



Epidemiology of coffee leaf rust under the effect of fungicides in the Northern and Northeastern Sierra of Puebla state

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ABSTRACT

Background/Objective. Coffee leaf rust is the primary disease affecting this crop. The objective was to evaluate the incidence and severity of coffee leaf rust under the influence of a systemic fungicide and two biofungicides on varieties cultivated in the Northern and Northeastern Sierra regions of Puebla.

Experimental development. The effects of Azoxystrobin, *Melaleuca alternifolia*, and *Bacillus subtilis* were evaluated across six shade-grown plots featuring the Typica, Costa Rica 95, Garnica, Marsellesa, and Oro Azteca varieties, located in Huehuetla, Ixtepec, and Zongozotla. Sixteen sampling events were conducted, for each, of evaluation rust incidence and severity, shade percentage, the weight of 100 ripe cherries, and altitude were assessed. Additionally, temperature and relative humidity were recorded in Huehuetla. Incidence and severity were compared using the Kruskal-Wallis test and the Dwass-Steel-Critchlow-Fligner (DSCF) method. Furthermore, the correlation of these variables with altitude and shade percentage was analyzed. Cherry weight data underwent analysis of variance and mean comparison, while a risk analysis was performed using the climatic variables.

Results. Rust reached maximum severity and incidence (19.9 and 100%, respectively) in the Typica variety (March 2025). Azoxystrobin significantly reduced the disease, followed by *M. alternifolia* and *B. subtilis*. No influence of shade on incidence ($r=0.33360$) or severity ($r=0.30735$) was identified. In contrast, altitude showed a moderate negative correlation with incidence ($r=-0.4632$, $p<0.0001$) and with severity ($r=-0.57433$, $p<0.0001$). The Typica and Garnica varieties were the most susceptible.

Conclusion. The fungicides demonstrated control of the disease compared to the control treatment; therefore, they could be considered for the management of *H. vastatrix*.

Keywords: *Hemileia vastatrix*, Epidemiology, Azoxystrobin, Biofungicides, Varieties



INTRODUCTION

Coffee (*Coffea arabica*) is produced in over 80 countries in Latin America, Africa and Asia, mainly in humid tropical regions (WFO Plant List, 2024; Rubio-Sosa *et al.*, 2025). On a global scale, its production is of economic, social and environmental importance (Flores-Vichi, 2015). However, coffee cultivation faces problems such as price instability, high costs of agricultural inputs and phytosanitary problems. In the latter case, the most important and harmful case is orange rust or coffee leaf rust (*Hemileia vastatrix*), which is found in the coffee-producing regions of the world (Díaz-Vicente *et al.*, 2014). Several countries have resorted to the development of varieties with high resistance to rust, including Catigua MG2, Centroamericano, Esperanza, IPR 103, IPR 107, Kartika 1, Milenio, Mundo Maya, Parainema, Ruiru 11, S4808, Starmaya, and others with intermediate resistance such as Marsellesa and Oro Azteca (WCR, 2025). The use of copper oxychloride has also been recommended (preventive action), as well as cyproconazole, azoxystrobin and triadimefon (curative action) (SENASICA, 2019). Other studies have pointed out the reduction of the disease with the use of antagonistic microorganisms such as *Bacillus* sp. or *Trichoderma* sp. (Ramírez-Rodríguez *et al.*, 2020) and plant oils such as *M. alternifolia* (Fajardo-Franco *et al.*, 2020). However, the response of each variety can vary according to rust races and the microclimate found in each region.

In Mexico, coffee is planted in tropical mountainous and temperate areas in altitudes between 300 and 2000 m a. s. l., in a wide variety of climates, soil and vegetation from the center to the south of the country (Anta-Fonseca, 2006). In this sense, coffee is part of the country's strategic products, produced in 14 entities with a contribution of 0.66% of the national GDP (SAGARPA, 2017; Ley de Desarrollo Rural Sustentable, 2024). The main coffee producing states are Chiapas, Veracruz and Puebla (SIAP, 2025), where it is mainly grown by native communities in traditional rustic systems and under shade-grown conditions, which leads to a beneficial microclimate for flora and fauna, carbon storage and nutritional balance (Benítez-García *et al.*, 2015; Libert-Amico and Paz-Pellat, 2018).

