

Temporal progress of coffee leaf rust and environmental conditions affecting severity in Veracruz State, Mexico

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ABSTRACT

Coffee is an important crop in Mexico. Unfortunately, coffee production has been affected by coffee leaf rust (CLR). For Veracruz, the second state in Mexico with the major production of coffee, there are available reports of weekly CLR severity, but these are only informative without in-depth inferential analysis. We analyzed variations of CLR severity along the year in Veracruz with data from municipal weekly reports provided by Mexico's federal government phytosanitary epidemiological monitoring coffee program. We selected reports dated in 2018 from nine municipalities and after calculations of mean monthly severity values, we conducted a one-way ANOVA (months as factors) of severity data. We compared this information with other coffee-producing regions. Additionally, we explored the association of temperature, rainfall, and altitude with CLR severity using Principal Component Analysis and multiple linear regressions. Temperature and rainfall data were obtained from Mexican National Meteorological Service. We found that CLR severity in October, November, December, and January (months of harvest period) was significantly higher than values from March-June. During the harvest period, coffee plants allocate resources mainly for fruiting which competes in resources for other tasks such as defense and leaf growth, so this competition of resources can explain the positive relationship found between fruit load and CLR severity. This monthly variation of severity was similar to those reported in Chiapas, Guatemala, Colombia, Uganda, and Ethiopia. Our model ($R^2 = 0.948$) showed a significant and negative effect of minimum and maximum temperature (in a range from 9.9 – 15.5 °C and 18.5 – 26.5 °C, respectively) on CLR severity, while the effect of rainfall (in a range from 32.0 – 359.9 mm) and medium temperature (from 14.3 – 20.5 °C) was positive. With our study, we suggest applications of fungicides in March-June when coffee plantations are in leaf phenophase.

Key words: Altitude; *Coffea arabica*; *Hemileia vastatrix*; phenophase.

1 INTRODUCTION

Coffee is an important commodity for developing countries. In Mexico, the production of coffee is approximately 953 682 tons yearly (Serviço de Informação Alimentar e Pesqueira SIAP, 2021), which ranks 12th on the list of producer countries (Food and Agriculture organization of the United Nations - FAOSTAT, 2020). Veracruz is the second state in Mexico with higher production of coffee, just behind Chiapas, contributing about one-quarter of national production (SIAP, 2021). Producers from Veracruz cultivate mainly varieties from two species: *Coffea canephora* P. and *Coffea arabica* L. Despite some producers are substituting varieties from *C. arabica* for resistant varieties to Coffee Leaf Rust (CLR) (Sánchez-Hernández et al., 2018), the most cultivated varieties in Veracruz are Typica, Bourbon, Mundo Novo, Garnica and Catuai (López-García et al., 2016; Hernández-Sánchez; Travieso-Bello, 2021), which are highly susceptible to CLR. These varieties are preferred for their better cup quality, so they are still important due to their higher economic value (Sánchez-Hernández et al., 2018).

CLR is caused by the fungus *Hemileia vastatrix* Berk. et Br which parasitizes *C. arabica* plants and their varieties (Avelino et al., 2015; Silva et al., 2022). Urediospores are the infectious

forms of *H. vastatrix*. In the infection cycle of *H. vastatrix*, urediospores reach the undersurface of coffee leaves at upper levels mainly by air and rain splashing in leaves of lower levels (Rayner, 1961; Avelino et al., 2020). Following the first contact with the leaf surface with appropriate humidity, urediospores increase their surface area contact and release adhesive components which allow them to attach and infect coffee leaves (Zuluaga; Butiricá-Céspedes; Marín-Montoya, 2009). In conditions of free water, temperature from 21 to 25 °C, and darkness, germinative tubes are generated from urediospores (SENASICA, 2019). Germinative tubes elongate over the epidermis until to reach the raised lips of subsidiary cells of stomata, then the appressorium is developed and a portion of it allows to start the infection (Coutinho; Rijkenberg; Van-Asch, 1993; Couttolenc-Brenis et al., 2020). After penetration of stomatal cells, *H. vastatrix* starts differentiation in infection hyphae and haustorium formation in the substomatal chamber. Hyphae arrive at mesophyll cells and haustoria still appear for the subtraction of nutrients, then chlorotic spots are observed in leaves (Ramiro et al., 2009; Duplessis et al., 2021; Lu et al., 2022). *H. vastatrix* continues with the maturation of sori where new urediospores are formed to be dispersed by rain or air (Chinnappa; Sreenivasan, 1968). With the last, the cycle is repeated, and new plants are infected.

The infection process results in a decreased photosynthetic rate and loss in the foliar area. Thus, if the damage is higher than 60 %, branches of coffee plants suffer early defoliation and eventually death. The last causes production losses in the year of infection due to lost fruits in dead branches (primary yield losses) and losses in subsequent years (secondary yield losses) which are more significant than the primary ones (Avelino et al., 2015). Secondary losses due to defoliation represent a high energy cost inversion, so plants do not allocate enough resources to fruits to recover the production of leaves affecting the allocation of resources for the reproduction process (Obeso, 1993). Thus, coffee production is affected until new leaves reach maturity.

