International Journal of Agriculture and Biosciences 2025 xx(x): xx-xx.
https://doi.org/10.47278/journal.ijab/2025.084

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Aggressiveness of Different Isolates of Coffee Leaf Rust from Cusco and Functioning of PSII of Coffee CV Bourbon

Ricardo Borjas Ventura [1,*], Leonel Alvarado Huamán [1], Deyna Valderrama Palacios [1], Fabiola León Rojas [1], Viviana Castro-Cepero [1,2], Segundo Bello Amez [1], Carolina Cedano Saavedra [3] and Alberto Julca-Otiniano [1]

[1]Departamento de Fitotecnia. Facultad de Agronomía. Universidad Nacional Agraria La Molina, Av. La Molina s/n, La Molina, Lima, Perú
[2]Departamento de Biología. Facultad de Ciencias. Universidad Nacional Agraria La Molina, Av. La Molina s/n, La Molina, Lima, Perú
[3]Departamento de Agronomía y Zootecnia. Universidad Nacional de Trujillo, Perú
*Corresponding author: rborjas@lamolina.edu.pe

ABSTRACT

One of the main threats for coffee production is coffee leaf rust (CLR) caused by *H. vastatrix*. CLR research have predominantly focused on qualitative host-pathogen interactions, however, studies of quantitative aspects of the interaction of coffee and *H. vastatrix* are less documented. Therefore, this information is crucial for understanding the potential impact of new CLR races, coffee resistance durability and preventing new CLR outbreaks. In this experiment, we evaluated the aggressiveness of different populations of *H. vastatrix* and the effect on some ecophysiological parameters on coffee cv. Bourbon. Urediniospores of *H. vastatrix* were collected from coffee farms in La Convencion, Cusco. A total of 15 populations were obtained (from 1RS to 15 RS). The urediniospores were inoculated on coffee cv Bourbon in San Ramón (Chanchamayo). The aggressiveness (in term of Period of incubation, Frequency of infection, Latent Period and Medium latent period) and fluorescence (through OJIP analysis) were examined. It was recorded that a mean period of incubation, latent period and medium latent period for all the coffee leaf rust populations ranged from 18-20.22, 28.1-33 and 32-38.14 days after inoculation (dds). The variation in the infection period was of 2.75-8 degrees. 3RS were also less aggressive than 5RS. At 41 degrees, we observed that the level of chlorophylls fell significantly, ranging from 4.6 to 31.15%. Regarding to OJIP analysis, the majority of coffee leaf rust populations did not cause significant modification in the variation of Fv/Fm displaying a range from 0.51 to 0.73, at 41 dds. In the case of populations 9RS, 10RS, 13RS, 14RS and 15RS, they presented significant fall in the value of Fv/Fm of 15, 27, 9, 18 and 32% at the end compared to the first sampling. On overall, the range of PI varied from 1.69 to 5.18. 6RS caused in significant increment of ETo/RC. 9RS, 10RS, 14RS and 15RS increased ($P \leq 0.05$) the value of ABS/RC in 23, 149, 74 and 108% (compared to the initial evaluation). 9RS showed a significant increment of TRo/RC of 23.5% in the last sampling. Finally, we conclude that weather conditions and plant material used in this assessment caused a rapid period of incubation (IP). It also detected a photosynthetic defense mechanism that consisted in the increment of ABS/RC, ETo/RC and TRo/RC when the levels of Fv/Fm have fallen.

Keywords: *Chrolophyll a* fluorescence, Chlrophyll index, incubation period, latent period, *Hemileia vastatrix*.

Article History
Article # 25-131
Received: 20-Mar-25
Revised: 31-Mar-25
Accepted: 13-Apr-25
Online First: 20-June-25

INTRODUCTION

Coffee is a prominent commodity in global trade (Fromm, 2023). This crop carries considerable economic, cultural, and social significance worldwide (Maspul, 2023; Bracken et al., 2023). It is cultivated in over 60 countries,

Cite this Article as: Borjas-Ventura R., Alvarado-Huaman L, Valderrama-Palacios D, León-Rojas F, Castro-Cepero V, Bello-Amez S, Cedano-Saavedra C and Julca-Otiniano A, 2025. Aggressiveness of different isolates of coffee leaf rust from Cusco and functioning of PSII of coffee cv Bourbon. International Journal of Agriculture and Biosciences xx(x): xx-xx. https://doi.org/10.47278/journal.ijab/2025.084

A Publication of Unique Scientific Publishers

with an estimated 25 million farmers primarily being smallholders (less than 5 hectares) (Bracken et al., 2023; Fromm, 2023). Additionally, around 125 million individuals globally are directly engaged in various stages of the coffee value chain (Fromm, 2023). Peru is the world's seventh largest coffee producing countries, and coffee farms occupy more than 400 thousand hectares (MIDAGRI, 2013). And, it is present in 17 regions (MIDAGRI, 2025). One of the most important Peruvian region that produces coffee is Cusco, in fact, it depicts 8% of total coffee produced (ComexPeru, 2023). Coffee production is hold by 223 482 of small farmers. Likewise, the employment generated by coffee production is the third part of total of agricultural employment (MIDAGRI, 2025). Coffee offers environmental services, especially when it is grown under agroforestry systems (Jezeer et al., 2019; Cerda et al., 2020).

Despite a remarkably economic, social and environmental importance, coffee production remains low in countries such as Peru (400 – 600kg ha-1) (MIDAGRI. 2013). Crop production is subject to various forms of biotic stress caused by a wide range of microorganisms, including fungi, bacteria, and nematodes (Gull et al., 2019). The cultivation of *Coffea arabica* is especially threatened by different pathogenic agents, such as *Hemileia vastatrix*, which is responsible for the disease known as "coffee leaf rust" (CLR). This disease is considered the most destructive affliction impacting coffee production globally (Silva et al., 2006; Pires et al., 2020).