In the Northern and Northeastern Sierra of Puebla, there are plantations with rust-susceptible to rust, in which agricultural work and the use of inputs for the management of the diseases are minimal, with a predominant use of workforce, which favors the occurrence of diseases (Fajardo-Franco *et al.*, 2020). Under favorable conditions, rust can attack during the entire cycle of the plant. In this sense, the importance of knowing the current epidemiology of rust in this season is prominent, thus the following aim was established: to evaluate the behavior of the incidence and the severity of coffee leaf rust under the effect of a systemic fungicide and two bio-fungicides, in shade-grown varieties in the Northern and Northeastern Sierra of Puebla.

EXPERIMENTAL DEVELOPMENT

Establishment in experimental plots. In 2024, six plots with traditional management belonging to participating producers from the municipal areas of Huehuetla, Ixtepec and Zongozotla (Northeastern and Northern Sierra of Puebla) were selected. For this purpose, the coffee variety was considered, along with plants in productive ages, shade-grown coffee production system in areas bordering plots without the use of fungicides. The plots were georeferenced using a GPS (Garmin, GPS MAP 64s) (Table 1).

Table 1. Characteristics of experimental plots for the evaluation of incidence, severity, and the effect of fungicides on coffee leaf rust (*H. vastatrix*) in the Northern and Northeastern Sierra regions of the state of Puebla.

Region	Plot	Site	Geographic coordinates	m.a.s.l.	Variety	Age (years)
Huehuetla	Lipuntahuaca 1	El Panteón	20° 04' 26.1'' 097° 38' 03.9'	508	Costa Rica 95	8
	Lipuntahuaca 2	El Manantial	20° 05' 08.0'' 097° 37' 31.8''	498	Typica	12
Iztepec	Escatachuchut 1	El Pozo	20° 03' 03.5'' 097° 38' 24.5''	912	Costa Rica 95	7
	Escatachuchut 2	Las Piedras	20° 03' 48.7'' 097° 37' 41.3''	819	Garnica	6
Zongozotla	Zongozotla 1	Camino Real	20° 04' 13.8'' 097° 38' 19.4''	1116	Marsellesa	6
	Zongozotla 2	La Curva	19° 58' 39.5'' 097° 42' 25.5''	1064	Oro Azteca	6

Experimental plot and treatment design. The experiments were held under field conditions in a randomized complete block design (RCBD), considering the slope as the gradient of variation. Four treatments were evaluated, with five repetition each: Azoxistrobin (Maxtrobin®, 0.4 L ha⁻¹), *Melaleuca alternifolia* (Timorex Gold®, 1.5 L ha⁻¹), *Bacillus subtilis* (Baktillis®, 2 L ha⁻¹) and the control (no application). The experimental design consisted in four plants, adequately identified; in each experiment, 80 plants, established in the center of each venue, were evaluated. Fungicides were applied with manual backpack sprayers (Antarix model AS-18) with fan-shaped nozzles, using one exclusively per treatment to avoid cross-contamination.

In the treatments with Azoxistrobin (AZ) and *M. alternifolia* (MA), a total of two sprays were carried out, with a 15-day interval, whereas *B. subtilis* (BS) was applied four times every 7 days. To stop the product from going adrift due to the wind, sprays were carried out from 6:00 to 10:00 am.

Evaluation of incidence and severity. Sixteen evaluations were carried out in 15-day intervals between August 2024 and March 2025. Severity was determined as the percentage of the foliar area affected by *H. vastatrix* using as a reference a diagrammatic scale that includes 7 classes: 0%, chlorotic spots, 2, 7, 20, 45, ≥70%. Meanwhile, the incidence on the plant was estimated as the proportion of diseased leaves in regard to the total of sampled leaves. For this purpose, three branches were selected from the middle third of the plant, evaluating a total of 10 leaves per individual, distributed in 3, 4 and 3 leaves per branch (SINAVEF, 2013).

Evaluation of shade and weight of cherries. In the central section of each experimental plot, the percentage of shade in every season was evaluated. The measurement was carried out using a concave spherical density meter (Forestry Suppliers-Model C). The weight of the cherries was obtained by gathering 100 mature cherries per plant in every treatment.

Climate variables. The temperature and relative humidity were recorded using a weather microstation (WatchDog, series 1650), located in Huehuetla, Puebla. The information was systematized and the daily mean temperature and relative humidity were calculated (April 2024 to March 2025). Considering the temperature range (17-26 °C) and the optimum relative humidity (>80%) reported for *H. vastatrix*, the period with the most favorable conditions for the development of the disease was analyzed (SENASICA, 2019; Parada-Molina *et al.*, 2020).