CLR severity in coffee plantations is different depending on environmental conditions, spatial factors, and the yield of plants (Talhinhas et al., 2017; Hinnah et al., 2018). More severe attacks have been reported on high-yielding coffee trees (Souza et al., 2011). Concerning altitude, there are some controversies, but it is generally known that coffee plantations growing in altitudes lower than 1000-1100 m are more susceptible to being infected (Hindorf; Omondi, 2011). However, in recent years the reports of severe attacks in higher altitudes have increased, even over 1800 m (Avelino et al., 2015; Talhinhas et al., 2017). Temperature plays an important role in disease development, with 22 °C as optimal for urediospores germination of *H. vastatrix*, mycelial growth, and sori formation. If a stimulation with a lower temperature followed by an increase of this occurs, then spore germination is higher than a constant regime of temperature at 22 °C (Arroyo-Esquivel; Sánchez; Barbosa, 2019). Rainfall patterns are also determinants for CLR severity. Despite urediospores tolerate longer seasons without rainfall (Hindorf; Omondi, 2011), higher rainfall contributes to higher humidity which is necessary for germination and spore dispersion, then more plants are infected and the incidence of the disease is increased (Avelino; Rivas, 2013). The effect of all these environmental conditions in CLR severity are not instantaneously observed in coffee plantations due the time required between germination and the presence of the first pustules in plants. This incubation period for sporulation can be from 17 to 50 days approximately (Maia et al., 2017), so this makes that the effect of climate on CLR severity can be observed after a latent period of some months.

Environmental conditions change along the year, promoting temporary variations in CLR severity. These variations have been reported in different coffee-producing regions with increased severity during months of the harvest period and slow development of disease in months of the vegetative phase (Rivillas et al., 2011). For example, in Guatemala, CLR severity is lower from May to September due to adverse conditions, leaf falls, and winds from the dry season, while higher levels of severity are present from December to February the next year (Orozco-Miranda et al.,

2011). In Colombia, monthly variation is very similar, with high severity in the last months of the year which correspond to harvest period (Rivillas et al., 2011). In Mexico, some preliminary reports carried out in the 1980s are available for Chiapas with a similar pattern (SENASICA, 2019). In recent years, also for Chiapas, it is reported a peak of CLR severity in December/January and remaining dormant during the dry season (Vandermeer; Hajian-Forooshani; Perfecto, 2018). Moreover, Mexico's federal government phytosanitary epidemiological monitoring coffee program (PVEF-Cafeto) weekly reports CLR severity in leaves, CLR severity in plants, and phenological stage of coffee plantations with the aid of the installation of fixed and mobile plots in the main coffee producers states in Mexico, including Veracruz (SAGARPA; SENASICA; DGSV-MEXICO, 2018).

However, these available reports are only informative, in other words, only weekly CLR severity levels are publicly presented for stakeholders, but the reports do not analyze variations of CLR severity in Veracruz (the second state with major coffee production) along the year. Therefore, studies about these temporary variations are necessary to know the specific conditions of CLR disease in the regional and local context so the application of preventive control can be carried out. Thus, our study aims to assess the monthly variation of CLR severity in Veracruz and compare it with the progress of the disease over the year from other coffee producing-regions worldwide. As these variations may be influenced by environmental factors, then we also assess the effect of maximum, medium, and minimum temperature, rainfall, and altitude in the temporal progress of CLR severity in Veracruz.

2 MATERIAL AND METHODS

2.1 Data sets

We obtained CLR severity data in Veracruz from municipal weekly reports provided by the PVEF-Cafeto (Mexico's federal government phytosanitary epidemiological monitoring coffee program) (SAGARPA; SENASICA; DGSV-MEXICO, 2018). This program reports CLR severity based on a diagrammatic scale for severity in plants and sampling plots established in the reference technical operations handbook by SAGARPA and SENASICA (2018). Parameters taken from this program for our analysis were CLR severity in plants and phenological stage of coffee plantations. We also collected coordinates of the fixed plots from CLR severity reports were obtained. These fixed plots were digitally mapped (Google Earth) to get their altitude and their distance to the nearest meteorological stations of the SMN (Mexican National Meteorological Service) (SMN, 2018). From these stations' data, we extracted the mean monthly temperature (maximum, medium, and minimum) and rainfall of each municipality.

2.2 Selection criteria

We selected weekly reports by municipality considering the following criteria: 1) the most recent year that had reports for all 52 weeks of the year; 2) municipalities with information for at least 47 weeks; and 3) municipalities with temperature (maximum, medium and minimum) and rainfall information for the selected year and the previous two months (this particular criterion is fully justified in Statistical analysis section) from a meteorological station within a 10 km radius from fixed plots and <160 m of difference in altitude. From 59 municipalities that had weekly reports for 2018, only 12 met the aforementioned selection criteria. However, some municipalities shared the same meteorological station, so in these cases, we considered only one municipality for each station. In this sense, only nine municipalities were considered (Figure 1): Naolinco, Jilotepec, Xalapa; Emiliano Zapata, Coatepec, Teocelo, Cosautlan, Tlaltetela, and Huatusco.