In Peru, this fungus was first reported in the 1979s (Julca-Otiniano et al., 2013). Currently, studies indicate an incidence rate of 30% in susceptible cultivars; however, this can vary depending on climatic conditions and genetic material (Alvarado-Huamán et al., 2020; Borjas-Ventura et al., 2020). Between 2010 and 2013, coffee production was reduced by a series of outbreaks in Colombia, Costa Rica, Guatemala, Peru, among others. In Central America, it was estimated that CLR affected 50% of coffee plantations and reduced in 17% the employment of the area (ICO, 2013). In Colombia and Peru was reported a loss of 30 to 50% of coffee production (Avelino et al., 2015; MIDAGRI, 2013). Four main hypotheses have been put forward to explain CLR outbreaks: fungus evolution (new races), climate change, land use change and low coffee prices (Koutouleas and Collinge, 2022).

One of the primary strategies for controlling CLR involves the use of tolerant genetic material, such as Catimors or Sarchimors, which are derived from Caturra x Timor and Villa Sarchi x Timor, respectively (Julca-Otiniano et al., 2023). However, in personal communications with various small-scale farmers, it has been noted that some Catimor varieties are losing their tolerance to CLR. Consequently, developing new, tolerant genetic material is crucial to ensure sustained high yields.

New coffee genetic material, whether produced domestically or imported from other countries, must possess resistance genes against CLR. The resistance of coffee to CLR can be explained by Flor's theory, which concludes that for each resistance gene in the coffee plant, there is a corresponding virulence gene in the pathogen (Quiroga-Cardona, 2021). Therefore, it is essential to identify the genetic traits of the different races of CLR to determine which resistance genes should be incorporated into new cultivars.

CLR is capable of infecting, germinating on, and colonizing coffee plants due to the presence of specific genes known as virulence genes (v1-v9) (Rodrigues Junior et al., 1975), whose combinations give rise to physiological races. In fact, there are currently 55 known physiological races (Silva et al., 2022). In Peru, Julca-Otiniano et al. (2024) have identified the following physiological races: XXIII (v1,2,4,5), I (v2,5), XXIV (v2,4,5), a new race (v1,2,4,5,7,8 or v1,2,4,5,7,8,9), XXXV (v2,4,5,7,9) and XXXIV (v2,5,7 or v2,5,7,9). It is important to note that *H. vastatrix* has the ability to mutate, resulting in new races that can overcome the resistance of existing genetic material. Additionally, Quispe-Apaza et al. (2017) observed greater CLR diversity in Quillabamba compared to Villa Rica, suggesting that this genetic diversity may indicate variability in the pathogenicity of different CLR isolates.

On the other hand, aggressiveness refers to the pathogenic capacity of microorganisms (Rozo et al., 2012; Suffert et al., 2018) and can be quantified using several parameters, including the incubation period, latency period, number of sporulated lesions, and infection intensity (Avelino and Rivas, 2013). Regarding the pathogenicity or aggressiveness of pathogen isolates or races, Eskes (1983) reported varying degrees of pathogenicity among different *H. vastatrix* isolates. Similar findings were reported by Morales and Grajea (2021) in Honduras. Under Peruvian conditions, the aggressiveness of different CLR isolates is not well understood, which limits effective disease control.

The organ affected by CLR is the leaf, where it causes chlorosis and defoliation. Once *H. vastatrix* reaches the coffee leaf and penetrates the plant cell, it alters metabolic processes to create an environment conducive to its development. *H. vastatrix* is also capable of suppressing the plant's defense mechanisms. Coffee plants have two primary defense systems: the first is a surface-level defense involving the activation of kinases, while the second includes an increase in the activity of enzymes such as peroxidases, superoxide dismutase, and catalases (Talhinhas et al., 2017; Silva et al., 2022; Honorato Júnior et al., 2015a). The overproduction of antioxidant enzymes indicates an excessive generation of reactive oxygen species (ROS), which can potentially damage various cellular structures, including proteins and membranes. Additionally, one of the most significant processes affected by *H. vastatrix* is photosynthesis. The presence of this pathogen has been shown to decrease Fv/Fm, indicating impaired health of photosystem II (PSII). Pigment production is also adversely affected (Honorato Júnior et al., 2015a). Despite this information, further research is needed to better understand the extent of the damage caused by *H. vastatrix* to coffee plants.

The OJIP test offers a quantitative evaluation of fluorescence kinetics (Moreno et al., 2008) and serves as a valuable method for assessing the impact of specific pathogens on plants. This analysis evaluates the health of photosystem II (PSII) and the components of the electron transport chain during photosynthesis (Toniutti et al., 2017). Several researchers highlight the importance of the

OJIP test, noting its utility in the early evaluation of plant responses to both biotic and abiotic stress (Ceacero et al., 2012; Mariño, 2014; Rodriguez et al., 2014).

Then, the objectives of this work are, for one hand, to quantify the aggressiveness of different isolates of *H. vastatrix* and to determine the health of Photosystem II when it is affected by the isolates of CLR collected in the province of Convención in Cusco.

MATERIALS & METHODS

Urediniospores Collection

The urediniospores were collected with gelatin capsules from well sporulated lesions on 4 districts (Huayopata, Santa Ana, Vilcabamba and Maranura) in La Convencion province, Cusco (Peru) (Fig. 1) (Várzea, 2016). Coffee plantations with high incidence of *H. vastatrix* were sampled and information of geographic reference, famer, cultivar, and severity were recorded (Table 1). The urediniospore collection was performed in May (2023).

The gelatin capsules with urediniospores were labelled and transported to the ¨Tropical Crops¨ laboratory of the National Agrarian La Molina University (UNALM) in San Ramon, Junin (Peru) (altitude: 965 m.a s. l.; latitude 11° 5'44.62"S and longitude 75°21'8.49"W) (Fig. 2).

