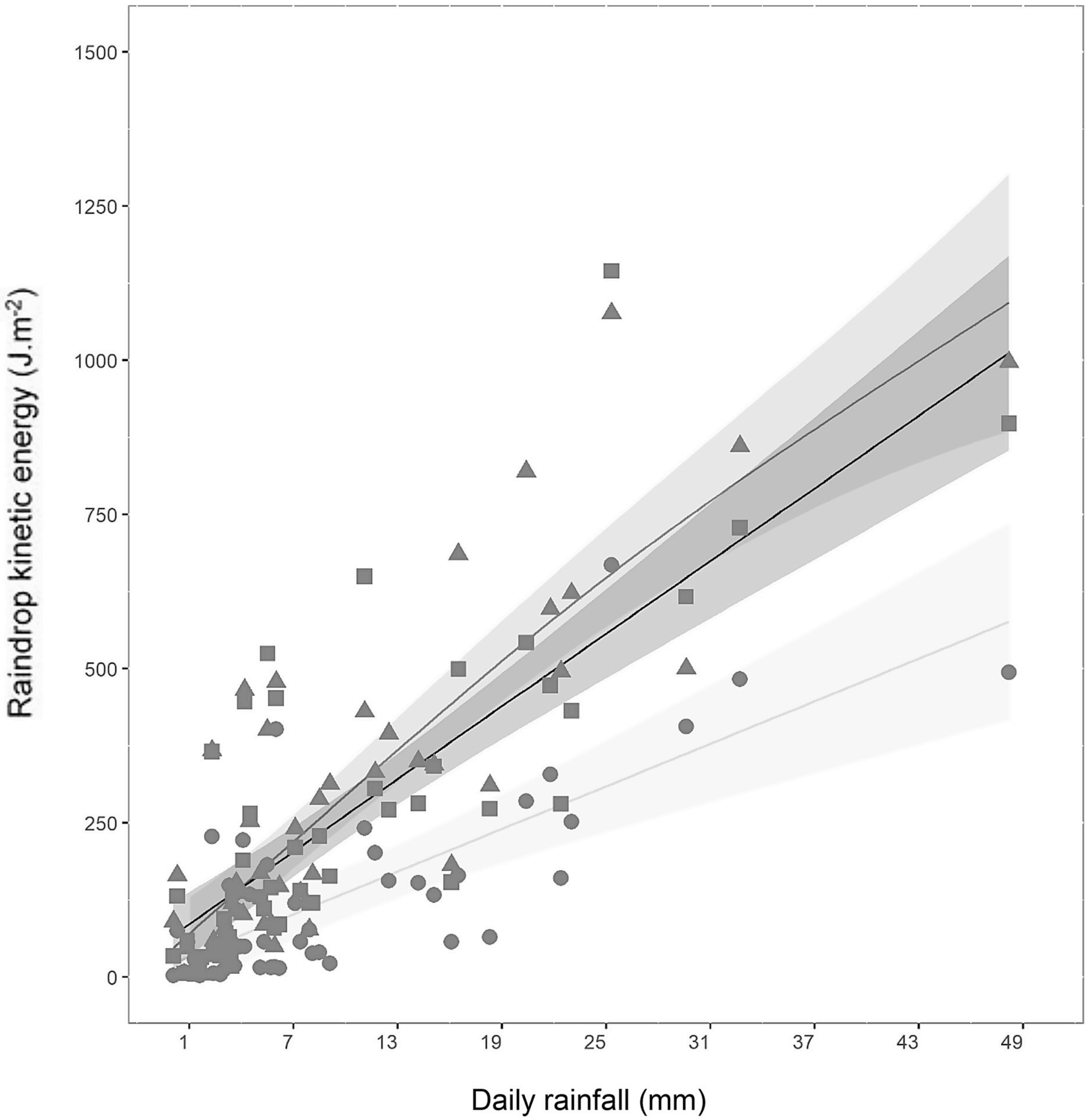

Fig. 7. Relationship between daily rainfall and raindrop kinetic energy as a function of shade condition and splashcup location. Darker gray and squares: Below shade trees, at the edge of the longest branch; Gray and triangles: Below shade trees, half distance from tree trunk and the edge of the longest branch; Lighter gray and solid circles: Full sun; Predicted values deduced from the generalized additive model with mixed effects (GAMM) on kinetic energy. Areas represent standard errors. Dots represent means of nine data in full sun and 12 in each shade condition.

the quantity of rainwater reaching the coffee bushes is reduced due to rain interception by shade trees and stemflow (Siles et al., 2010). Throughfall below coffee bushes was on average 21% lower below shade trees than in full sun. However, throughfall was equivalent below shade trees and in full sun in the interval between coffee bush rows, suggesting an additional mechanism. Similar trends were obtained by Siles et al. (2010). Our results can help explain this situation. We hypothesize that the higher kinetic energy of the raindrops below shade trees increased their splash projection distance after hitting coffee leaves (Madden, 1997). Below shade trees, part of the raindrops hitting coffee bushes were therefore recovered in the containers located in the interval between coffee bush rows. This phenomenon probably did not occur to the same extent in full sun where raindrop kinetic energy is lower. We deduced that the reduced throughfall below coffee bushes and below shade trees was not only due to the interception of rainwater by shade trees and stemflow but also by a reduced interception of the rainwater by the coffee bushes. Raindrops interception by plant foliage is higher when the raindrop size is smaller (Calder, 1996). The higher kinetic energy of the raindrops below shade trees compared with full sun indicates that the size of raindrops was larger below shade trees than in full sun (Vis, 1986). This higher kinetic energy limited the interception by coffee bush foliage, and so contributed to reduce throughfall below coffee bushes and uredospores washing-off.

Shade trees are essential for adapting to increasing temperatures, but shade trees have unwanted effects on CLR. Increasing throughfall and reducing raindrop kinetic energy below shade trees appears to be crucial to improved CLR regulation, by increasing wash-off of uredospores by rain and reducing uredospore dispersal in the air during rainfall. Some shade tree functional traits can help, such as small, pinnate, flexible leaves and low leaf-area index that will reduce rainwater interception and accumulation on the canopy, or small trees that will reduce the height of fall of raindrops, and therefore their kinetic energy (Goebes et al., 2015). In addition, shade tree management seems necessary. Pruning of shade trees during the rainy season will open the canopy and increase throughfall.

5. Conclusions

By studying each CLR stage separately, we identified key influencing factors along with action levers that can be mobilized to manage the disease. The selection of appropriate shade tree functional traits increasing throughfall and reducing raindrop kinetic energy in the understory is a promising research area for improving CLR management.

Author contributions

Jacques Avelino: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing-Original draft, Writing-Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **Sergio Vílchez:** Methodology, Software, Formal analysis, Resources, Data Curation, Visualization. **Marta Beatriz Segura-Escobar**: Methodology, Investigation, Data curation. **Marvin Alejandro Brenes-Loaiza:** Investigation, Data curation. **Elías de Melo Virginio Filho:** Investigation, Resources. **Fernando Casanoves:** Methodology, Validation, Formal analysis, Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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