2.3 Statistical analysis

We calculated the mean monthly severity values for these municipalities. To test for significant differences

in CLR severity among months, we carried out one-way ANOVAs considering the months as a factor with 12 levels (independent variable) while the dependent variable was monthly severity data. Additionally, a post hoc Tukey test was performed to make multiple comparisons. The dependent variable did not fulfill assumptions of a normal distribution, so it was transformed into ranks suitable for parametric statistical analysis (Conover; Iman, 1981). In our ANOVAs, each municipality was considered a sampling unit with nine replicates per level (month). A confidence interval of 95 % was analyzed ($P < 0.05$) using R statistical free software 3.5.2 version (R Development Core Team, 2018).

We carried out a Principal Component Analysis (PCA) in a correlation matrix to explore plausible relationships between altitude, rainfall, temperature (maximum, medium, and minimum), and CLR severity. Taking into account that the latency of incubation period for sporulation may last for up to 50 days (Maia et al., 2017), we considered as observations the mean monthly temperature (maximum, medium, and minimum) and rainfall of each municipality with a lag of two months, while for CLR severity we considered values from each month from the selected year (2018).

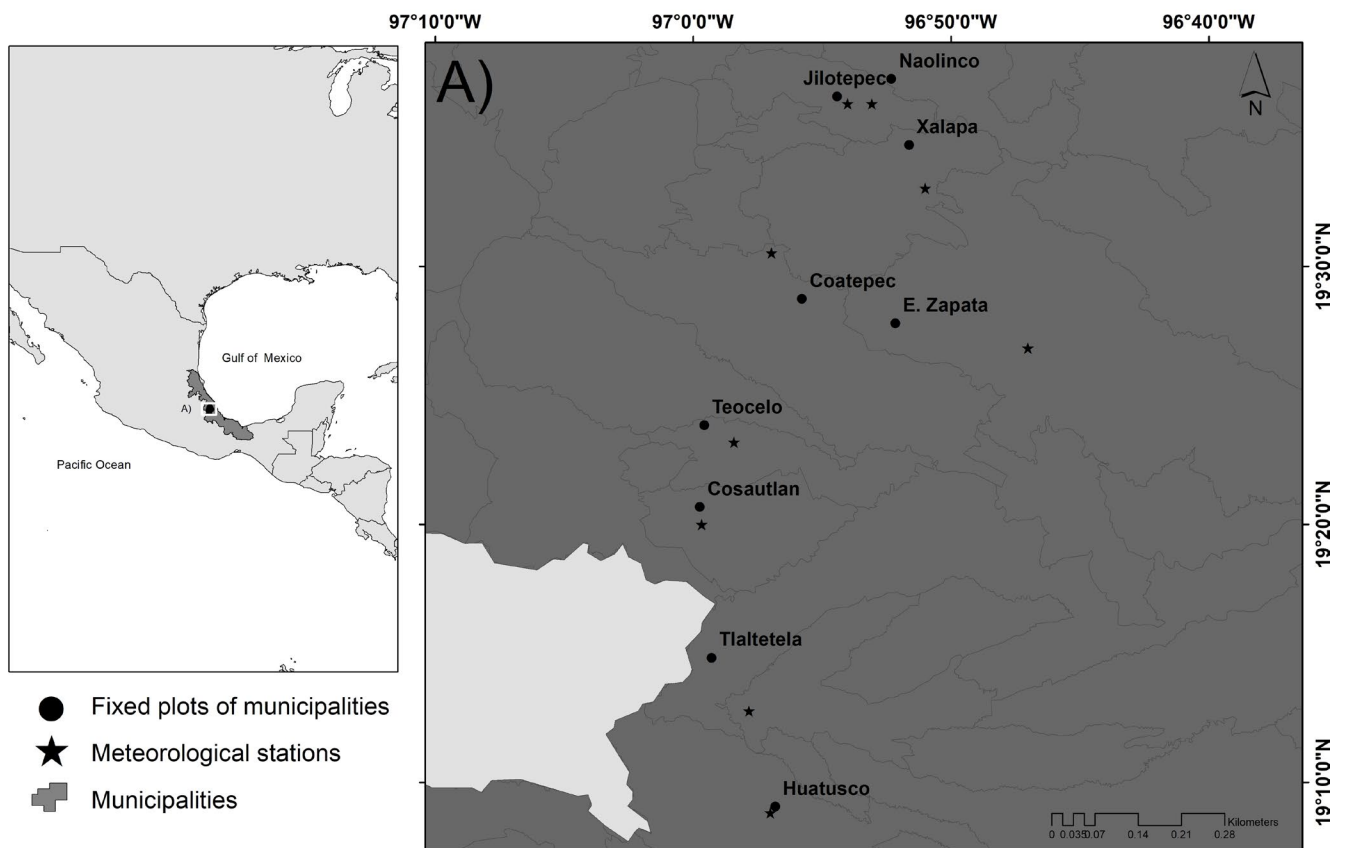


Figure 1: Location of fixed plots in municipalities with CLR severity reports and climatological information for 2018. Area in dark gray corresponds to Veracruz state. Asterisks depict the closest meteorological stations, located within a 10 km radius and <160 m difference in altitude.

