Shade Effects on the Dispersal of Airborne *Hemileia vastatrix* Uredospores

Audrey Boudrot, Jimmy Pico, Isabelle Merle, Eduardo Granados, Sergio Vílchez, Philippe Tixier,
Elías de Melo Virginio Filho, Fernando Casanoves, Ana Tapia, Clémentine Allinne, Robert A. Rice, and Jacques Avelino

First author: Agrocampus Ouest, 65, rue de Saint Brieuc 35000 Rennes; first, third, and twelfth authors: CIRAD, UPR Bioagresseurs, F-34398, Montpellier, France; second author: INIAP, Estación experimental Joya de los Sachas 220350, Orellana, Ecuador; second, fifth, sixth, seventh, eighth, tenth, and twelfth authors: CATIE, 7170, Cartago, Turrialba, 30501, Costa Rica; third author: ENSAIA, Protection des cultures, 2 Avenue de la Forêt de Haye, TSA 40602, 54518 Vandæuvre-lès-Nancy, France; fourth and ninth authors: Universidad de Costa Rica, sede del Atlántico, Turrialba, Costa Rica; sixth author: CIRAD, Persyst, UPR 26, TA B-26/PS4, Boulevard de la Lironde, 34398, Montpellier Cedex 5, France; tenth author: CIRAD, UMR System, SupAgro Montpellier, 2 place P. Viala, 34060 Montpellier, France; eleventh author: Migratory Bird Center, Smithsonian Conservation Biology Institute MRC 5503, Washington, DC, 20013-7012; and twelfth author: IICA-PROMECAFE, AP. 55, 2200 Coronado, San José, Costa Rica.

Accepted for publication 26 January 2016.

ABSTRACT

Boudrot, A., Pico, J., Merle, I., Granados, E., Vílchez, S., Tixier, P., Virginio Filho, E. M., Casanoves, F., Tapia, A., Allinne, C., Rice, R. A., and Avelino, J. 2016. Shade effects on the dispersal of airborne *Hemileia vastatrix* uredospores. Phytopathology 106:572-580.

Hemileia vastatrix caused a severe epidemic in Central America in 2012–13. The gradual development of that epidemic on nearly a continental scale suggests that dispersal at different scales played a significant role. Shade has been proposed as a way of reducing uredospore dispersal. The effect of shade (two strata: *Erythrina poeppigiana* below and *Chloroleucon eurycyclum* above) and full sun on *H. vastatrix* dispersal was studied with Burkard traps in relation to meteorological records. Annual and daily patterns of dispersal were observed, with peaks of uredospore capture obtained during wet seasons and in the early afternoon. A maximum of 464 uredospores in 1 day (in 14.4 m^3 of air) was recorded in October 2014. Interactions between shade/full sun and meteorological conditions were found. Rainfall, possibly intercepted by tree cover and redistributed by raindrops of higher kinetic energy, was the main driver of uredospore dispersal under shade. Wind gusts reversed this effect, probably by inhibiting water accumulation on leaves. Wind gusts also promoted dispersal under dry conditions in full sun, whereas they had no effect under shaded conditions, probably because the canopy blocked the wind. Our results indicate the importance of managing shade cover differentially in rainy versus dry periods to control the dispersal of airborne *H. vastatrix* uredospores.

Additional keywords: agroforestry, *Coffea arabica*, coffee rust, Costa Rica.

Coffee leaf rust (CLR) ranks as one of the most serious diseases of coffee worldwide. The great epidemic of 2012–13 in Central America resulted in a 16% reduction in production levels that year compared with the 2011–12 harvest, and a subsequent reduction of 10% for the 2013–14 growing season (Avelino et al. 2015; McCook and Vandermeer 2015). CLR is caused by *Hemileia vastatrix*, an obligate parasite that affects the living leaves of the genus *Coffea* and particularly the cultivated species *C. arabica*. CLR induces defoliation which, if severe, can result in the death of branches, thereby causing high primary and secondary losses affecting the yield of the current and following years (Zadoks and Schein 1979).

The main trigger of this great epidemic seemed to be meteorological. Indeed, the meteorological conditions were unusual compared with climate records. Early onset of the rainy season, which likely led to early development of the epidemic by stimulating the sporulation of necrotic and latent lesions, occurred simultaneously with a reduction in the daily thermal amplitude, which possibly shortened the CLR latent period and explained the high epidemic intensity (Avelino et al. 2015; McCook and Vandermeer 2015). Although this coffee rust epidemic reached levels never seen before, differences in the levels of attack were reported among affected zones and even among farms located very close to one another (Avelino et al. 2015; Villarreyna 2014). The great diversity of coffee production systems (Toledo and Moguel 2012) could partly explain these differences (Avelino et al. 2006, 2015). The reduction and misuse of inputs such as fertilizers and fungicides have been highlighted as important factors in the variability of the 2012–13 epidemic (Avelino et al. 2015; Villarreyna 2014). Fertilizers applications help to replace lost infected leaves with new healthy ones, preventing branch death and heavy losses (Avelino et al. 2006, 2015; Cristancho et al. 2012).

Shade is another factor creating heterogeneity between farms and related to the management of CLR (Avelino et al. 2004, 2006). However, shade effects on CLR are still controversial and the details unresolved (McCook and Vandermeer 2015). Some authors have reported that shade increases CLR incidence (Staver et al. 2001), whereas others have found that shade reduces it (Soto-Pinto et al. 2002). Still others maintain that shade effects depend on shade tree species (Salgado et al. 2007) or on the fruit load of coffee trees (Avelino et al. 2004, 2006; Lopez-Bravo et al. 2012). These controversies can be explained by the fact that shade stimulates multiple ecological mechanisms resulting in opposing effects, which also interact with meteorological variables (Avelino et al. 2004). The balance of these effects is difficult to establish (Avelino et al. 2011; Schroth et al. 2000). For instance, shade helps to control CLR by regulating fruit load and decreasing leaf receptivity to the fungus (Lopez-Bravo et al. 2012). Shade also reduces CLR by favoring its hyperparasite, the entomopathogenic fungus *Lecanicillium lecanii* (Staver et al. 2001; Vandermeer et al. 2014). However, shade can facilitate CLR development by creating a more favorable microclimate for the pathogen (Lopez-Bravo et al. 2012). Increased leaf wetness under shade during the rainy season, for example, favors *H. vastatrix*

Corresponding author: J. Avelino; E-mail address: jacques.avelino@cirad.fr

*The *e*-Xtra logo stands for "electronic extra" and indicates that two supplementary tables are published online.

---

http://dx.doi.org/10.1094/PHYTO-02-15-0058-R

germination, which is free-water dependent. Similarly, more stable temperatures in shaded plantations shorten the latent period of the disease (Lopez-Bravo et al. 2012). As shade effects can vary depending on the process considered, a reductionist approach by assessing the effect of shade on CLR prevalence seems inadequate. CLR prevalence is the ultimate result of all the shade effects on all the disease processes. A process-based approach (Zadoks and Schein 1979) is required to understand and quantitatively integrate the effects of shade on CLR epidemiology.

The recent epidemic affected a large region of Latin America, originating in 2008 in Colombia, then reported in 2012 in Central America, Mexico, and the Caribbean, and finally in Peru and Ecuador in 2013 (Avelino et al. 2015; Cristancho et al. 2012; McCook and Vandermeer 2015). This gradual development over several years in a changing weather regime might indicate that aerial transport of uredospores at different scales, among leaves and among plants (Skelsey et al. 2010; Zadoks and Vandenbosch 1994; Zawolek and Zadoks 1992) and especially among farms and possibly among landscapes, played a key role in the epidemic development (Avelino et al. 2015; Vandermeer and Rohani 2014). In that respect, based on positive relationships found between landscape openness and coffee rust incidence (Avelino et al. 2012), it has been suggested that deforestation and removal of shade trees in coffee plantations might favor spore dispersal at medium and large distances, by eliminating the windbreak function of trees (McCook and Vandermeer 2015; Vandermeer et al. 2014).