Data analysis. Due to the non-compliance of the assumptions of normality, the data of incidence and severity were analyzed using the Kruskal-Wallis test, using the Dwass-Steel-Critchlow-Fligner (DSCF) method to compare multiple ranges. Additionally, a correlation analysis was carried out to determine the relation between the altitude and the percentage of shade with the incidence and severity of the disease. On the other hand, the data on the

weight of the cherries were submitted to an Analysis of Variance (ANOVA) followed by Tukey's for mean comparisons ($p \leq 0.05$). Finally, based on the climate characterization, an analysis was conducted using graphical methods and descriptive statistics, using exclusively the data of the weather microstation (Lipuntahuaca 2). All statistical analyses were carried out using the Statistical Analysis System software (SAS Studio, OnDemand for Academics).

Temporary behavior of rust in control plants. The Marsellesa variety (Zongozotla 1) displayed the lowest percentages of both incidence and severity, whereas Typica presented the highest ones. Regarding severity the initial inoculant (Y_i) in Marsellesa was of 0.1% in October 2024, and the maximum intensity (Y_{max}) in Typica reached 19.9% in March 2025. For incidence, Y_i was also recorded in Marsellesa with 1% on the same date, whereas Y_{max} was observed in Typica (Lipuntahuaca 2) on March 2025, with a value of 100%, indicating that the entirety of the evaluated plants presented symptoms.

The Typica variety has low resistance to rust, whereas Costa Rica 95, Oro Azteca and Marsellesa are considered varieties with an intermediate resistance (Hernández-Martínez and Velázquez-Premio, 2016; WCR, 2025). This tolerance is still found in the study area, since the plots with Costa Rica 95, Marsellesa and Oro Azteca presented low levels of the disease (0.01% severity 1% incidence) (Figure 1). However, they can lead to sources of infection for nearby plots with susceptible varieties such as Typica and Garnica (Fajardo-Franco *et al.*, 2020). The exponential phase coincided with the period of relative humidity of over 80%. The period of highest growth was between December 2024 and February 2025; subsequently, a stationary phase was observed (Figure 1).

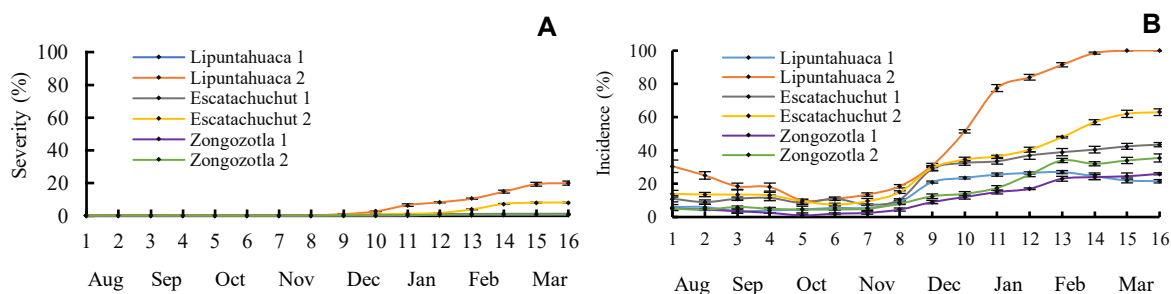


Figure 1. Temporal dynamics of rust (*H. vastatrix*) severity on coffee plants grown in Huehuetla, Ixtotec, and Zongozotla, Puebla, without fungicide application. A) Severity, B) Incidence. The lines represent disease progression over time: Costa Rica 95 (blue), Typica (orange), Costa Rica 95 (gray), Garnica (yellow), Marsellesa (purple), and Oro Azteca (green).

This behavior coincides with what was mentioned by the DGSV (2014) and Díaz-Vicente *et al.* (2014), where rust had a sigmoidal growth and the highest growth rate was presented in the months of January and February, which is attributed to warm temperatures and prolonged periods of humidity (Santiago-Elena *et al.*, 2020). During the study period (April 2024 - March 2025), the mean temperature was 24.9 °C, with an absolute minimum of 9.4 °C recorded in January 2025 and an absolute maximum of 34.6 °C reached in May of 2024.

On the other hand, the relative humidity (RH) fluctuated between 28.2 and 100%. Between August 2024 and January 2025, the temperature remained within a range from 15 °C to 28 °C, and RH surpassed 70%, (occasionally reaching 100%). This coincides with the temperature (17 °C-26 °C) and relative humidity (>80 %) ranges reported as optimal

for the development of *H. vastatrix* (SENASICA, 2019; Parada-Molina *et al.*, 2020). The period with optimum temperatures for the development of *H. vastatrix* was from August to January (standing out as of October). However, from January to March, there were also favorable periods, although temperatures occasionally decreased as far as 9.4 °C (Figure 2).