Based on the results of our PCA, we modeled the effect of altitude, rainfall, and temperature with a lag of two months on CLR severity through multiple linear regressions. For this analysis, we transformed the response variable (monthly CLR severity) to natural logarithm values. The models were conducted stepwise and without intercept. We used model selection to compare the complete model with reduced models. For that purpose, we used the Mallows' Cp criterion. Variables removed in the model were those that were not significant. For PCA, we employed XLSTAT software (2020.1 version), while multiple linear regressions were carried out in SAS OnDemand for Academics (2022).

3 RESULTS

We found significant monthly differences in CLR severity reported during 2018. The high severity of January, October, November, and December were significantly different with respect to the low values reported in March-June. The months of increased severity coincide mainly with the ripe and consistent fruit phenological stage (with immature fruits in October), in other words, with the harvest period. On the other hand, coffee plantations were mainly in leaf phenophase from March to June, starting with the occurrence of immature coffee beans in June (Figure 2).

Regarding environmental conditions, altitude values were 952, 988, 1074, 1105, 1165, 1206, 1214, 1261, and 1405 m for Xalapa, Naolinco, Emiliano Zapata, Jilotepec, Cosautlan, Teocelo, Coatepec, Huatusco, and Tlaltetela, respectively. In these municipalities, the medium temperatures

with a lag of two months were on average from 14.3 – 20.4 °C throughout the year, while the range from minimum and maximum temperatures were 9.9 – 15.5 °C and 18.5 - 26.5 °C, respectively. For rainfall, the values were from 24.7 to 359.9 mm. The monthly variation of temperature and rainfall with a lag of two months in these municipalities are presented in Figure 3.

Graphically, we observed a point of decrease in temperatures affecting to March, which represents the beginning of the period of lower CLR severity values. From the temperatures related to May to December, average temperatures maintained their values close to 20 °C. According to rainfall, increasing values related to months with higher CLR severity levels were observed, except for January which its rainfall regime with a lag of two months had a low value. Additionally, we observed that rainfall affecting August were also high.

To explore the association between these variables and CLR severity, we carried out a PCA. We selected two components that account for 62.33 % of the total variation (42.16 % by PC1 and 20.17 % by PC2). When the PC1 axis is considered, which consisted of the meaningful contribution of maximum, medium and minimum temperatures (with high square cosines of 0.58, 0.94, and 0.67, respectively), we can notice that the majority of observations for months with CLR severity levels (January, October, November, and December) are distributed along with positive values in this axis, except for some observations of January. On the other hand, all the observations for March and the majority for April (months of the beginning of the period with lower CLR severity levels)

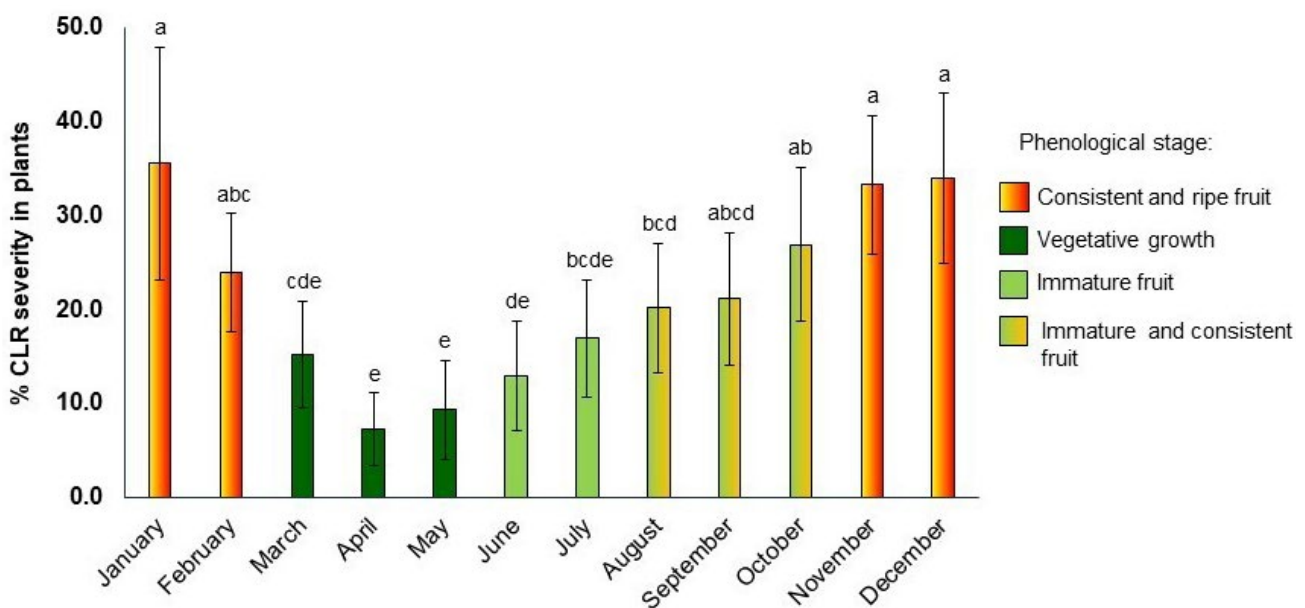


Figure 2: Monthly CLR severity in Veracruz during 2018 reported by PVEF-Cafeto. Data are average values of CLR severity from the nine selected municipalities. Error bars represent the standard deviation. Different letters indicate significant differences (Tukey's HSD, $\alpha \leq 0.05$).