In the 1970s, wind and rainfall were identified as the main meteorological factors influencing dispersal (Becker 1977a; Becker and Kranz 1977; Becker et al. 1975). Despite in-depth information of meteorological, biological and human factors contributing to *H. vastatrix* dispersal being gathered during those years, there is no available knowledge about shade effects.

The objective of this study was to contribute to the understanding of the effects of shade trees on the dispersal of the airborne *H. vastastrix* uredospores at the plot scale. This is the first step in the process-based approach we propose. We hypothesized that shade trees interact with meteorological variables for the aerial dispersal of *H. vastatrix* and that shade attributes of interest for fighting coffee rust can be deduced from these interactions.

MATERIALS AND METHODS

Climatic conditions at the study site and studied period. The experiment was conducted from August 2013 to December 2014 at the experimental station belonging to the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), located in Turrialba, Costa Rica (9°53′N, 83°38′W). The climatic conditions in the area are conducive to coffee rust development. The area is characterized as a low-elevation (600 m above sea level) wet zone (2,996 mm annual rainfall on average over the last 72 years) with no marked dry season due to a strong Caribbean influence. However, rainfall is less abundant between February and April, with 364 mm on average over these 3 months. In 2013, the minimum monthly rainfall was reached in February with 21 mm. In 2014, the minimum was recorded in March with 10 mm. The mean annual temperature is 22°C (56-year average), showing very little variation. The monthly averages varied from 21 to 23°C in 2013 and from 21 to 24°C in 2014. Our study spanned two rainy seasons and one dry season, starting during the first rainy season, which finished at the end of January 2014. The second rainy season started at the beginning of April 2014, and extended through to the end of the experiment. The period between these two wet seasons, from 21 January 2014 to 8 April 2014, was a well-defined dry season (Fig. 1A), in which daily rainfall did not exceed 8 mm.

Experimental design and treatments. The study was carried out in a long-term experiment described in Haggar et al. (2011), comparing different agroforestry systems: 20 treatments resulting from the combination of six types of shade and full sun exposure, with two management strategies, conventional and organic, and two intensity levels of input application each. All treatments were replicated three times, in an incomplete randomized block design. This experiment of approximately 9.2 ha was set up in 2000 with the dwarf cultivar Caturra of the species *Coffea arabica*, susceptible to most coffee rust races, at low plant spacing (2 × 1 m^2, 5,000 coffee bushes ha^{-1}). Two coffee plants were planted in each hole. The experiment showed a reduction in coffee production in 2013–14 compared with the 2012–13 season, explained by the large number of dead branches and severe pruning of the coffee trees after the 2012–13 epidemic. However, the 2014–15 season showed an improvement in production.

Only 2 out of the 20 treatments were considered for our study. They received the same management but differed in the presence or absence of shade: (i) dense shade and (ii) full sun exposure, both managed in the least intensive conventional system. Dense shade and full sun plots had an average area of 775 m^2. The dense shade treatment involved a combination of *Erythrina poeppigiana* (164 trees ha^{-1}), also known as the *poró* tree, and *Chloroleucon eurycyclum* (*cashá* tree) (78 trees ha^{-1}). Both are leguminous and are considered to be service trees due to their N-fixing properties. *Chloroleucon eurycyclum*, which is also used for its wood, is characterized by a high (14 to 16 m) and wide canopy, whereas *E. poeppigiana* displays a more compact canopy that reaches 4 to 5 m in height and exhibits substantial biomass production. Percent shade cover was assessed each month with a spherical densiometer used to measure forest overstory foliage density (Lemmon 1957). The average shade cover was 66 and 64% for the second half of 2013 and 2014, respectively. Drastic pruning (pollarding) of the *poró* tree was carried out in January to February and consisted in eliminating the tree cover at 2 m in height, maintaining only three branches. This pruning was followed by a second one in June or July, at a height of 4 m, in order to regulate the shade. The least intensive conventional management was characterized by the application of soil fertilizers (500 kg ha^{-1} of the complete formula 18-5-15, N-P-K; 180 kg ha^{-1} of a nitrogen fertilizer, 33.5-0-0, N-P-K) and foliar mineral fertilizers (B, Cu, Zn, Ca, Mg, Fe, Mn, and Mo). Use of a synthetic insecticide (chlorpyrifos) controlled the coffee berry borer (*Hypothenemus hampei*). Weed control was based on selective management by manual clearing and applications of glyphosate between the coffee rows. To control diseases, especially CLR, the technical staff in charge of this long-term experiment used a copper-based fungicide (50% Cu) in 1 kg ha^{-1} doses combined with a systemic product (cyproconazole 10% WG) in 0.4 liter ha^{-1} doses. In 2013, the copper-based fungicide was applied twice, in March and April, and cyproconazole once in June. In 2014, copper was applied once in March and cyproconazole twice, in January and July.

Quantification of the *Hemileia vastatrix* inoculum stock. The inoculum quantity was assessed every 21 days by collecting all the diseased leaves from six branches randomly selected from six different plants in each study plot. Branches were selected from different levels on the coffee plant (two from the upper level, two from the middle and two from the lower level). The uredospores were scraped from the sporulating lesions using small gelatinous capsules and then transferred to microtubes to which 1 ml of sterilized water was added with 2.5% Tween. Tween enabled the dispersion of uredospores in the suspension, as they aggregate due to hydrophobic properties. Samples were then placed for 5 min at 30°C in an ultrasonifier. Finally, each sample was counted three different times using a Neubauer counting chamber with the 10× power objective lens. The average count of the six branches was calculated to obtain the inoculum quantity per branch.

Volumetric spore trapping. To monitor the dispersal of rust uredospores, we used two SporeWatch electronic spore and pollen samplers (Burkard Scientific Ltd., Uxbridge, Middx, UK), whose design is based on the traditional 7-day Burkard trap (Wili 1985). The main difference is that the rotation speed of the drum can be adjusted via the control panel. Trapping was carried out over a total of 42 weeks, first between August 2013 and July 2014, in which the traps were installed 3 weeks out of 4 weeks, and then after July 2014, in which they were installed 2 weeks out of 4 weeks. Traps were placed in the center of each of the two studied treatments in the same block during a trapping week. The block was randomly selected between the two blocks that were not used in the previous trapping week (except for the first trapping week, where random selection was carried out using the three blocks). The intake orifice of the trap was installed 1.5 m above the ground, corresponding to the upper level of coffee trees. Air was sampled at 10 liter min^{-1} and was checked on each trapping day using a Burkard flowmeter in order to detect any variation in the aspiration rate. The tape, which was prepared and mounted according to the instructions for the