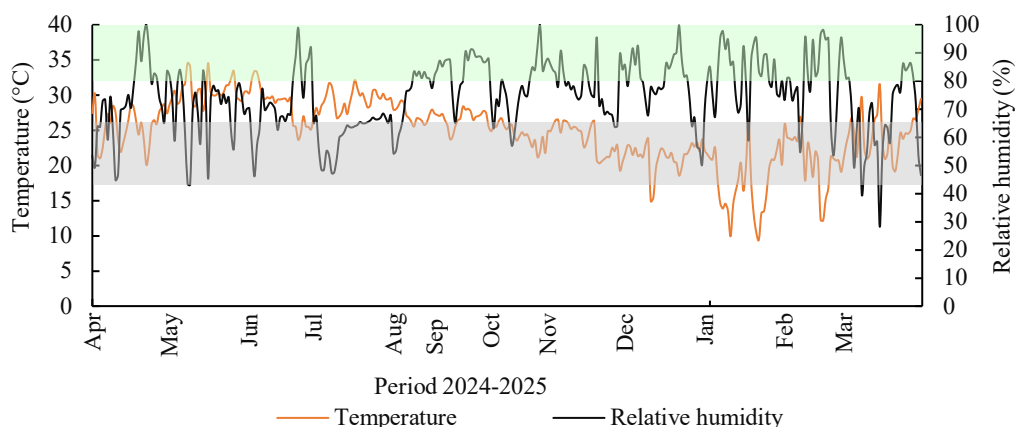


Figure 2. Temperature and relative humidity in Huehuetla, Puebla. Period: April 2024 to March 2025. The gray band indicates the optimal temperature range for coffee leaf rust (17 to 26°C), and the green band indicates the optimal relative humidity range (80–100%) reported by SENASICA (2019) and Parada-Molina *et al.* (2020).

As of November, the days with $HR \geq 80\%$ were more frequent, which, associated to optimum temperatures, could favor the development of the disease, since it was precisely in November when the increases in incidence and severity were observed. Therefore, it is important to identify the favorable periods for the development of the disease in order to design management strategies to help minimize their damages (Guerrero-Carrera *et al.*, 2020).

The correlation analysis indicates that the incidence and severity presented a high positive correlation ($r=0.92928$, $p<0.0001$), similar to what was reported by Julca-Otiniano *et al.*, (2019) in a study on rust in coffee plantations in San Ramón, Chanchamayo, Peru. However, the altitude and damage variables presented a moderate negative correlation: incidence with $r=-0.4632$, $p<0.0001$ and severity with $r=-0.57433$, $p<0.0001$. These results indicate that the highest levels of incidence and severity were found in the plots located at lower altitudes, which may be due to favorable weather conditions with warm temperatures and prolonged periods of humidity (Santiago-Elena *et al.*, 2020).

The percentage of shade varied between 10 and 65%, with maximum values in the summer and spring of 2024 and 2025, respectively. In addition, a low correlation was observed between shade with both incidence ($r=0.33360$) and severity ($r=0.30735$). Therefore, the expression of the disease was not significantly influenced by the shade.

Effect of fungicides on severity and incidence. The three fungicides evaluated significantly reduced the severity and incidence of *H. vastatrix* in all plots, with significant differences between treatments starting on the ninth sampling and until the end of the evaluation. In all sites, Azoxistrobin (AZ) was the most efficient treatment, followed by *M. alternifolia* (MA) and *B. subtilis* (BS), whereas the control plants (TE) recorded the highest values (Figures 3 and 4). The gradual reduction of the disease occurred between days 15 and 30 after the second application.