are distributed along with the decrease in temperature. When phenological stage and PC1 is considered, the pattern is not clear. According to the PC2 axis (consisting in the meaningful contribution of CLR severity with a high square cosine of 0.79), most months with higher severity levels are plotted along with the increment in this axis, except for one observation of January, two from November and two from December. From these five observations not aligned with the increment in PC2 axis, three of them correspond to values reported in Tlaltetela, a municipality which atypically presented low CLR values during all the year. In addition, a similar pattern is observed when phenological stage is considered due to the majority of observations from months in which coffee plantations presented consistent and ripe fruits are distributed to the increment in CLR severity. Despite score plot for PC1 against PC2 showed that rainfall is aligned in the same direction with CLR severity and altitude is diametrically opposed to the increment of severity, these two variables did not meaningfully contribute to PC1 nor PC2, so we found no determinant pattern between any single environmental variable and CLR severity (Figure 4). Because we found no relationship between single variables and CLR severity, we combined all environmental variables in multiple regression models.

After model comparison with the complete model, temperatures (maximum, medium, and minimum) and rainfall were selected in the model because these variables had a significant influence on CLR severity, and the model presented the lowest value of Mallows' Cp criterion in relation

to the others evaluated. The parameters of this regression are shown in Table 1.

Adjusted R^2 showed a value of 0.948. Thus, the combination of these four variables explains 94.8 % of the variability in CLR severity. Regression coefficients were -0.131, +0.451, and -0.190 for maximum, medium and minimum temperature, respectively; and +0.001 for rainfall, so this indicates a negative correlation of CLR severity with minimum and maximum temperature (in ranges from 9.9 – 15.5 and 18.5 – 26.5 °C, respectively), while the correlation with medium temperature (in a range from 14.3 – 20.4 °C) and rainfall was positive. According to ANOVA (Table 2), the information provided for this model is significant.

4 DISCUSSION

We found among-month differences in CLR severity in Veracruz during 2018. The variation in our data is similar to the previous reported for Southern Mexico (Soconusco region in Chiapas) from 2013 to 2016, where CLR severity had a regular seasonality with an increase beginning in May/June (rainy season) and reaching a peak in December/January, remaining more or less dormant during the dry season and then repeating the cycle (Vandermeer; Hajian-Forooshani; Perfecto, 2018). This trend is also reported in other countries from Central and South America such as Guatemala which has a phase of slow development of CLR severity from May to September and a starting increase in October with a maximum peak from December to February in the next year.

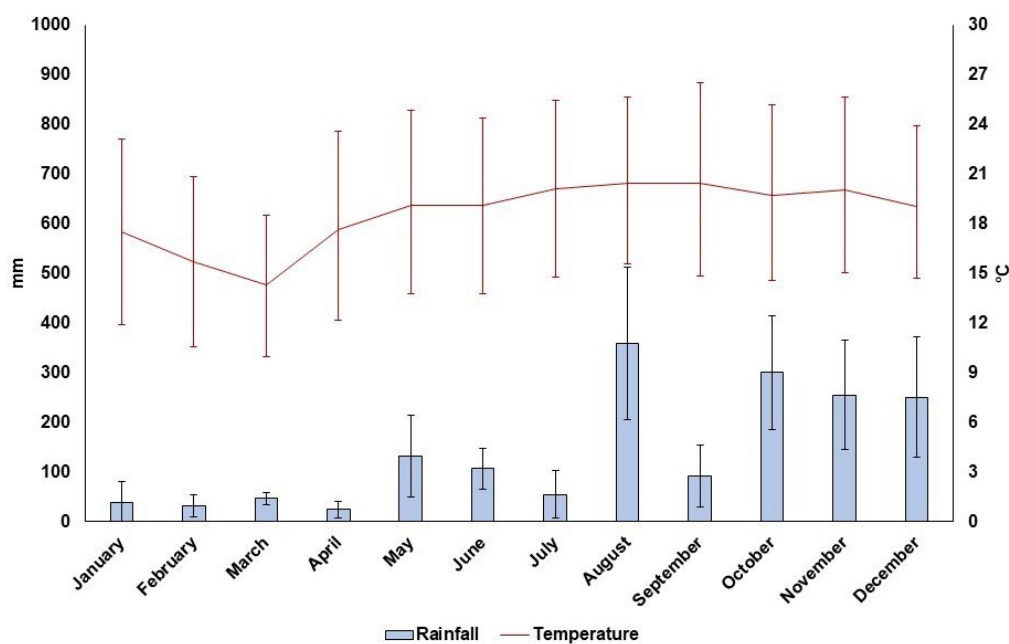


Figure 3: Monthly rainfall (mm) and temperature (°C) presented with a lag of two months in the central Veracruz coffee region during 2018. Rainfall data are aggregated mean of the nine municipalities considered in this study (error bars are standard deviation). Medium temperature is presented while error bars represent maximum and minimum temperatures for the nine municipalities.