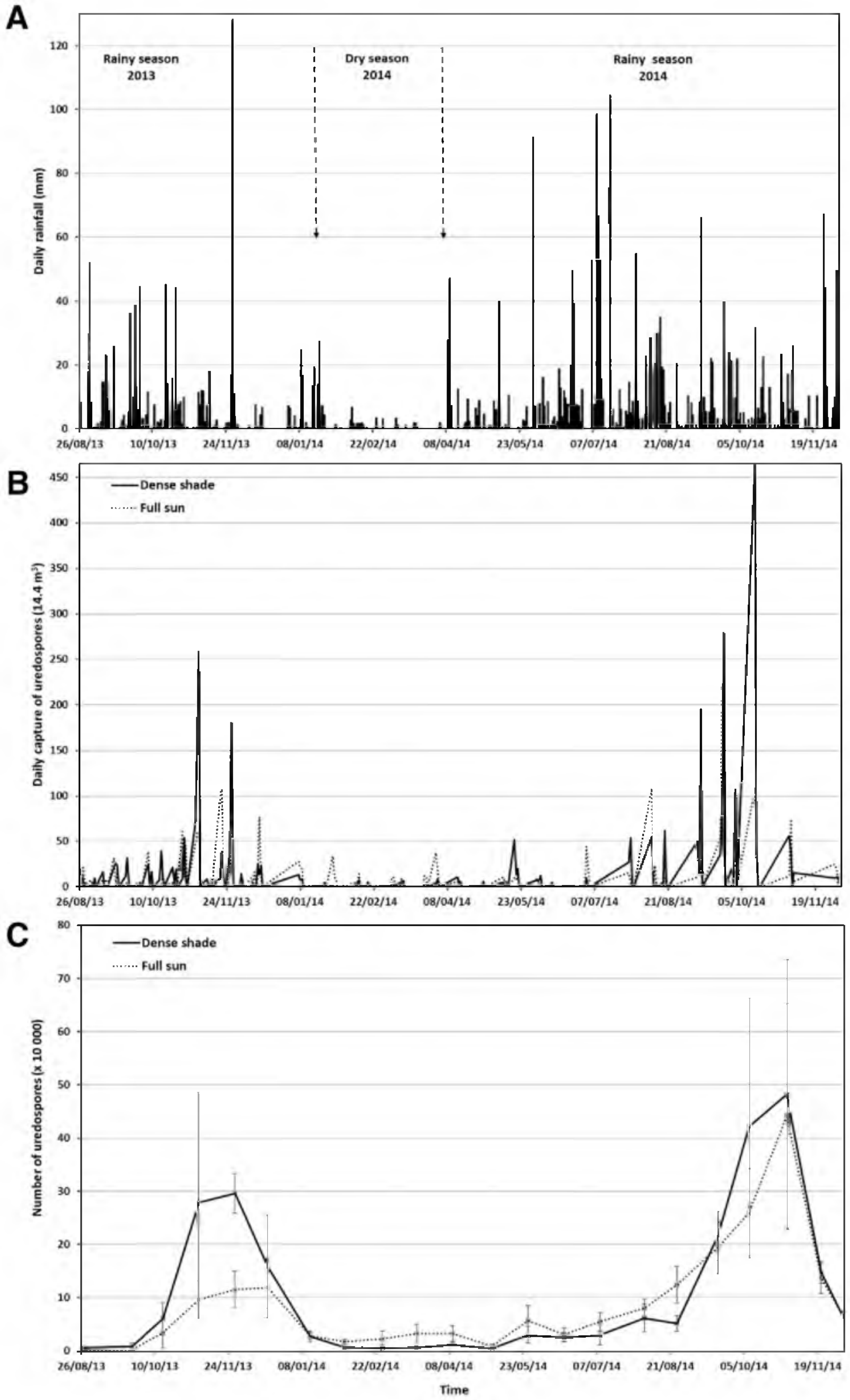

Fig. 1. Time course of **A,** daily rainfall, **B,** daily capture of *Hemileia vastatrix* uredospores, and **C,** inoculum stock per branch (total number of uredospores averaged over three replicates; error bars represent standard errors) from August 2013 to December 2014.

Burkard sampler, was collected at the end of each trapping period. The trapping period lasted three days each week. However, traps were programmed to operate for 4 days in order to obtain strips of 42 mm, corresponding to 12 h of capture. *Hemileia vastatrix* uredospores were counted under a compound microscope at 100× magnification and 85% of each strip was read. Uredospores measure 25 μm on average, have an orange color and a cuneiform shape with the convex side bearing spines or warts (Kushalappa and Eskes 1989).

Microclimate recording. Two Hobo H21-001 weather stations (Onset Computer Corporation, Bourne, MA) were positioned in one of the three blocks of the experiment, for each treatment, with one relative humidity sensor (S-THB-M00x) and one wind speed sensor (S-WSA-MO003). Sensors were positioned identically in both treatments. Anemometers measuring wind speed and wind gusts were placed above the coffee trees, 2.2 m above the ground. The data logger was programmed to record every 30 s and the mean of the data were calculated every 15 min. Maximum wind gusts were sampled every 2 s and an average was also calculated every 15 min. The relative humidity sensors were previously calibrated and corrections were applied to homogenize the data. HOBOware Pro software was used to collect the data from the data logger every 8 to 12 days. Rainfall data were recorded by a pluviograph in the CATIE weather station located on the CATIE campus.

Biological and meteorological predictors of uredospore dispersal (Table 1). *Daily inoculum quantity per branch (Inoc).* The quantity of inoculum was assessed every 21 days, not necessarily coinciding with the trapping days. Therefore, the daily inoculum quantity on each trapping date was estimated by calculating the time-weighted mean of the values obtained in the two evaluations closest to each considered trapping date. This provided an estimate of the inoculum values for each trapping day in the two treatments.

Hourly relative humidity (%) (RH_DS and RH_FS). Relative humidity (*RH*) was measured every 15 min, providing a relative humidity value for each hour of the day by averaging the four readings in the hour. This same calculation was done for both treatments. The resulting variables were *RH_DS* for dense shade and *RH_FS* for full sun.

Hourly wind gusts (Wind Gust). The maximum wind gust values were recorded every 15 min. The spatial organization of the trial, with different treatments close to one another, led to great variability in the wind speeds. The hourly wind gust (the wind gust value in each hour of the day) represented the maximum of the eight values recorded in both shade and sun conditions.

Hourly rainfall (Rainfall). The millimetric bands obtained from the pluviograph were read to obtain the rainfall data for each hour of the trapping days.

Hours with rain (HR) and hours without rain (HWR). Two additional variables were used to deepen the study of the influence of rain on dispersal. Time without rain corresponds to the number of hours of the day accumulated since the last rain (≤ 0.1 mm h^{-1}). Time with rain is the inverse variable; it is accumulated from the moment where rainfall >0.1 mm h^{-1}. $HR = 0$ ($HWR > 0$) means that it was not raining in the hour of trapping. $HWR = 0$ ($HR > 0$) means that it was raining in the hour of trapping.

Statistical analyses. Pairwise Pearson correlations were first calculated with the meteorological variables in order to select those that were not or less correlated for modeling uredospore dispersal.

Due to nonnormal distribution, the response variable, i.e., the number of uredospores dispersed, in absence or presence of shade, under different meteorological conditions, was modeled using a generalized linear mixed model (GLMM) (Bolker et al. 2009), with a Poisson distribution and log link, which are appropriate for analyzing counts (O'Hara and Kotze 2010). This modeling approach has already been proposed for analyzing epidemiological data in plant diseases (Garrett et al. 2004; Madden et al. 2002; Stroup 2015). To account for the repeated measures and plot and sampling structures, we included in the model the following random effects (Table 1): (i) the variable *Season*, which has three possible values (two wet seasons and one dry season); (ii) the crossed variables *Block_week* and *Block_week_Shade*, which represent the trapping week and the experimental unit (plot in which the Burkard trap was installed each week), respectively (Stroup 2015); and (iii) the variable log(*Inoc*). Adding a random unit-level effect (*Block_week_Shade*) allowed us to take into account overdispersion (Stroup 2015). Including log(*Inoc*) as a random factor allowed us to study the effects of shade and weather on uredospore dispersal corrected by the evolution with time of the inoculum stock.

To facilitate disentangling the effect of rainfall in interaction with other meteorological factors and shade, modeling was divided into the study of dispersal for positive values of *Rainfall* (>0.1 mm h^{-1}) in a first model (model 1) and the study of dispersal for positive values of *HWR* in a second model (model 2). In model 1, the explanatory variables were set as *Shade* (*Dense Shade* or *Full Sun*), *hour* and its quadratic term $hour^2$, and the meteorological variables selected using the pairwise Pearson correlation analysis. All the double and triple interactions between *Shade* and the meteorological variables were also included, as well as the double and triple interactions between meteorological variables. The exact same strategy was adopted for model 2.

Model selection was conducted by comparing the complete model with reduced models. For that purpose, we used the Akaike information criterion (AIC), Bayesian information criterion (BIC), and likelihood ratio tests (LRTs). The best model had lowest values of AIC and BIC. Variables removed in the model were those that were not significant (Zuur et al. 2009). However, when interactions were significant, simple factors involved in these interactions as well as lesser order interactions with these same factors were automatically kept in the model, even if their effect was not significant. The goodness of fit of each model was assessed by correlating predicted and observed values (pseudo-R^2). In addition, we tested the null hypotheses that slope and intercept of the obtained regression lines were equal to 1 and 0, respectively (Y = X), by using linear combinations of parameters (Harrell 2001).