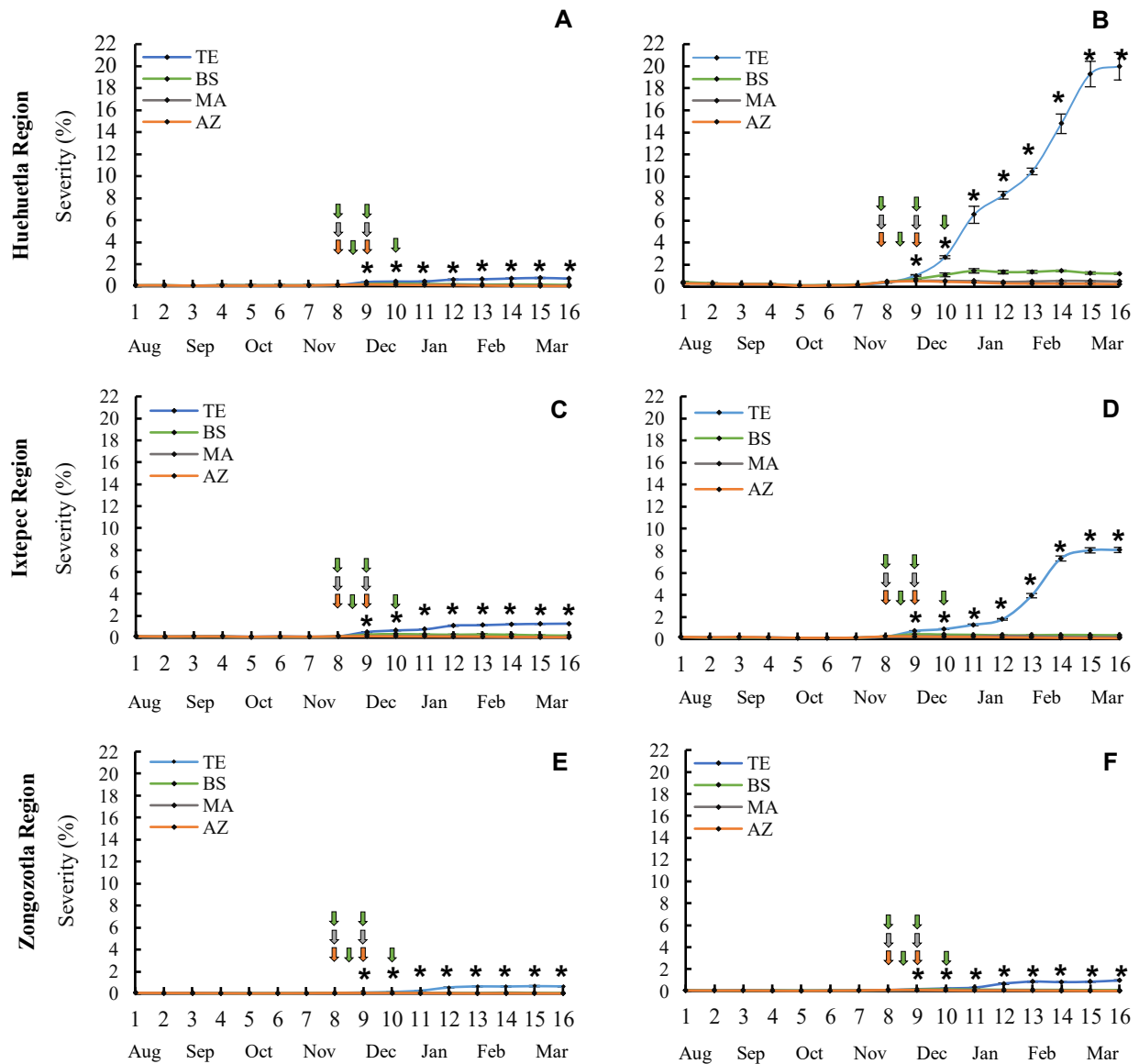


Figure 3. Effect of fungicides on the dynamics of rust (*H. vastatrix*) severity in coffee plants grown in Huehuetla, Ixtepe, and Zongozotla, Puebla. **A)** Lipuntahuaca 1 (Costa Rica 95), **B)** Lipuntahuaca 2 (Typica), **C)** Escatachuchut 1 (Costa Rica 95), **D)** Escatachuchut 2 (Garnica), **E)** Zongozotla 1 (Marsellesa), and **F)** Zongozotla 2 (Oro Azteca). Arrows indicate the timing of fungicide application: orange: Azoxystrobin, gray: *M. alternifolia*, and green: *B. subtilis*. The asterisk (*) indicates significant differences between treatments at each sampling point, according to Tukey's mean comparison test.