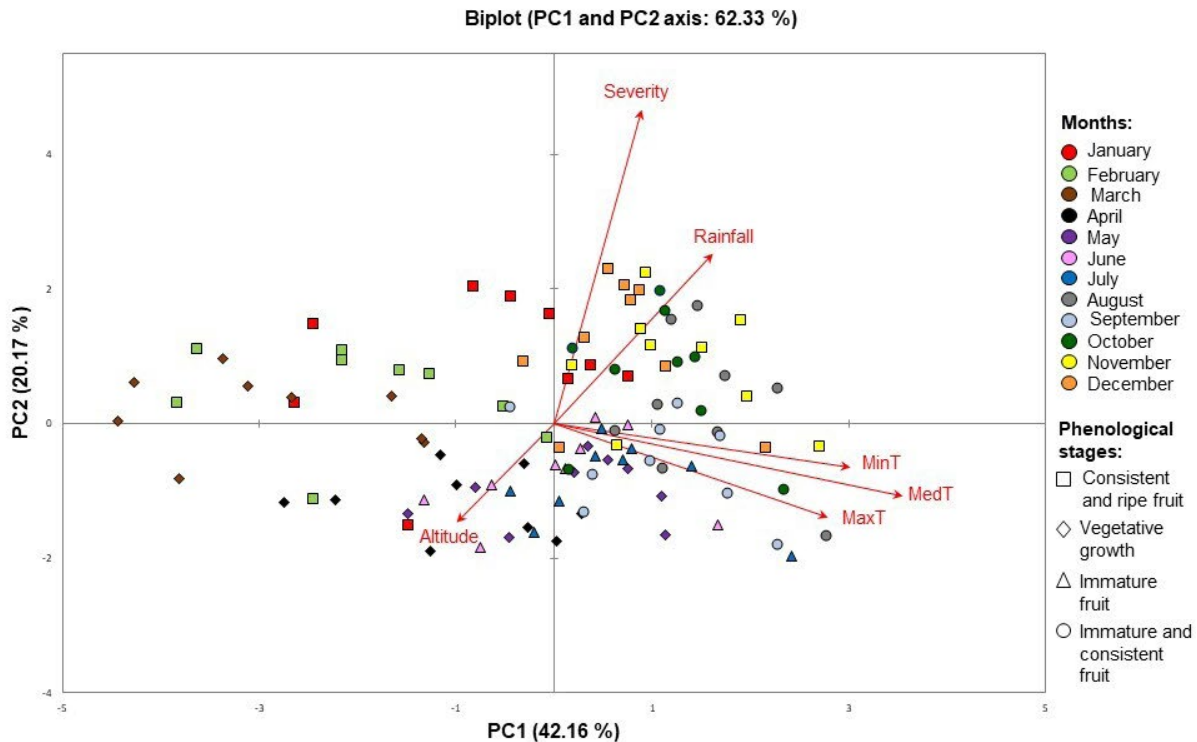


Figure 4: PCA of CLR severity and environmental conditions (altitude, rainfall and maximum, medium, and minimum temperature with the considered lag) in Veracruz. It is shown the score plot of PC1 against PC2 and the observations with the correlation between variables. MaxT, MedT, and MinT represent maximum, medium, and minimum temperature respectively. Phenological stages in each month are also shown by different markers.

Table 1: Parameters for each predictor variable in linear regression model of environmental conditions (with the considered lag) explaining CLR severity in Veracruz.

Variable	Coefficient	Standard error	<i>p</i>
Maximum temperature (Tmax)	-0.131	0.067	0.055
Medium temperature (Tmed)	0.451	0.132	0.009
Minimum temperature (Tmin)	-0.190	0.067	0.005
Rainfall	0.001	0.000	0.019

These variables explain CLR severity as is shown in the next equation:
 $\ln(\text{CLR severity}) = -0.131(\text{MaxT}) + 0.451(\text{MedT}) - 0.190(\text{MinT}) + 0.001(\text{Rainfall})$.

Table 2: ANOVA for Regression model of CLR severity explained by temperatures and rainfall (with a lag of two months).

Source of variation	Degrees of Freedom	Sum of Squares	Mean squares	F	Pr > F
Model	4	897.22	224.30	497.58	< 0.0001
Error	104	46.88	0.45		
Total	108	944.10			

Same as in Veracruz, the period of higher CLR severity coincides with the phenological stage of fruit ripening and

harvest period (Orozco-Miranda et al., 2011). In Colombia, a similar monthly variation of CLR severity is reported, with an increase from August and a peak from the last months of a year and the first ones of the next year, also in the main harvesting period (Rivillas et al., 2011). In Costa Rica, an experiment of urediospores capture of *Hemileia vastatrix* also showed a similar tendency for the annual progression of CLR disease, with higher levels of spores from October-December followed by a stage of decreasing captures that ends until July. Following that, the number of urediospores started to increase from July-November. This period with higher spore captures coincides with the period when CLR epidemics reach their highest levels (Boudrot et al., 2016).