Table 1: Collection data from coffee farms in La Convención, Cusco

Farm	Date	Producer	Altitude	East (m)	North (m)	Locality	District	Province	Region	Cultivar	Plant age
1RS	15/05/23	Alberto Aucapuro	1316	759279	8560982	Chuyamayo	Huayopata	La Convencion	Cuzco	Caturra	8
2RS	15/05/23	Teodoro Quispe	1450	759440	8561243	Chalanque	Huayopata	La Convencion	Cuzco	Typica	8
3RS	15/05/23	Washintong Saive	1553	762267	8561176	Huayopata	Huayopata	La Convencion	Cuzco	Geisha	10
4RS	16/05/23	Frank Palomino	1510	742732	8573172	Esmeralda	Santa Ana	La Convencion	Cuzco	Typica	15
5RS	16/05/23	Orlando Tupayachi	1470	743063	8573076	Cacaopampa	Santa Ana	La Convencion	Cuzco	Typica	30
6RS	16/05/23	Ricardo Quintanilla	1484	743354	8572798	Cacaopampa	Santa Ana	La Convencion	Cuzco	Typica	30
7RS	16/05/23	Javier Tupegachi	1408	744087	8572914	Ipal	Santa Ana	La Convencion	Cuzco	Typica	30
8RS	17/05/23	Walter Gonzales	1591	748053	8558737	Mesacancha	Vilcabamba	La Convencion	Cuzco	Typica	20
9RS	17/05/23	Porfirio Quispe Quispe	1582	747717	8559140	Moyomonte	Vilcabamba	La Convencion	Cuzco	Typica	20
10RS	17/05/23	Noemi Pumacayo	1595	746746	8559354	Ipal Bajo	Vilcabamba	La Convencion	Cuzco	Typica	15
11RS	17/05/23	Cesar Carrillo Mar	1899	735054	8557983	Oyara	Vilcabamba	La Convencion	Cuzco	Typica	20
12RS	17/05/23	Sergio Vilxhag	1865	736334	8557547	Oyara	Vilcabamba	La Convencion	Cuzco	Typica	20
13RS	17/05/23	Eusebio Fuentes	1584	743927	8558203	Accorcona	Vilcabamba	La Convencion	Cuzco	Typica	25
14RS	18/05/23	Adele Avenas	1363	755058	8566521	Kcosñipata	Maranura	La Convencion	Cuzco	Typica	10
15RS	18/05/23	René Escalante	1753	751315	8570421	Huayllapata	Maranura	La Convencion	Cuzco	Typica	1

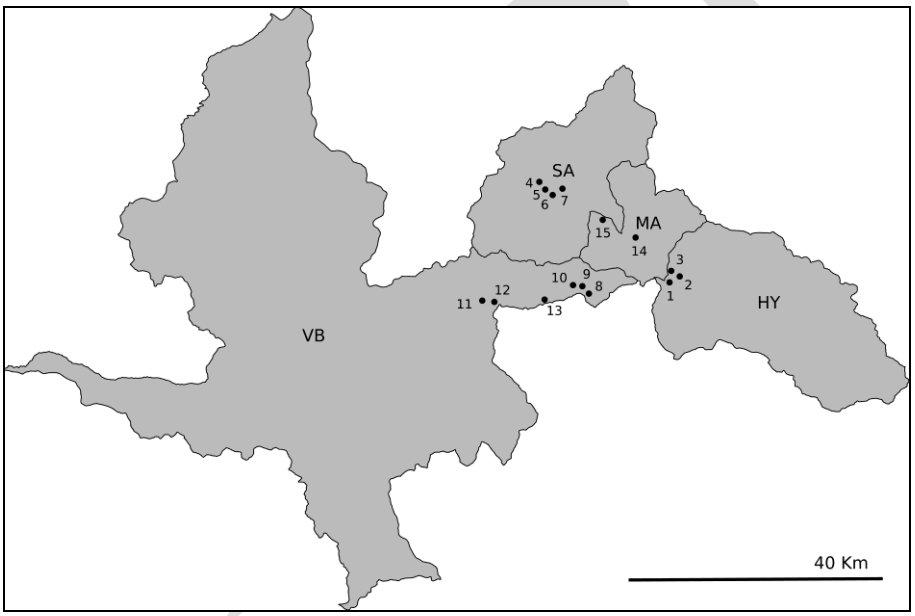

Fig. 1: Map of some districts of La Convención province where the *H. vastatrix* islates were collected; HY: Huayopata, SA: Santa Ana, VB: Vilcabamba, MA: Maranura.

Fig. 2: Urediniospores collection with gelatin capsules in La Convencion, Peru.

Inoculation

Two leaves of 6-months coffee seedlings cv. Bourbon was inoculated with urediniospores of *H. vastatrix* (about 1mg per pair of leaves) in the ¨Tropical Crops¨ greenhouse of the National Agrarian La Molina University (UNALM) in San Ramon, Junin (Peru) (Fig. 3). The inoculation was performed with a camel´s hairbrush on the lower surface of the leaf at 5:00 pm when the temperature was around of 23°C and the relative humidity was of 87% (Fig. 5). The inoculated leaves were sprayed with distilled water and enveloped with a humid plastic bag for 24h. To avoid the direct incidence of the sun rays, the plastic bags were covered with newspaper sheets (Várzea et al., 2023).

Fig. 3: Inoculation of *Hemileia vastatrix* on attached leaves; A: Brushed with a camel´s hairbrush B: Enveloped with a humid plastic bag during 24 h C: Covered with newspaper sheets.

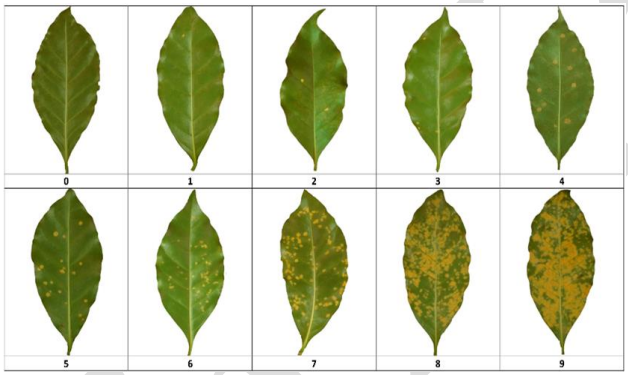

Fig. 4: Infection frequency scale of coffee leaf rust (0-9) adapted from Eskes (1983).