Models were fitted by the Laplace approximation using the 'glmer' function in the 'lme4' package (Bates et al. 2014). All graphical representations were obtained using the "Lattice" package (Deepayan 2008). The prediction graphs were generated in the ranges in which most data had been observed to avoid any inappropriate extrapolation. All statistical analyses were

TABLE 1. Studied predictors of airborne uredospore dispersal, codes, and units

Predictors	Code	Unit
Shade provided by *Erythrina poeppigiana* and *Chloroleucon eurycyclum*	*Dense Shade*	–
Full sun exposure	*Full Sun*	–
Hour of the day	*hour*	H
Daily inoculum quantity per branch or inoculum stock	*Inoc*	Number of spores
Hourly relative humidity in dense shade	*RH_DS*	%
Hourly relative humidity at full sun	*RH_FS*	%
Hourly wind gusts	*Wind Gust*	m.s^{-1}
Hourly rainfall	*Rainfall*	mm
Period of time with continuous rain	*HR*	H
Period of time with continuous dry condition (with no rain)	*HWR*	H
Block of the field experiment	*Block*	–
Trapping week	*week*	Counting
Dry or wet season	*Season*	–

performed with R 2.15.2 (R Development Core Team 2014) with an alpha level of 0.05.

RESULTS

Airborne uredospore dispersal throughout the year. In all, 2,505 and 3,224 uredospores were captured in full sun and under shade, respectively, during the 172-day trapping period. The daily capture of uredospores from 26 August 2013 to 4 December 2014 revealed a period of high values in the months of October, November, and December 2013, followed by a near-absence of uredospore capture (all the more noticeable under dense shade) until July 2014 (Fig. 1B). A new increase in captures was observed from July to November 2014. The maximum was seen in that period with 464 uredospores collected on 13 October 2014 (14.4 m^3) under shade. The 2014 high dispersal period showed higher daily capture values than the same period in 2013. The same pattern of aerial dispersal was observed in full sun and dense shade, but with some variation in the capture values (Fig. 1B). The maxima of inoculum production per branch in the three successive climatic seasons we studied (26 August 2013 to 20 January 2014, 21 January 2014 to 8 April 2014, and 9 April 2014 to 4 December 2014) were 296,185 (28 November 2013), 6,519 (19 March 2014), and 482,111 (31 October 2014) uredospores, on average under shade, and 118,704 (18 December 2013), 32,370 (19 March 2014), and 441,926 (31 October 2014) in full sun (Fig. 1C).

Airborne uredospore dispersal throughout the day. A high frequency of uredospore capture occurred during daytime hours (>6% of hours with uredospore captures from 7:00 a.m. to 7:00 p.m.), whereas nighttime showed very little capture (≤6%) (Fig. 2A). The highest dispersal was observed between 11:00 a.m. and 3:00 p.m., with a peak at midday. However, it remained low with a maximum of only 37% of hours with uredospore captures. Wind gusts followed the same pattern with the highest values recorded during daytime (>2 m s^{-1} between 9:00 a.m. and 4:00 p.m.), whereas the hourly average relative humidity showed an opposite pattern with the lowest values over the same time period, particularly in hours with no rain (Table 2, Fig. 2B). Relative humidity was slightly higher under dense shade (Table 2). The highest values of hourly rainfall were obtained between 2:00 and 6:00 p.m., shortly after the wind gust peak, revealing that high values of precipitation and wind gusts rarely overlapped temporally. The statistical analyses were conducted using only the period of the day where dispersal occurred the most, from 7:00 a.m. to 7:00 p.m.

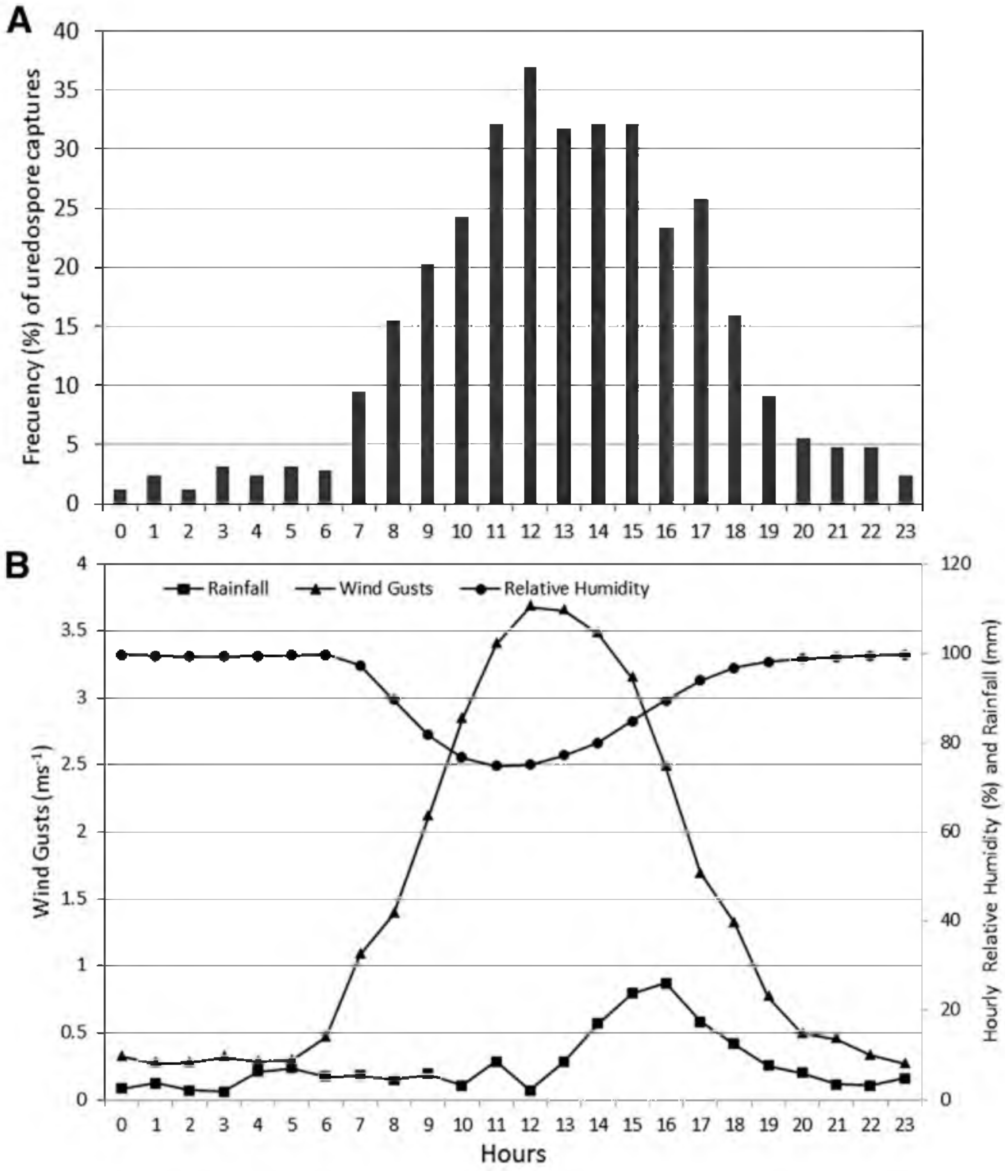

Fig. 2. Hourly frequency of **A,** *Hemileia vastatrix* uredospore captures and **B,** hourly means of meteorological variables over the 17-month study.

Relationships between meteorological variables. *Rainfall* and *Wind Gust* were the only two variables that were not significantly correlated with each other (Table 3). These two variables were then retained in the following modeling stage. All the other variables were significantly correlated to either *Rainfall* or *Wind Gust.* The highest correlations with these variables were found for *RH_DS* and *Wind Gust* ($r = -0.64$), *RH_FS* and *Wind Gust* ($r = -0.63$), *HR* (hours with rain), and *Rainfall* ($r = 0.43$) (Table 3). We then decided not to include *RH_DS*, *RH_FS*, and *HR* in the modeling stage. In addition, we adopted the variable *HWR* (hours without rain), which had a weak relationship, despite being significant, with *Rainfall* ($r = -0.14$) and *Wind Gust* ($r = 0.27$) (Table 3). For model 1, during rainy events, the selected variables were therefore *Rainfall* and *Wind Gust* (*HWR* was not included in this model as *HWR* = 0 when raining). For model 2, during dry events, the selected variables were therefore *Wind Gust* and *HWR* (*Rainfall* = 0 in dry events and then was not included in this model).