In Brazil, in turn, CLR intensity has the opposite trend due to its different annual season that occurs at the South cone, with lower levels in December/January, an exponential increase in March/May, and a maximum peak in June/July (Zambolim, 2016). In other regions like Southwestern Ethiopia (the geographic origin of coffee and leading African country producer), monthly variations of CLR severity are also different (North cone). The levels of CLR severity decrease in January (dry season) and reach higher levels in November, with zero values from April to July (rainy season) (Daba et al., 2019). In the Ethiopian case, the stage of higher levels of severity also coincides with the harvest period. In contrast to

the latter, CLR annual variation in eastern Uganda is reported with symptoms that appear two months after the rainy season (June/July) with an exponential increase in August/September peaking in the dry season from December-February (Liebig et al., 2019).

A possible explanation for the association between CLR severity and dry/harvest season has been explored in several studies. It is known that plants allocate resources to reproduction, growth, and defense in a constant dynamic equilibrium to survive (Fine et al., 2006; Seiwa, 2007). During the harvest period, coffee plants allocate resources mainly for fruiting (reproduction) which competes in resources for other tasks such as defense and leaf growth (enhancing leaf fall). Since the higher levels of CLR severity are observed in this phenological stage, the competition between fruiting and defense explains the positive relationship reported between fruit load and CLR severity (Merle et al., 2020) and the more severe attacks of CLR on high yielding coffee trees (Avelino et al., 2015). Furthermore, lower levels of CLR severity during the leaf-out stage are attributed to the rust dilution effect of leaf renewal by incorporating healthy leaves into coffee plants (Ferrandino, 2008; Merle et al., 2020). These changes in phenology over time are an important criterion to determine regimes of fungicide application (Zambolim, 2016).

The key to successful coffee rust control seems to lie in preventively applying fungicides (Avelino et al., 2015). This means that its application should be done during the leaf phenophase (the period of lower levels of CLR severity) and avoiding application during the harvest period due to it is a practice that is not recommended (Rivillas et al., 2011; SENASICA, 2019). According to our results in the studied municipalities, we suggest fungicide application from March-June (months with lower CLR severity). This suggestion is similar to the one proposed by the Regional Early Warning of Coffee Leaf Rust for Veracruz state in 2018-2019 (SAGARPA; SENASICA; LANREF, 2018) in which preventive management is suggested by fungicide application from April-June, except for Cosautlan where fungicide application is proposed from May-July.

In addition to phenological stages, other studies have found that CLR severity is importantly influenced by climate, the altitude effect, shade, soil fertility, and others (Talhinhas et al., 2017). The literature reports that many meteorological factors drive CLR disease, such as temperature, light, humidity, and rainfall (Avelino; Willocquet; Savary, 2004; Liebig et al., 2019). After exploring the possible effect of altitude, temperature (maximum, medium, and minimum), and rainfall on CLR severity in our study in Veracruz, in PCA we observed a plausible positive relation with temperatures and rainfall, while a opposite pattern with altitude was shown. By multiple linear regression, our selected model with the lowest value of Mallows' Cp criterion showed a significant effect of rainfall,

and medium and minimum temperature, while the significance of maximum temperature was marginal.

According to temperature, the life cycles of fungal pathogens are particularly determined by this factor (Bebber; Castillo; Gurr, 2016; Le-May et al., 2020). For *Hemileia vastatrix*, temperature directly affects the process of urediospores germination and appressoria formation, with optimal values of 21.4 and 11.6 °C respectively (De Jong et al., 1987; Bebber; Castillo; Gurr, 2016). A temperature regime from 22 to 27 °C has been reported as more favorable for CLR than the alternative from 18 to 23 °C which limits the sporulation of *H. vastatrix* (Toniutti et al., 2017). Another study showed that a range from 22 to 28 °C is optimal for the latency of urediospores shortening this period of the disease (Avelino et al., 2015). This fully coincides with the pattern observed between temperatures (maximum, medium and minimum) and CLR severity in PCA analysis (Figure 4), but it is partially consistent with our model because we found a positive correlation between CLR severity and only medium temperature in a range from 14.3 – 20.4 °C. This can be noticed with lower medium temperatures at the beginning of months with lower levels of CLR severity (specially in March) and an increment in medium temperatures for January and from May – December (Figure 3). As we consider a lag of two months for the effect of temperature on CLR severity, the months with higher temperatures act as an incubation period for sporulation because the process from germination to the presence of urediospores in leaves can take up to 50 days (Maia et al., 2017), so these temperatures regimes promote higher levels of CLR severity in subsequent months. According to minimum and maximum temperatures, our model showed a negative significant correlation with CLR severity (in ranges from 9.9 – 15.5 and 18.5 – 26.5 °C, respectively). Since we expected higher CLR severity levels with an increment in temperature, this is not consistent with literature.