Aggressiveness Assessment

Aggressiveness components were measured on each inoculated leaf. The period of incubation was measured as the time (days) between the inoculation and the appearance of the early symptoms (Santacreo, 1989). The infection frequency (Fig. 4) as the number of sporulated lesions per leaf in the 35th day after inoculation, using an arbitrary scale (0-9) proposed by Eskes (1983) (Fig. 45). The latent period as the time (days) between the inoculation and the production of urediniospores (Leguizamón et al., 1998) and medium latent period as the time (days) between the inoculation and the production of urediniospores of 50% of the lesions (Costa et al., 2007).

Ecophysiological Assessment

Chlorophyll content was estimated by a chlorophyll meter SPAD-502 PLUS KONICA MINOLTA. Likewise, for the OJIP test, the fluorometer OP-30p OPTI-SCIENCE was used to quantify the parameters as Fv/Fm (maximal photochemistry activity), PI (Performace Index), ABS/RC (Mean absorbed photon flux per PS II reaction center), TR_O/RC (maximum trapped exciton flux per PS II) and ET_O/RC (Electron transport flux from Q_A to Q_B per PS II) (Stirbet and Govindjee, 2011). The measurements were performed on the 1 st, 19th, 35th, and the 41th day after inoculation.

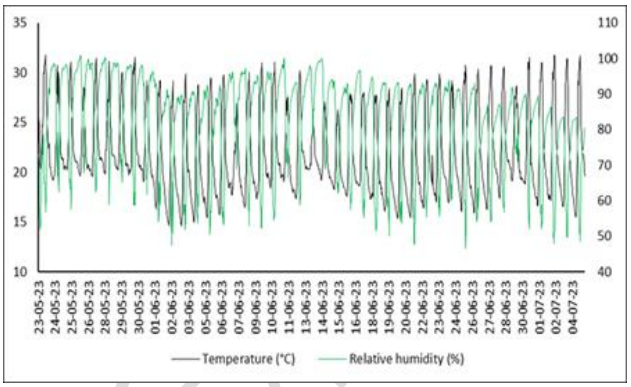

Fig. 5: Temperature (℃) and relative humidity (%) during the experiment in San Ramón, Perú.

Data Analysis

The treatments consisted in fifteen isolates of *H. vastatrix* from La Convencion province, Cusco. Each treatment consisted on 5 replicates (1 replicate = 1 seedling). The experimental design was completely randomized. One-way analysis (ANOVA) was used and a multiple comparison test was done using Tukey (95%).

RESULTS

Aggressiveness Assessment

The incubation period (IP), latent period (LP) and medium latent period (LPM) varied significantly among the isolates, ranging from 18 to 20.22 for IP, 28.1 to 33 for LP and 32 to 38.14 for LPM (days). The infection frequency (IF) ranged from 8 in the most highly aggressive isolates and 2.75 for the least aggressive (Fig. 6).

The differences among isolates for the aggressiveness components measured are shown in Fig. 6. For instance, the isolate 3RS was the least aggressive for all isolates with 21 days for IP, 33 days for LP, 36 days for LPM and 2.2 for IF. Similar tendency was found in the isolate 1RS. However, there is not consistent results for the most aggressive isolate in all four components. For the IP, the isolates 4RS, 7RS, 11RS, 12RS, and 14RS reported the lowest values around 18 days. However, for the IF, the isolates 5RS presented the highest value 8.

Content of Chlorophylls

The level of chlorophylls showed a decrease during the experiment, especially after the LP. At the end of the evaluation (41dds), we observed that the level of chlorophylls fell significantly in a range of 4.6%-31.15% in most of the isolations (except the 1RS and 13RS) (Fig. 7)

compared to the initial evaluation. 1RS and 13RS showed the same content of chlorophylls during the experiment (P≤0.05).

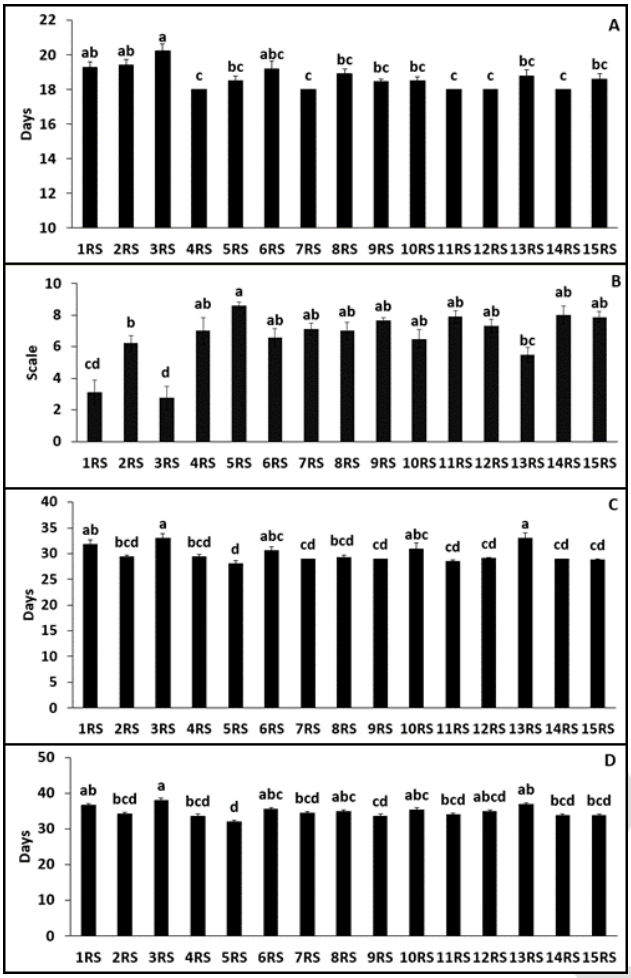

Fig. 6: Aggressiveness of different population of *H. vastatrix* collected in Cusco; Incubation period B) Infection frequency C) Latent period D) Medium latent period. Different letters indicate statistical differences (Tukey 95%).