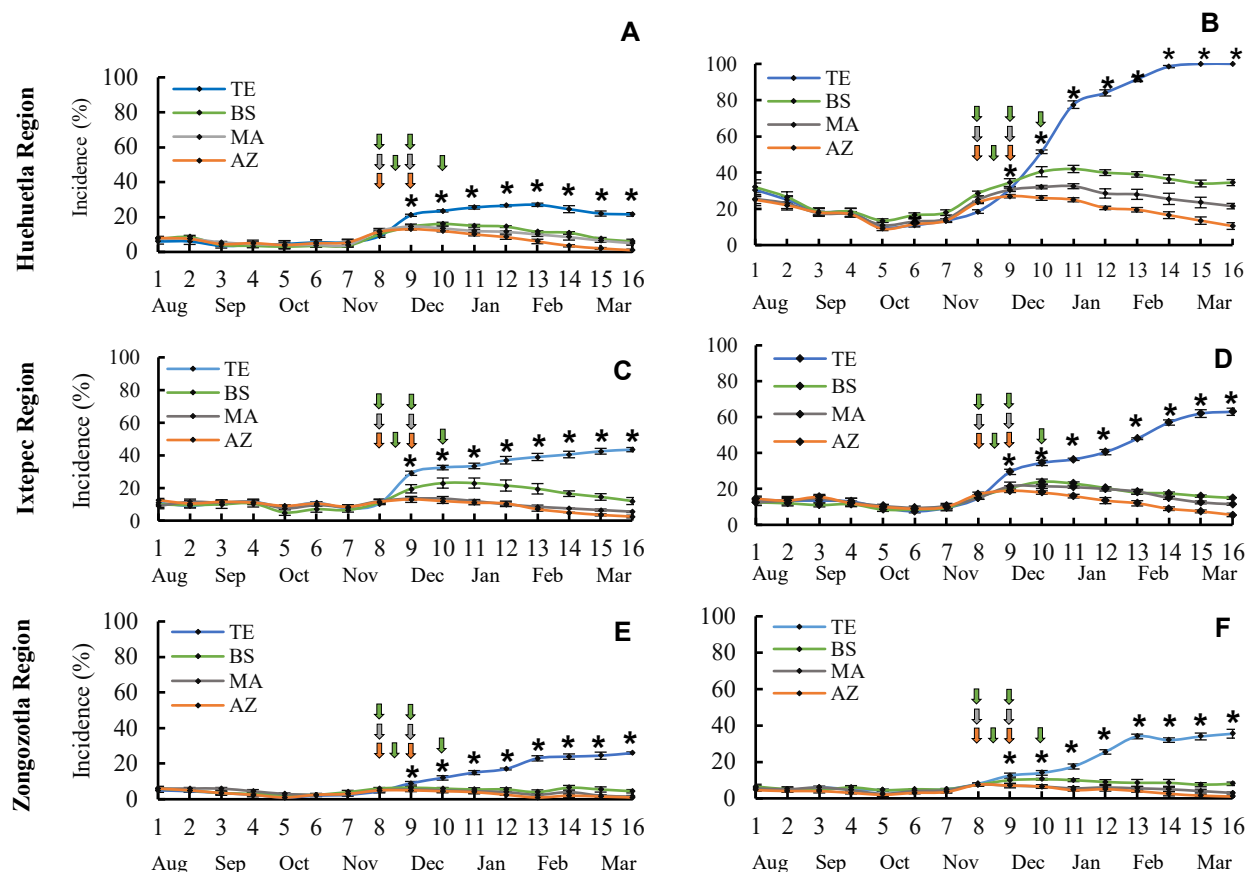


Figure 4. Effect of fungicides on the incidence dynamics of rust (*H. vastatrix*) in coffee plants grown in Huehuetla, Ixtepec, and Zongozotla, Puebla. **A)** Lipuntahuaca 1 (Costa Rica 95), **B)** Lipuntahuaca 2 (Typica), **C)** Escatachuchut 1 (Costa Rica 95), **D)** Escatachuchut 2 (Garnica), **E)** Zongozotla 1 (Marsellesa), and **F)** Zongozotla 2 (Oro Azteca). Arrows indicate the timing of fungicide application: orange: Azoxystrobin, gray: *M. alternifolia*, and green: *B. subtilis*. The asterisk (*) indicates significant differences between treatments at each sampling point, according to Tukey's mean comparison test.

Huehuetla Region (Lipuntahuaca 1 and 2). In this region, the epidemiological extremes of the study converged. Lipuntahuaca 2 (Typica, 498 m a. s. l.) presented the highest disease levels in the control plants, reaching a severity of 19.9% and an incidence of 100%. By contrast, Lipuntahuaca 1 (Costa Rica 95, 508 m.a.s.l.) displayed substantially lower values with only 0.69% of severity and 21.5% of incidence.

Azoxistrobin drastically reduced the disease in both sites, with final severity values of 0.01 and 0.23% and incidence values of 1 and 10.5%, respectively. Treatments MA and BS also presented significant reductions, particularly in Lipuntahuaca 2, where *B. subtilis* only reduced the incidence to 34.5%.