Shade effects on airborne uredospore dispersal during rainy events. All the variables were adopted in model 1 (Supplementary Table S1). However, due to the presence of the triple interaction *Shade* × *Rainfall* × *Wind Gust* in the model, we did not interpret simple and double interaction effects of factors involved in the triple interaction. The variable *hour* ($\chi^2 = 136.7$, $P < 0.001$) and its quadratic term, *hour*^{2} ($\chi^2 = 120.6$, $P < 0.001$) were the best predictors of uredospore dispersal. This effect reflected the daily pattern of uredospore capture, with higher captures during daytime and a peak at noon (Fig. 2A). The triple interaction *Shade* × *Rainfall* × *Wind Gust* showed a less significant effect on uredospore dispersal ($\chi^2 = 4.9$, $P < 0.05$). Airborne uredospore dispersal was higher overall under shade than in full sun (Fig. 3). Under dense shade, predicted dispersal increased with increasing rainfall for low *Wind Gust* values (<3 m s^{-1}), but this effect disappeared for higher *Wind Gust* values (>4 m s^{-1}). At high *Wind Gust* values, dispersal remained virtually constant (slight decrease) with the increase in *Rainfall* (Fig. 3). However, as high values of both variables rarely coincided, as seen in the daily pattern, the decrease observed in high *Rainfall* and *Wind Gust* values had to be carefully interpreted. An opposite effect of wind gusts was observed, as predicted dispersal decreased with increasing wind gusts for high *Rainfall* values (trend observed for rainfall >8 mm h^{-1}) and remained constant in the case of low *Rainfall* values (<5 mm h^{-1}). In full sun, predicted dispersal showed values close to zero that remained virtually constant for all variations of *Wind Gust* and *Rainfall* (Fig. 3). The resulting model achieved an acceptable description of the available data, with a pseudo-R^2 of 0.73 (Fig. 4). The regression line between observed and predicted values did not differ from the identity line Y = X (null intercept was not rejected, $P = 0.63$; slope = 1 was not rejected, $P = 0.32$).

Shade effects on uredospore dry dispersal during dry events. All the variables and interactions, including the triple interaction *Shade* × *HWR* × *Wind Gust,* were kept in model 2 (Supplementary Table S2). As in model 1, *hour* ($\chi^2 = 109.9$, $P < 0.001$) and *hour*^{2} ($\chi^2 = 101.3$, $P < 0.001$) were the best predictors. The triple interaction *Shade* × *HWR* × *Wind Gust* also showed a significant effect on uredospore dispersal ($\chi^2 = 6.5$, $P < 0.05$), but once again less than for *hour* and *hour*^{2}. This confirmed the great importance of the diurnal pattern of dispersal. Predicted dispersal values were slightly higher under dense shade for low values of *HWR* (this means a short time after the rain stopped) compared with full sun (Fig. 5). Under dense shade, dispersal decreased with the increase in *HWR* and this trend was not influenced by changes in wind gusts. The same effect was observed in full sun for low *Wind Gust* values, but rising wind gusts (>4 m s^{-1}) resulted in a strong increase in dispersal (Fig. 5). The model considered fitted the data poorly with a pseudo-R^2 of only 0.27. This might be explained by an underestimation of several high dispersal values (>30 uredospores h^{-1}) that was apparent when comparing predicted and observed values (Fig. 6). However, as with the previous model, the regression line between observed and predicted values did not differ from the identity line Y = X (null intercept was not rejected, $P = 0.70$; slope = 1 was not rejected, $P = 0.33$).

DISCUSSION

General pattern of uredospore dispersal in the air. The pattern of aerial dispersal observed from this 17-month experiment was consistent with the observed CLR epidemics. The two periods of high uredospore captures were associated with the two rainy seasons when CLR epidemics grew and reached their highest level, and low captures with the dry season when the epidemic was at its lowest point. This behavior confirmed the relationship between sporulation and dispersal as already shown by Becker (1977b) in Kenya.

TABLE 2. Mean relative humidity (and standard error) under dense shade and at full sun exposure in different periods of the day and rain conditions

Variables	From 10:00 a.m. to 1:00 p.m. in dry hours	From 7:00 a.m. to 7:00 p.m. in rainy hours	From 7:00 a.m. to 7:00 p.m.
Dense shade	78.1 (3.7)	92.3 (6.6)	87.5 (2.2)
Full sun	73.1 (3.4)	89.7 (6.4)	84.2 (2.1)

TABLE 3. Pearson correlation coefficients (r) between meteorological variables[a]

Variables	Rainfall	HR	HWR	RH_DS	RH_FS	Wind gust
Rainfall	1.00	**0.43****	–0.14**	0.09**	0.10**	–0.05^{NS}
HR		1.00	–0.22**	0.17**	0.17**	–0.14**
HWR			1.00	–0.25**	–0.21**	0.27**
RH_DS				1.00	**0.91****	**–0.64****
RH_FS					1.00	**–0.63****
Wind gust						1.00

[a] **, $P < 0.01$; NS, not significant ($P > 0.05$); highest correlations between different variables are in bold; and meaning of variables are provided in Table 1.

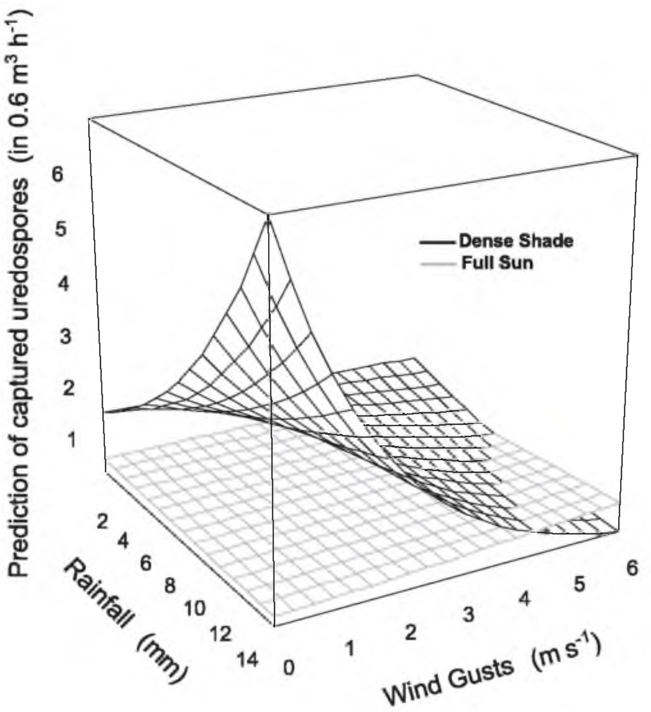

Fig. 3. Predicted effects of wind gusts and rainfall on hourly average dispersal of airborne *Hemileia vastatrix* uredospores (rainy events), as a function of shade—model 1 (generalized linear mixed model with a Poisson distribution).