OJIP Test

In general, we observed a trend of diminishing the value of Fv/Fm as the appearance of sings incremented. The majority of CLR populations did not cause significant effect on Fv/Fm showing a range from 0.51 to 0.73, at 41 dds. In the case of populations 9RS, 10RS, 13RS, 14RS and 15RS, they presented significant fall in the value of Fv/Fm of 15%, 27%, 9%, 18% and 32% at the end compared to the first sampling (Fig. 8).

Likewise, for Performance Index (PI), most of isolates provoked a decrease of PI in the last evaluation; although, this fall was not significant. The range of PI varied from 1.69 to 5.18 (Fig. 9).

ETo/RC was not affected by populations of *H. vastatrix* (P≤0.05) except in 6RS where a significant increase was recorded in the last sampling. In the case of ABS/RC, just the populations 9RS, 10RS, 14RS and 15RS increased their value in 23%, 149%, 74% and 108% (compared to the initial evaluation), respectively. Only the isolate 9RS showed a significant increment of TRo/RC of 23.5% in the last measurement (Table 2).

DISCUSSION

Aggressiveness

The aggressiveness of different isolates of *H. vastatrix* from Peruvian regions has been few studied (Palacios et al., 2025). This character is associated to incomplete resistance, it means that low aggressiveness can be associated to multiples resistance gens in coffee plants (Várzea et al., 2023). In Peruvian conditions, Quispe-Apaza et al. (2017) and Quispe-Apaza et al. (2021) reported genetic variability of *H. vastatrix*. In the last year, Julca-Otiniano et al. (2024b) stated the presence of different races of *H. vastatrix*.

The genetic variability of different pathotypes of *H. vastatrix* can explain the different degrees of aggressiveness found in this work (Fig. 6). In general, the results obtained for IP were less than report by Pozza et al. (2021) and Pires et al. (2020) who found IP of 25-30 days, even Kushalappa and Martins (1980) reported that IP can extend until 65 days. On contrary, the values of LP noticed in this work were in range of those ones reported by Toniutti et al. (2017) (21-37 days) and Maia et al. (2017) (17-50 days).

The frequency of infection is the most effective method for assessing the damage caused by coffee leaf rust, as it involves counting pustules, providing a more precise and less subjective measurement (Gallego-Sánchez et al., 2020). The IF is an scale that is associated to the severity of the disease. The severity, at the same time, is a character heavily associated to incidence (Julca et al., 2019), as well. In peru, the severity has been studied by Borjas-Ventura et al. (2020) and Alvarado-Huamán et al. (2020), in both cases an increase in the precipitation increases the severity of *H. vastatrix*. Furthermore, the severity is high in susceptible cultivars and when the coffee plant is fruiting.

In particular, it was noticed that as 1RS as 3RS were the least aggressive (Fig. 6). Both 1RS and 3RS displayed low value of Infection Fequency (IF). The behaviour of the isolates 1RS and 3RS imply that the establishment and development of *H. vastatrix* intra and intercellularly can vary slightly when the pathogen is inside the cell. It is reported that when the pathogens are inside the plant cell, they are able to create suitable conditions, through certain proteins (effectors), for successful colonization (Lovelace et al., 2023). Subit et al. (2021) indicated that disease progression may vary when the pathogen is within the cell, as fungal maturation requires differentiation into sori.

Content of Chlorophylls

Chlorophylls are essential pigments in plants as they absorb energy from sunlight for photosynthesis, a crucial process for growth (Ebrahimi et al., 2023; Zulkarnaini et al., 2019; Jin et al., 2023). Leaf greenness serves as an indicator of chlorophyll content, as it reflects the photosynthetic capacity and overall health of plants (Zulkarnaini et al., 2019). This variable is considered a physiological indicator, with higher levels associated with optimal conditions for growth and development, and low levels with stress conditions (Motyka et al., 2020).

Table 2: ETo/RC, ABS/RC, and TRo/RC after the inoculation of *H. vastatrix* from Cusco on coffee seedlings cv Bourbon