Ixtepec Region (Escatachuchut 1 and 2). In this region, the disease had moderate to low levels. The control in Escatachuchut 2 (Garnica, 819 m a. s. l.) presented the highest damage levels in the region (severity: 8.07%; incidence: 63%), whereas Escatachuchut 1 (Costa Rica 95, 912 m.a.s.l.) presented a quite low natural development of rust (severity 1.28%; incidence 43.5%) without the action of fungicides. Azoxistrobin controlled rust efficiently in both sites, with final severity values of 0.045 and 0.065% and incidence values of 2.5 y 5.5%, respectively. *M. alternifolia* and *B. subtilis* also presented significant reduction, albeit less pronounced, particularly in Escatachuchut 1, where *B. subtilis* only reduced incidence to 12%.

Zongozotla Region (Zongozotla 1 and 2). In Zongozotla 1 (Marsellesa, 1116 m a. s. l.), the control presented a final severity of only 0.65% and an incidence of 26%, whereas Zongozotla 2 (Oro Azteca, 1064 m a. s. l.), the values of the control were slightly higher (severity: 0.97%; incidence: 35.5%). The Azoxistrobin significantly reduced the disease in both sites with final severity values of 0.01% and an incidence of 1%. *M. alternifolia* and *B. subtilis* also displayed significant reductions, particularly in Zongozotla 1, where *M. alternifolia* reduced incidence to 1.5%.

Globally, Azoxistrobin displayed a high consistency, as it reduced final severity between 96.48 and 99.19%, and incidence between 91.26 and 97.18%. This high efficiency in the in the control coincides with previous reports for other rusts, such as the yellow barley rust (*Puccinia striiformis* f.sp. *hordei*) (Rodríguez-García *et al.*, 2021). Nevertheless, due to the negative impact of the agrochemicals in the environment, the need has arisen to generate sustainable alternatives for the management of phytopathogens (Villarreal-Delgado *et al.*, 2017). Under this approach, the *M. alternifolia* extract proved to be a competitive and viable tool, as it achieves reductions in severity between 92.75 and 97.77%, and for incidences, between 76.74 and 94.23%. Likewise, biological control agent *B. subtilis* mitigated final severity between 83.3 and 95.78%, and incidence, between 65.5 and 82.69% (Table 2).

Table 2. Reduction of coffee leaf rust (*H. vastatrix*) through the use of fungicides in the Northern and Northeastern Sierra of Puebla state.

Plot	Active ingredient/Microorganism	Final severity (%)	Reduction in severity (%)	Final incidence (%)	Reduction in incidence (%)
Lipuntahuaca 1	<i>Bacillus subtilis</i>	0.115	83.3	6	72.09
	<i>Melaleuca alternifolia</i>	0.05	92.75	5	76.74
	Azoxistrobin	0.01	98.55	1	95.34
	Control	0.69	-----	21.5	-----
Lipuntahuaca 2	<i>Bacillus subtilis</i>	1.19	94.04	34.5	65.5
	<i>Melaleuca alternifolia</i>	0.46	97.69	21.5	78.5
	Azoxistrobin	0.23	98.84	10.5	89.5
	Control	19.995	-----	100	-----
Escatachuchut 1	<i>Bacillus subtilis</i>	0.19	85.15	12	72.41
	<i>Melaleuca alternifolia</i>	0.055	95.70	5.5	87.35
	Azoxistrobin	0.045	96.48	2.5	94.25
	Control	1.28	-----	43.5	-----
Escatachuchut 2	<i>Bacillus subtilis</i>	0.34	95.78	15	76.19
	<i>Melaleuca alternifolia</i>	0.18	97.77	11.5	81.74
	Azoxistrobin	0.065	99.19	5.5	91.26
	Control	8.075	-----	63	-----
Zongozotla 1	<i>Bacillus subtilis</i>	0.045	93.07	4.5	82.69
	<i>Melaleuca alternifolia</i>	0.015	97.69	1.5	94.23
	Azoxistrobin	0.01	98.46	1	96.15
	Control	0.65	-----	26	-----
Zongozotla 2	<i>Bacillus subtilis</i>	0.085	91.23	8	77.46
	<i>Melaleuca alternifolia</i>	0.03	96.90	3	91.54
	Azoxistrobin	0.01	98.96	1	97.18
	Control	0.97	-----	35.5	-----

Impact on yield (weight of cherries). In the regions of Huehuetla and Ixtpec, applications of Azoxistrobin, *B. subtilis* and *M. alternifolia* significantly increased the weight of 100 mature cherries in comparison with their respective controls, although no statistical differences were recorded between treatments. This positive effect on yield was consistently observed in the plots in both locations, covering the varieties of Costa Rica 95, Typica and Garnica.