Temperature is related to altitude, so it is important to consider that it is generally reported that coffee plantations in lower altitudes present higher levels of CLR severity. In Southwestern Ethiopia CLR severity was more intense at an altitude of 1450 m compared to 2063 m (Daba et al., 2019). In Eastern Uganda, CLR severity was also higher in lower altitudes (1100-1400 m) than in higher altitudes (1700-2200 m) (Liebig et al., 2019). In the Americas, Brazilian coffee plantations at an altitude above 1200 m do not require fungicides because CLR prevalence at these altitudes does not exceed 20 % (Zambolim, 2016). In the past, the limits in altitude for severe CLR disease were 1000-1100 and 1600 m for Central America and Colombia, respectively (Avelino et al., 2015).

These decreases in CLR intensity with increasing altitude have been linked to the temperature that affects rust spore germination. The lower temperatures of higher altitudes

result in extended latent period and slower disease development (Daba et al., 2019). All the foregoing is overall consistent with the pattern that we observed in our PCA analysis, where the score plot showed that CLR severity and altitude were diametrically opposed (Figure 4). However, altitude did not have a meaningful contribution to PC1 nor PC2. In addition, in multiple linear regression, this variable also was not significant in our selected model. This contrast may be due to the altitude of fixed plots for the municipalities included in our study (from 952 to 1405 m) can fall only within the range of lower altitudes (higher levels of CLR severity) in previous reports. Despite this contrast, Tlaltetela, the municipality with the highest altitude in our study (1405 m), atypically presented low CLR values during all the year.

On the other hand, in the last years, very high intensities of CLR diseases have been also observed even at altitudes above 1800 m (Avelino et al., 2015). The latter can be explained by climate change which has generated increases in minimum temperatures that have shifted the elevational boundaries to higher altitudes and moved the optimal temperature for germination of urediospores upward (Libert-Amico; Ituarte-Lima; Elmqvist, 2020; Rozo et al., 2012; Torres-Castillo et al., 2020). Additionally, climate change seems to have also reduced the diurnal temperature range shortening the disease's latency period and increasing CLR severity (McCook; Vandermeer, 2015). Many studies confirm that climate change disturbs plant-pathogen interactions promoting more severe infections (Bebber, 2015; Garrett et al., 2006). Nevertheless, a study carried out by Bebber, Castillo and Gurr (2016) rejected the hypothesis that climate change increased CLR intensity.

Regarding rainfall, it is one of the main factors that strongly influence the life cycles of pathogens. It has been established that high unusual levels of precipitation triggered the outbreak of CLR in Colombia (Roza et al., 2012) and irregular patterns of rainfalls in Central America (McCook; Vandermeer, 2015). Urediospores germination requires high levels of humidity in leaves and this leaf wetness can be adequately estimated from rainfall values (Alves et al., 2011), so rainfall can give accurate levels of humidity for the development of CLR disease. Leaf wetness by rainfall is mainly caused by raindrops rebounding on the upper surface of lower leaves and some additional wetting is due to raindrops landing on the undersurfaces when the leaves are disturbed by winds (Rayner, 1961). When such splashes pass through a lesion, this mechanism also promotes the dispersion of spores. The last is mainly important in coffee farms under dense shade where the water from rainfall is accumulated on leaves of shade tree foliage, resulting in an increase of raindrops size and more kinetic energy, so drop impacts on the upper surface of leaves release urediospores and disperse them into the air (Boudrot et al., 2016). In altitudes from 1100-1700 m, when germination is favored by rainfall, symptoms of the disease appear

approximately after two months after the rainy season (Liebig et al., 2019), so this is consistent with our study since in Figure 3 it can be observed that rainfall with a lag of two months were high for October, November and December, which are months with higher CLR severity levels. Despite in PCA analysis the rainfall did not have a meaningful contribution to PC1 nor PC2 (squared cosines of 0.196 and 0.228, respectively), we observed that CLR severity and rainfall are aligned in the same direction (Figure 4). The last is consistent with the relevance of rainfall in the development of CLR disease (Boudrot et al., 2016; Liebig et al., 2019). Moreover, it coincides with our model for multiple linear regression, which showed a highly significant positive effect of rainfall on CLR severity.

5 CONCLUSIONS

CLR severity in Veracruz during 2018 was significantly higher in January, October, November, and December with respect to low values from March-June. The months with higher CLR severity levels were mainly with ripe and consistent fruit phenological stage (harvest period), while months with lower CLR severity levels presented mainly leaf phenophase. According to environmental conditions, temperatures (maximum, medium, and minimum) and rainfall had a significant effect on CLR severity. Minimum and maximum temperature presented a negative relationship with CLR development throughout the year (with a marginal significance for maximum temperature), while medium temperature and rainfall had a positive effect on the disease. Other aspects are relevant in the progress of CLR disease such as varieties cultivated, shade management, edaphic conditions, and others. Thus, as a perspective, the effect of these factors should be analyzed in future investigations.

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7 AUTHORS' CONTRIBUTION

IPE wrote the manuscript and performed the experiment, RM and LRSV supervised the experiment and co-worked the manuscript, and MRPL, EAG and LRSV reviewed and approved the final version of the work, IPE, RM and LRSV conducted all statistical analyses.

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