	ETo/RC				ABS/RC				TRo/RC			
	1dds	19dds	35dds	41dds	1dds	19dds	35dds	41dds	1dds	19dds	35dds	41dds
1RS	4.72±0.13 a	4.56±0.14 a	4.62±0.13 a	4.87±0.16 a	1.69±0.04 a	1.72±0.05 a	1.75±0.05 a	2.33±0.48 a	1.28±0.03 a	1.31±0.04 a	1.28±0.03 a	1.31±0.06 a
2RS	4.51±014 a	4.64±0.07 a	4.95±0.17 a	4.78±0.18 a	1.74±0.06 a	1.68±0.03 a	2.04±0.35 a	2.04±0.35 a	1.29±0.04 a	1.29±0.03 a	1.27±0.04 a	1.5±0.19 a
3RS	4.35±0.21 b	4.48±0.09 b	5.21±0.14 a	4.65±0.16 ab	1.73±0.07 a	1.68±0.07 a	1.74±0.06 a	1.83±0.08 a	1.29±0.05 a	1.28±0.05 a	1.29±0.03 a	1.3±0.03 a
4RS	4.4±0.06 a	4.7±0.06 a	4.74±0.38 a	4.86±0.27 a	1.68±0.05 a	1.59±0.02 a	2.00±0.37 a	2.13±0.21 a	1.26±0.03 ab	1.23±0.01 b	1.27±0.06 ab	1.4±0.04 a
5RS	4.43±0.17 b	4.57±0.07 ab	5.11±0.17 a	4.38±0.13 b	1.79±0.04 a	1.64±0.02 b	1.73±0.04 ab	1.73±0.04 ab	1.33±0.03 a	1.26±0.01 a	1.31±0.03 a	1.27±0.02 a
6RS	4.28±0.12 b	4.78±1.17 ab	5.48±0.3 a	5.23±0.21 a	1.71±0.04 a	1.68±0.05 a	1.87±0.07 a	1.92±0.21 a	1.28±0.02 a	1.28±0.04 a	1.36±0.04 a	1.36±0.06 a
7RS	4.54±0.21 a	4.72±0.08 a	4.94±0.59 a	4.59±0.12 a	1.71±0.03 a	1.67±0.05 a	1.68±0.24 a	2.05±0.29 a	1.29±0.03 a	1.27±0.03 a	1.23±0.16 a	1.37±0.08 a
8RS	4.19±0.06 b	4.7±0.1 b	5.69±0.45 a	4.59±0.14 b	1.63±0.04 a	1.71±0.06 a	1.75±0.08 a	1.76±0.07 a	1.24±0.03 a	1.3±0.04 a	1.35±0.07 a	1.31±0.04 a
9RS	4.27±0.1 b	4.8±0.11 b	5.63±0.23 a	4.85±0.15 b	1.66±0.03 b	1.73±0.03 b	1.94±0.11 ab	2.05±0.12 a	1.25±0.02 b	1.3±0.02 b	1.38±0.04 ab	1.43±0.07 a
10RS	3.99±0.13 a	4.74±0.09 a	5.11±0.71 a	4.51±0.42 a	1.62±0.04 b	1.65±0.05 b	1.9±0.32 b	4.03±1.37 a	1.22±0.25 a	1.24±0.04 a	1.37±0.09 a	4.47±1.17 a
11RS	4.13±0.11 a	4.58±0.01 a	4.88±0.65 a	4.88±0.26 a	1.71±0.04 a	1.61±0.05 a	1.97±0.27 a	2.24±0.51 a	1.28±0.03 a	1.21±0.04 a	1.33±0.15 a	0.94±0.44 a
12RS	3.95±0.08 b	4.96±0.12 a	5.49± 0.27 a	3.77±0.07 b	1.63±0.04 a	1.78±0.06 a	1.78± 0.07 a	1.9±0.16 a	1.22±0.02 a	1.31±0.03 a	1.32± 0.04 a	1.27±0.05 a
13RS	4.91±0.15 b	5.07±0.13 b	6.63±0.61 a	4.12±0.13 b	1.8±0.05 a	1.8±0.06 a	2.10±0.08 a	2±0.19 a	1.36±0.04 ab	1.31±0.05 ab	1.35±0.06 a	1.44±0.05 b
14RS	4.12±0.12 b	4.55±0.08 ab	4.74±0.14 a	4.12±0.17 b	1.72±0.04 b	1.76±0.05 b	1.95±0.11 ab	2.99±0.84 a	1.31±0.02 a	1.33±0.02 a	1.95±0.05 a	2.99±0.13 a
15RS	4.1±0.01 b	4.56±0.09 ab	4.98±0.2 a	4.42±0.36 ab	1.73±0.04 b	1.71±0.05 b	1.84±0.08 b	3.59±0.8 a	1.31±0.03 ab	1.3±0.04 b	1.35±0.05 ab	1.75±0.24 a

Different letters indicate statistical differences (Tukey 95%) in the same row. dds: days after inoculation

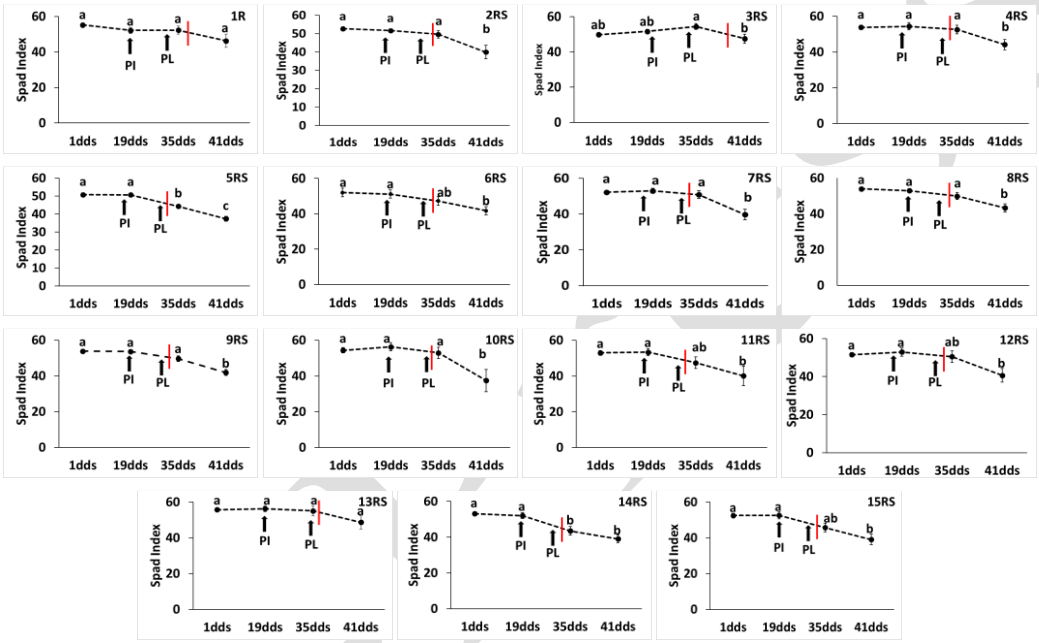

Fig. 7: Chlorophyll content after the inoculation of *H. vastatrix* from Cusco on coffee seedlings cv Bourbon; dds: Days after inoculation. Red line: Medium latent period. PI: Incubation period. PL: Latent period. Different letters indicate statistical differences (Tukey 95%). dds: days after inoculation.