The lowest weight for 100 mature cherries was obtained in control plants ($\bar{x}$ =165.45 g) of the Typica variety in the Lipuntahuaca 2 plot (Figure 5B), whereas the highest weight was obtained in plants sprayed with Azoxistrobin ($\bar{x}$ =173.6 g) in the Marsellesa variety in the Zongozotla 1 plot (Figure 5F). However, the plants treated with *M. alternifolia* and *B. subtilis* also displayed an increase in the weight of cherries. In this sense, Rodríguez-García *et al.* (2021) reported a similar behavior when increasing grain yield in barley treated with Azoxistrobin and Tebuconazole.

On the contrary, in the region of Zongozotla, no significant differences were observed between the treatments and the control (Figure 5), possibly due to the security levels being so low (<1 %), therefore, the pathogen did not reduce the weight of the cherries. This low pressure of the disease in Zongozotla can be explained by the synergy between the agroecological conditions of altitude (above 1000 m.a.s.l.) and the use of genetically tolerant varieties such as Marsellesa and Oro Azteca, as well as the low initial inoculant load in the entire region.

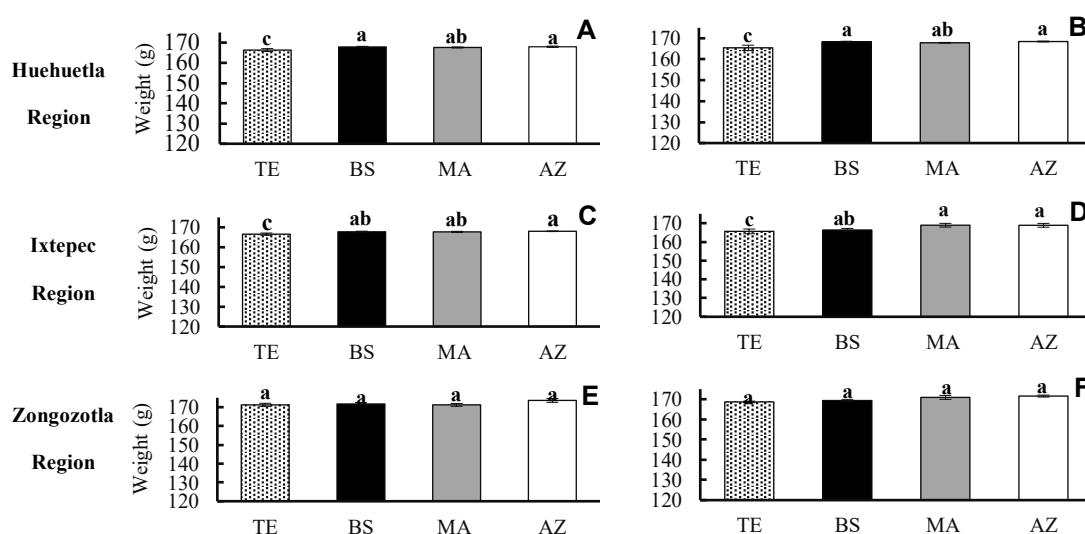


Figure 5. Effect of fungicides on the yield of coffee grown in Huehuetla, Ixtpec, and Zongozotla, Puebla. **A)** Lipuntahuaca 1: Costa Rica 95, **B)** Lipuntahuaca 2: Typica, **C)** Escatachuchut 1: Costa Rica 95, **D)** Escatachuchut 2: Garnica, **E)** Zongozotla 1: Marsellesa, and **F)** Zongozotla 2: Oro Azteca. The bars indicate the treatments: TE = Control, BS = *Bacillus subtilis*, MA = *Melaleuca alternifolia*, and AZ = Azoxystrobin. Bars with the same letter are statistically equal (Tukey, $p \leq 0.05$).

CONCLUSIONS

The epidemic manifested itself between November 2024 and March 2025, reaching a maximum incidence and severity in the months of February to March 2025. The fungicides decreased the incidence and severity of coffee leaf rust in comparison with the control and also had an influence on the weight of the coffee cherry, mainly with the use

of Azoxistrobin, followed by the bio-fungicides produced with *M. alternifolia* and *B. subtilis*, which could, therefore, be considered in the integral management of *H. vastatrix*.

Limitations. The study was carried out in shade-grown coffee plantations in the Northern and Northeastern Sierra of the state of Puebla, and results are applicable to similar conditions.

Conflict of interest. The authors declare to have no conflict of interest regarding the investigation, authorship and/or publication of this Note.

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Contribution of the authors. Conceptualization, methodology, data analysis, writing, review: MJLA, MLFF, and MAT. Experimentation: MJLA and MLFF.

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