As mentioned earlier, yields recovered in 2014, after the severe epidemic that occurred in 2012 and the near-zero yield in 2013. Association of high CLR incidence and severity with high coffee fruit load has been well documented (Avelino et al. 2006; Lopez-Bravo et al. 2012; Zambolim et al. 1992). The physiological resistance of coffee leaves is reduced by the presence of many fruits. The maximum capture was recorded in October 2014 (464 uredospores in 1 day under dense shade). This maximum in spore capture coincides with a year of high disease compared with 2013, possibly due to the increase in yield in 2014. The range of maximum daily capture recorded in our study was similar to the value reported in Brazil (Kushalappa and Eskes 1989), but much lower than the 4,404 uredospores recorded in Kenya at a height of 1.25 m (versus 1.5 m here) in 1 day (Becker and Kranz 1977). Our study provides new evidence of the small amount of aerially dispersed *H. vastatrix* uredospores compared with other rusts (Kushalappa and Eskes 1989). Our analyses also showed a strong effect of the hour of the day (which is the best predictor in both models) on the dispersal of *H. vastatrix* uredospores. This diurnal pattern of pathogen dispersal was similarly observed in Kenya (Becker 1977a; Becker et al. 1975), where the highest dispersal occurred late in the morning and in the early afternoon and was linked to the decrease in relative humidity and the highest recorded wind speed, which is also what our results showed. Most rust fungi have the same circadian cycle (Vuorinen and Helander 1995) with respect to spore dispersal. Such overall agreement between the present results and earlier reports provides support for further conclusions of this work. The very low dispersal levels predicted by the models may be explained by the challenge of predicting null values of dispersal (more than 70% of the values in both models). However, although the average predicted value was low, dispersal was fairly well predicted as the regression lines of each model were close to the concordance line between observed and predicted values (Figs. 4 and 6).

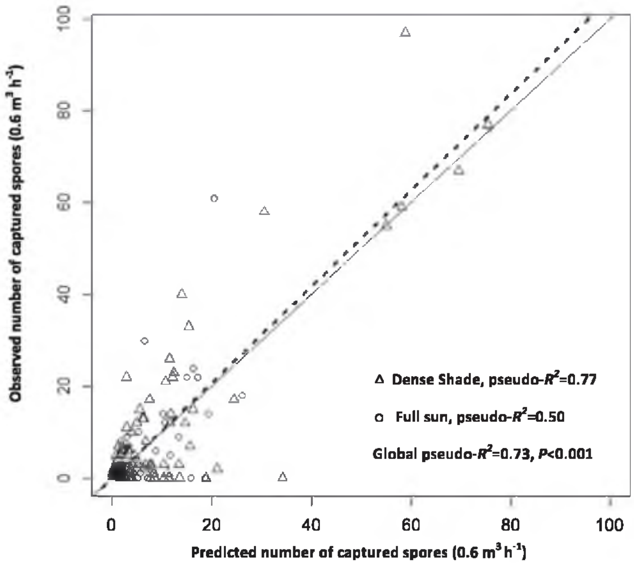

Fig. 4. Predicted versus observed values of aerially dispersed uredospores per hour under rainy conditions as a function of shade. The dotted line represents the regression line between both variables, considering all the data. The solid line represents Y = X.

Shade effects on airborne uredospore dispersal during rainy events. Rainfall appeared as the best predictor of uredospore dispersal in the air under dense shade. This effect may be explained by the interception of rainfall by shade tree foliage, which induced an accumulation of water on leaves, resulting in an increase in raindrop size and kinetic energy, as proposed by Avelino et al. (2004) and already shown in forests (Mosley 1982), including tropical forests (Vis 1986). The increased air movement due to drops and greater strength of drop impacts on the upper surface of coffee leaves probably facilitated the dry release of uredospores from lesions and their dispersal into the air. Such a mechanism of release and dry dispersal of propagules by rain has already been demonstrated for coffee rust and other pathogens (Hirst and Stedman 1963; Rayner 1961b; Savary and Janeau 1986; Savary et al. 2004). The first drops hitting the dry surface of leaves are responsible for this dry dispersal (Hirst and Stedman 1963; Savary and Janeau 1986). Rain can also be involved in propagule dispersal through wash-out from the air, splashing, dripping and run-off (Hirst and Stedman 1963; Savary and Janeau 1986). Although coffee rust lesions are located on the lower leaf surface, so that uredospores are not directly exposed to raindrops, *H. vastatrix*

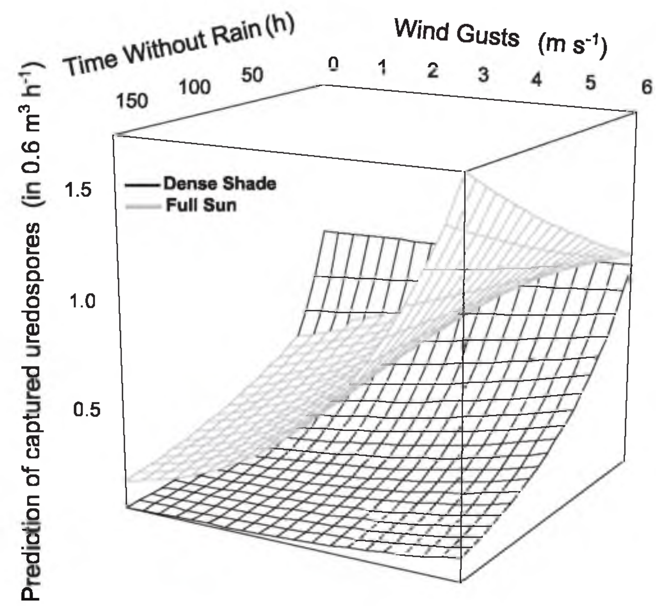

Fig. 5. Predicted effects of wind gusts and time with no rain on hourly average dry dispersal of *Hemileia vastatrix* uredospores (dry periods), as a function of shade—model 2 (generalized linear mixed model with a Poisson distribution).

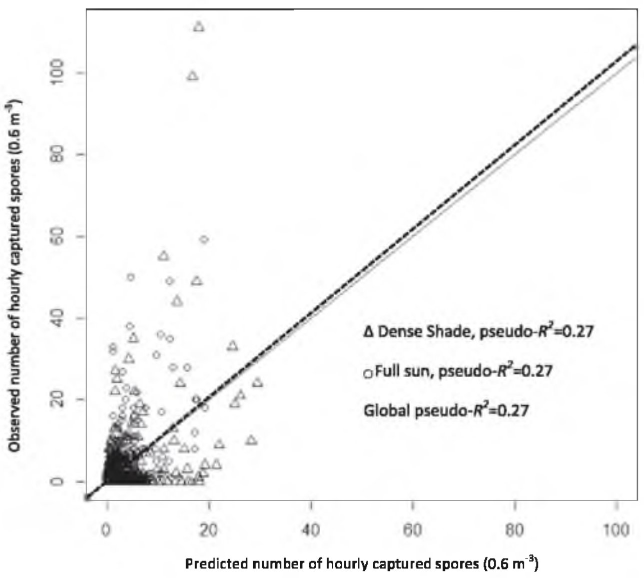

Fig. 6. Predicted versus observed values of dry dispersed uredospores per hour under dry conditions, as a function of shade. The dotted line represents the regression line between both variables, considering all the data. The solid line represents Y = X.

uredospores may also be dispersed through splashing (Savary et al. 2004). Splashing is possible when released uredospores reach the upper surface of other leaves (Rayner 1961a). Splashing has also been reported when water droplets with dense suspensions of uredospores are located on the tip or the margin of the leaf (Bock 1962). However, Burkard traps are not particularly designed for capturing propagules dispersed by splashing, especially when droplets are large. Mainly dry dispersed uredospores were then likely monitored in our study, even during rainy events. Shade effects on uredospore dispersal in the air might depend on fall velocities and consequently fall heights (Mosley 1982; Vis 1986). In our study, the higher canopy level was formed by trees of >14 m in height (*C. eurycyclum*). Drops intercepted by this upper canopy layer will always reach their terminal velocities (Mosley 1982; Vis 1986), at the coffee level, if not intercepted by the lower shade canopy layer of *poró* trees at a height of 4 to 5 m. This shade condition is therefore particularly conducive to high values of kinetic energy and thus strong dispersal. Wind alone did not have any significant impact on uredospore dispersal under dense shade, but showed an indirect effect by reversing the increase in uredospore dispersal caused by high rainfall values. We put forward the hypothesis that wind gusts reduced the collection of rainfall droplets by the shade tree cover, by shaking leaves, leading to smaller and lighter raindrops falling from the tree canopy. The scant dispersal predicted in full sun might be explained by processes similar to those operating under dense shade. The reduced impact caused by numerous but small raindrops in absence of shade trees may result in a lack of uredospore release from lesions. Another hypothesis is the wash-off of uredospores by rain, reducing the amount of uredospores dispersed into the air (Savary et al. 2004). Both mechanisms probably play important roles in the reduction of dispersal in full sunlight compared with dense shade when raining. Our results provide new insights into the aerial dispersal of *H. vastatrix* uredospores, indicating that rainfall is an important factor under shade. This result is complementary to what was reported in Kenya, with full sun exposure, where rainfall was considered as a secondary factor affecting uredospore dispersal (Becker and Kranz 1977; Becker et al. 1975).