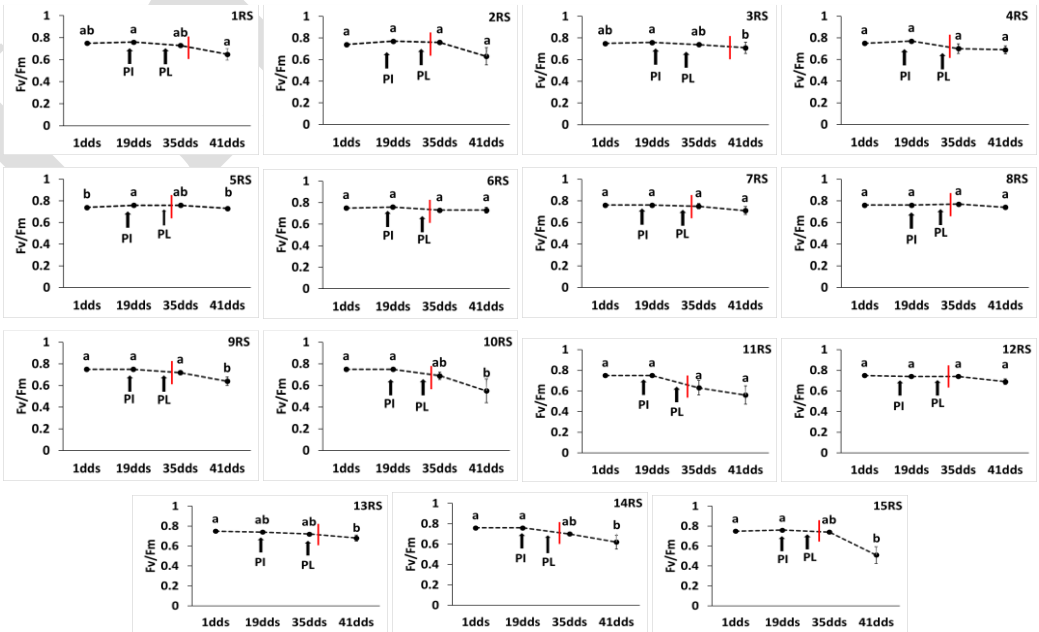

Fig. 8: Fv/Fm ratio after the inoculation of *H. vastatrix* from Cusco on coffee seedlings cv Bourbon; dds: days after inoculation. Red line: Medium latent period. PI: Incubation period. PL: Latent period. dds: days after inoculation. Different letters indicate statistical differences (Tukey 95%).

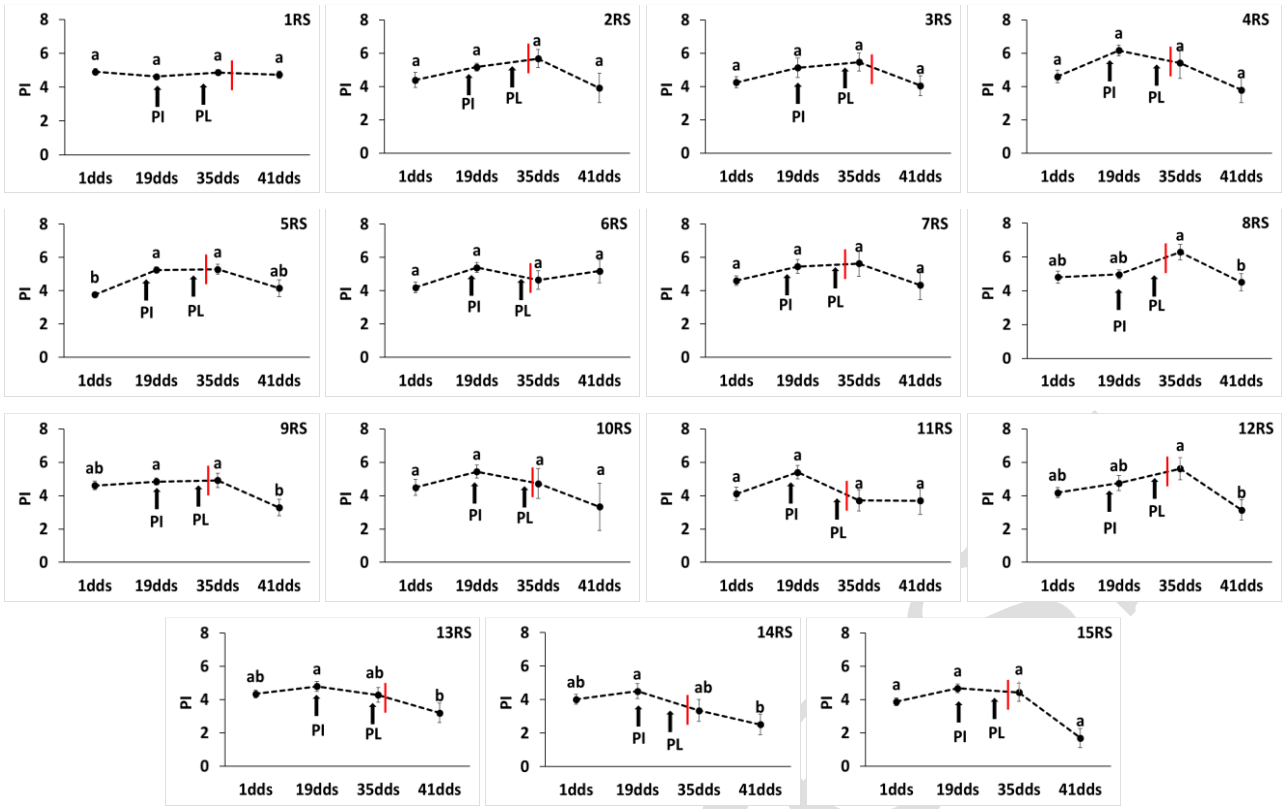

Fig. 9: Performance Indez (PI) after the inoculation of *H. vastatrix* from Cusco on coffee seedlings cv Bourbon; dds: days after inoculation. Red line: Medium latent period. PI: Incubation period. PL: Latent period. dds: days after inoculation. Different letters indicate statistical differences (Tukey 95%).