Shade effects on uredospore dry dispersal during dry events. The absence of rainfall induced a completely different pattern of dispersal in both treatments. The increase in dispersal caused by wind gusts in full sun, with no equivalent observation under dense shade, reflects the role played by shade trees in wind interception (Jaramillo-Robledo and Gómez-Gómez 1989). By blocking wind gusts at the coffee level, shade trees prevented dispersal of uredospores by wind. In addition, relative humidity, which was particularly low on dry days in full sun, possibly facilitated uredospore dispersal under these conditions. This result also indicates that wind is the main factor affecting uredospore dry dispersal during dry events in full sun as shown in Kenya (Becker 1977a; Becker and Kranz 1977; Becker et al. 1975). Such a pattern was not observed under shade, where the general decrease in dispersal with increasingly long dry periods is not easily explained. One hypothesis is that uredospore stock depletion (Savary et al. 2004) caused by previous rain reduced the quantity of uredospores available for wind dispersal and that dry conditions were not conducive to a renewal of the inoculum stock.

The underestimation of high dispersal values by the model may be due to interplot interferences. Uredospores coming from more infected neighboring plots (particularly plots under organic management) could have been transported by wind to the Burkard traps located in the studied treatments (with fungicides). In addition, the poor quality of prediction might indicate that variables explaining dispersal in dry periods have been omitted. The conditions of the study, in small experimental plots closely located to one another, with diverse shade types and varied CLR attack intensities, can partly explain this situation. Therefore, taking into account the quantity of inoculum in neighboring plots, as well as the direction of the wind, could have enabled us to explain more of the observed variability. However, the aim of this study was not to predict dispersal values but to identify the effects of the presence or absence of shade on uredospore dispersal, which was achieved by the modeling, as significant effects were revealed.

Modeling approach. Our modeling approach enabled an analysis of the aerial dispersal of coffee rust uredospores, corrected for the variation of the inoculum stock over time, the seasonal dispersal pattern and the circadian rhythm of dispersal. Our analysis thus isolates significant effects of shade on spore dispersal.

GLMMs have proven useful in plant pathology (Krauss et al. 2013; Madden et al. 2002; Stroup 2015). This statistical approach allowed us to model complex data structures involving repeated measures and overdispersion. Overdispersion is a difficult challenge to overcome (Garrett et al. 2004; Madden et al. 2002; Stroup 2015), which in our study was associated with the existence of some extreme values and more than 70% of null values in the dataset. By assuming variability among sampling units, i.e., adding a random effect at the level of the sampling unit (Stroup 2015), data overdispersion was satisfactorily addressed. Excess of null values and outliers can be dealt with by using alternative quantitative methods such as zero-inflated and zero-truncated Poisson models (Zuur et al. 2009), with, however, an increase in complexity of the modeling approach, and more limited availability of algorithms for the inclusion of random factors. Overdispersion also could have been corrected by categorizing data and using descriptive multivariate analyses (Savary et al. 1995) or logistic and multinomial regressions (Esker et al. 2006; Yee 2010). These methods entail, however, a loss of information due to the categorization process.

What type of shade reduces airborne uredospore dispersal? Our analyses showed the existence of interactions between treatments and meteorological variables on the aerial dispersal of *H. vastatrix*. This implies shading practices may help to suppress, or inversely may enhance, the aerial dispersal of this pathogen, depending on the rainfall regime. To our knowledge, this is the first time that such complex effects of shade on *H. vastatrix* dispersal have been described. However, further investigation would be needed to study the fate of the released uredospores.

Recommendations can be put forward for the management of shaded coffee to help manage CLR epidemics. Shade can be considered as a regulation instrument to rapidly adapt a coffee crop to annual or interannual variations in meteorological conditions, since increased shade may suppress dry spore dispersal under dry conditions, while decreased shade (pruning) may provide some control against rain-induced spore dispersal, which is mediated by shade trees. Other shade characteristics should also be considered, such as canopy height. For instance, according to coffee farmers, high timber trees increase American leaf spot incidences, a disease caused by *Mycena citricolor*, because these trees induce drops of high kinetic energy promoting the pathogen dispersal in the coffee plantation (Beer et al. 1998; Cerdán et al. 2012). Canopy shape, size, as well as the shape and specific leaf area of leaves are also important. For instance, a low canopy of shade trees with small and flexible leaves seems better adapted to preventing airborne *H. vastatrix* uredospore dispersal via raindrop interception/accumulation.

ACKNOWLEDGMENTS

This work, financed by the CIRAD core budget through the SAFSE project "Search for trade-offs between production and ecosystem services provided by agroforestry systems", is part of the Scientific Partnership Platform: Agroforestry Systems with Perennial Crops (PCP Mesoamerica). We thank E. Salas Valverde for helping in the field and laboratory activities, the anonymous reviewers for their valuable contributions, and P. Biggins for reviewing the English.

LITERATURE CITED

Avelino, J., Cristancho, M., Georgiou, S., Imbach, P., Aguilar, L., Bornemann, G., Läderach, P., Anzueto, F., Hruska, A. J., and Morales, C. 2015. The coffee rust crises in Colombia and Central America (2008–2013): Impacts, plausible causes and proposed solutions. Food Sec. 7:303-321.

Avelino, J., Romero-Gurdián, A., Cruz-Cuellar, H. F., and Declerck, F. A. J. 2012. Landscape context and scale differentially impact coffee leaf rust, coffee berry borer, and coffee root-knot nematodes. Ecol. Appl. 22:584-596.

Avelino, J., Ten Hoopen, G. M., and DeClerck, F. A. J. 2011. Ecological mechanisms for pest and disease control in coffee and cacao agroecosystems of the Neotropics. Pages 91-117 in: Ecosystem Services from Agriculture and Agroforestry Measurement and Payment. B. Rapidel, F. A. J. DeClerck, J.-F. Le Coq, and J. Beer, eds. Earthscan Publications, London, UK.

Avelino, J., Willocquet, L., and Savary, S. 2004. Effects of crop management patterns on coffee rust epidemics. Plant Pathol. 53:541-547.

Avelino, J., Zelaya, H., Merlo, A., Pineda, A., Ordonez, M., and Savary, S. 2006. The intensity of a coffee rust epidemic is dependent on production situations. Ecol. Modell. 197:431-447.

Bates, D., Maechler, M., Bolker, B., and Walker, S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. J. Stat. Software 67:1-48. http://CRAN.R-project.org/package=lme4

Becker, S. 1977a. Diurnal periodicity in spore dispersal of *Hemileia vastatrix* in relation to weather factors Z. Pflanzenk. Pflanzens. J. Plant Dis. Prot. 84: 577-591.

Becker, S. 1977b. Relation between disease progress and spores catches of *Hemileia vastatrix* in Kenya. Z. Pflanzenk. Pflanzens. J. Plant Dis. Prot. 84: 691-698.

Becker, S., and Kranz, J. 1977. Comparative studies on the dispersal of *Hemileia vastatrix* in Kenya. Z. Pflanzenk. Pflanzens. J. Plant Dis. Prot. 84: 526-539.

Becker, S., Mulinge, S. K., and Kranz, J. 1975. Evidence that uredospores of *Hemileia vastatrix* Berk. and Br. are wind-borne. Phytopathol. Z. 82:359-360.

Beer, J., Muschler, R., Kass, D., and Somarriba, E. 1998. Shade management in coffee and cacao plantations. Agrofor. Syst. 38:139-164.

Bock, K. R. 1962. Dispersal of uredospores of *Hemileia vastatrix* under field conditions. Trans. Br. Mycol. Soc. 45:63-74.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and White, J. S. S. 2009. Generalized linear mixed models: A practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127-135.

Cerdán, C. R., Rebolledo, M. C., Soto, G., Rapidel, B., and Sinclair, F. L. 2012. Local knowledge of impacts of tree cover on ecosystem services in smallholder coffee production systems. Agric. Syst. 110:119-130.

Cristancho, M. A., Rozo, Y., Escobar, C., Rivillas, C. A., and Gaitán, A. L. 2012. Outbreak of coffee leaf rust (*Hemileia vastatrix*) in Colombia. New Dis. Rep. 25:19.

Deepayan, S. 2008. Lattice: Multivariate Data Visualization with R. Springer, New York.

Esker, P. D., Harri, J., Dixon, P. M., and Nutter, F. W. 2006. Comparison of models for forecasting of Stewart's disease of corn in Iowa. Plant Dis. 90: 1353-1357.

Garrett, K. A., Madden, L. V., Hughes, G., and Pfender, W. F. 2004. New applications of statistical tools in plant pathology. Phytopathology 94: 999-1003.

Haggar, J., Barrios, M., Bolaños, M., Merlo, M., Moraga, P., Munguia, R., Ponce, A., Romero, S., Soto, G., Staver, C., and Virginio, E. M. F. 2011. Coffee agroecosystem performance under full sun, shade, conventional and organic management regimes in Central America. Agroforest Syst. 82: 285-301.

Harrell, F. E., Jr. 2001. Regression Modeling Strategies with Applications to Linear Models, Logistic Regression, and Survival Analysis. Springer Series in Statistics. Springer-Verlag, New York.

Hirst, J. M., and Stedman, O. J. 1963. Dry liberation of fungus spores by raindrops. J. Gen. Microbiol. 33:335-344.

Jaramillo-Robledo, A., and Gómez-Gómez, L. 1989. Microclima en plantaciones a libre exposición solar y bajo sombrío. Cenicafe 15:65-79.

Krauss, U., ten Hoopen, M., Rees, R., Stirrup, T., Argyle, T., George, A., Arroyo, C., Corrales, E., and Casanoves, F. 2013. Mycoparasitism by *Clonostachys byssicola* and *Clonostachys rosea* on *Trichoderma* spp. from cocoa (*Theobroma cacao*) and implication for the design of mixed biocontrol agents. Biol. Control 67:317-327.

Kushalappa, A. C., and Eskes, A. B. 1989. Advances in coffee rust research. Annu. Rev. Phytopathol. 27:503-531.

Lemmon, P. E. 1957. A new instrument for measuring forest overstory density. J. For. 55:667-668.

Lopez-Bravo, D. F., Virginio-Filho, E. D. M., and Avelino, J. 2012. Shade is conducive to coffee rust as compared to full sun exposure under standardized fruit load conditions. Crop Prot. 38:21-29.

Madden, L. V., Turechek, W. W., and Nita, M. 2002. Evaluation of generalized linear mixed models for analyzing disease incidence data obtained in designed experiments. Plant Dis. 86:316-325.

McCook, S., and Vandermeer, J. 2015. The Big Rust and the Red Queen: Long-term perspectives on coffee rust research. Phytopathology 105:1164-1173.

Mosley, M. P. 1982. The effect of a New Zealand beech forest canopy on the kinetic energy of water drops and on surface erosion. Earth Surf. Processes 7:103-107.

O'Hara, R. B., and Kotze, D. J. 2010. Do not log-transform count data. Methods Ecol. Evol. 1:118-122.

R Development Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rayner, R. W. 1961a. Germination and penetration studies on coffee rust (*Hemileia vastatrix* B. & Br.). Ann. Appl. Biol. 49:497-505.

Rayner, R. W. 1961b. Spore liberation and dispersal of coffee rust *Hemileia vastatrix* Berk. et Br. Nature 191:725.

Salgado, B. G., Macedo, R. L. G., Carvalho, V. L. d., Salgado, M., and Venturin, N. 2007. Progress of rust and coffee plant cercosporiose mixed with grevilea, with ingazeiro and in the full sunshine in Lavras-MG. Cienc. Agrotec. 31:1067-1074.

Savary, S., and Janeau, J. L. 1986. Rain-induced dispersal in *Puccinia arachidis*, studied by means of a rainfall simulator. Neth. J. Plant Pathol. 92: 163-174.

Savary, S., Janeau, J. L., Allorent, D., Escalante, M., Avelino, J., and Willocquet, L. 2004. Effects of simulated rainfall events on spore dispersal and spore stocks in three tropical pathosystems. (Abstr.) Phytopathology 94 (suppl.):S92.

Savary, S., Madden, L. V., Zadoks, J. C., and Klein-Gebbinck, H. W. 1995. Use of categorical information and correspondence analysis in plant disease epidemiology. Pages 213-240 in: Advances in Botanical Research, Vol. 21. Academic Press Ltd., London.

Schroth, G., Krauss, U., Gasparotto, L., Aguilar, J. A. D., and Vohland, K. 2000. Pests and diseases in agroforestry systems of the humid tropics. Agroforest Syst. 50:199-241.

Skelsey, P., Rossing, W. A. H., Kessel, G. J. T., and van der Werf, W. 2010. Invasion of *Phytophthora infestans* at the landscape level: How do spatial scale and weather modulate the consequences of spatial heterogeneity in host resistance? Phytopathology 100:1146-1161.

Soto-Pinto, L., Perfecto, I., and Caballero-Nieto, J. 2002. Shade over coffee: Its effects on berry borer, leaf rust and spontaneous herbs in Chiapas, Mexico. Agroforest Syst. 55:37-45.

Staver, C., Guharay, F., Monterroso, D., and Muschler, R. G. 2001. Designing pest-suppressive multistrata perennial crop systems: Shade-grown coffee in Central America. Agroforest Syst. 53:151-170.

Stroup, W. W. 2015. Rethinking the analysis of non-normal data in plant and soil science. Agron. J. 106:1-17.

Toledo, V. M., and Moguel, P. 2012. Coffee and sustainability: The multiple values of traditional shaded coffee. J. Sustain. Agric. 36:353-377.

Vandermeer, J., Jackson, D., and Perfecto, I. 2014. Qualitative dynamics of the coffee rust epidemic: Educating intuition with theoretical ecology. Bioscience 64:210-218.

Vandermeer, J., and Rohani, P. 2014. The interaction of regional and local in the dynamics of the coffee rust disease. arXiv. 407.8247v1.

Villarreyna, R. 2014. Análisis de las condiciones de manejo que propiciaron el impacto de la roya (*Hemileia vastatrix*) en la zona cafetalera de los municipios de Jinotega, el Tuma-La Dalia y San Ramón, Nicaragua, Sistemas Agrícolas Sostenibles. Catie, Turrialba, Costa Rica.

Vis, M. 1986. Interception, drop size distributions and rainfall kinetic energy in four Colombian forest ecosystems. Earth Surf. Processes 11:591-603.

Vuorinen, P., and Helander, M. 1995. Variation in airborne urediniospore concentration of *Melampsoridium betulinum*. Aerobiologia 11:259-264.

Wili, G. M. 1985. Comparison of the designs of 2 volumetric spore traps. Phytopathology 75:380.

Yee, T. W. 2010. The VGAM package for categorical data analysis. J. Stat. Softw. 32:1-34.

Zadoks, J. C., and Schein, R. D. 1979. Epidemiology and Plant Disease Management. Oxford University Press, New York.

Zadoks, J. C., and Vandenbosch, F. 1994. On the spread of plant-disease: A theory on foci. Annu. Rev. Phytopathol. 32:503-521.

Zambolim, L., Silva-Acuña, R., Do Vale, F. X. R., and Chaves, G. M. 1992. Influencia da produção do cafeeiro sobre o desenvolvimento da ferrugem (*Hemileia vastatrix*). Fitopatol. Bras. 17:32-35.

Zawolek, M. W., and Zadoks, J. C. 1992. Studies in focus development— An optimum for the dual dispersal of plant pathogens. Phytopathology 82: 1288-1297.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M. 2009. Mixed Effects Models and Extensions in Ecology with R. Vol. XXII. Statistics for Biology and Health. Springer, New York.