In the context of *H. vastatrix* infection, pathogen-induced chlorosis markly after latency period (LP). This results in a decrease in the photosynthetic area and interruption of the photosynthetic process (Gortari et al., 2018). When pathogens enter to plant cell, they release effectors and phytotoxins that alter the structure and functions of chloroplasts (including the production of chlorophylls) provoking chloroses (Lu and Yao, 2018).

Particularly, at the end of the evaluation, 3RS provoked a fall in the content of chlorophylls less dramatic than 5RS. This difference among 3RS and 5RS might be related to different mechanisms of colonization and manipulation of cellular activities (Lovelace et al., 2023). Our results confirm that the population 3RS is less pathogenic than 5RS (Fig. 7).

OJIP Test

The OJIP test facilitates the assessment of the physiological status of photosystem II (PSII) and electron transport chain components during photosynthesis (Toniutti et al., 2017), although it has rarely been used in the analysis of *H. vastatrix* (Honorato Júnior et al., 2015a; Salcedo-Sarmiento et al., 2021; Vitória et al., 2023). In dark-adapted plants, Fv/Fm values typically range between 0.75 and 0.85, reflecting an estimate of photosystem II (PSII) quantum yield performance. A decrease in this value indicates damage by photoinhibition (Carrasco and Escobar, 2002).

It was notice that just five populations of *H. vastatrix* caused a decrease of this variable (Fig. 8). The fall of the level of Fv/Fm has been reported by Honorato Júnior et al. (2015a), Honorato Júnior et al. (2015b) and Vitória et al. (2023). Likewise, non-inoculated coffee plants can have values of Fv/Fm around of 0.8-0.82 (Honorato Júnior et al., 2015b). Our result implies that coffee leaf rust can affect negatively the PS II to not allow the maximum use of the photons to trigger photosynthesis. The same effects are reported for *Fusarium* sp. (Kopacki et al., 2016; Bandara et al., 2019) and *Melanpsora* sp. (Gortari et al., 2018).

According to León-Burgos et al. (2022), Fv/Fm is influenced by chlorophyll content. A decrease in Fv/Fm occurs when oxidative damage affects photosynthetic pigments, such as chlorophylls, leading to physiological alterations in PSII in stressed plants. Therefore, the presence of higher levels of chlorophyll may help buffer photosynthetic damage caused by *H. vastatrix*.

On the other hand, it was noticed that PI (Performance Index) is related to the "density of reaction centers, the quantum efficiency of primary photochemistry and conversion of excitation energy in electron transport" (Strasser et al., 2000). This indicator showed just trends to decrease under the presence of different populations of coffee leaf rust (Fig. 9). This suggests that PI could not be an adequate indicator of the infection of *H. vastatrix*. *Fusarium* and *Colletotrichum graminicola* did not cause significant variations of PI in in wheat (Spanic et al., 2017) and corn (Campos et al., 2021), respectively. However, Yan et al. (2018) reported that *Fusarium solani* decreased PI in apple. On contrary, PI is widely used to detect the effect of abiotic stressors such as waterlogging (Saravia-Castillo et al., 2022), heat, drought (Barboričová et al., 2022) and salinity (Salim Akhter et al., 2021). Therefore, it would be very important to continue researching and improving the use of this tool for *H. vastatrix* in coffee.

It was noticed a tendency to augment the value of ETo/RC even though this trend was significant just for 6RS (Table 2). This result implies an improvement of electrons moving from Q_A to Q_B. Likewise, a small group of populations of coffee leaf rust (9RS, 10RS and 15RS) presented significant increase in the levels of ABS/RC. This variable depicts the quantity of light energy absorbed per reaction center (Khan et al., 2021) or the apparent size of the antenna of PSII (Ajigboye et al., 2016). This result is consistent with other one reported by Baghbani et al. (2019), Liu et al. (2023) and Marques et al. (2024) who found that *Fusarium verticillioides, Bursaphelenchus xylophilus* and *Fusarium equiseti* may increase the value of ABS/RC. In the case of TRo/RC, *H. vastatrix* increased its value just in the population 9RS. Li et al. (2022) reported similar result by examining the effect of *Puccinia graminis* f. sp. avenae on oat. This indicates a rise in the capacity to reduce Q_A.

The reduction in levels of chlorophylls and the decline in value of Fv/Fm has been accompanied by the increment of ABS/RC, ETo/RC and TRo/RC caused by certain populations of *H. vastatrix* might mean a defense mechanism to maintain, for one hand, the entrance constant of photons by increasing the size of the antenna and, for another hand, an adequate flux of electrons from PSII to PSI. This type of adaptation has been observed in other conditions by Tomar and Jajoo (2013) in wheat.

Conclusion

Weather conditions and the coffee genotype in this assessment caused a rapid period of incubation (IP) (18-20.22 days) even though the number of days to the appearance of the signs (latent period-LP) was similar to other ones reported. The populations 3RS and 5RS were different, in other words, 3RS was less aggressive than 5RS. The isolate 3RS presented long incubation period, latent period and medium latent period. 3RS also showed lower scale of infection frequency as well. Some populations of *H. vastatrix* caused interesting responses in the levels of chlorophylls and in different variables associated to OJIP test. On overall, the medium latent period was determinant because after that time the levels of chlorophyll fell although the fall was more considerable in 3RS than 5RS. We also detected a photosynthetic defense mechanism that consisted in the increment of ABS/RC, ETo/RC and TRo/RC when the levels of Fv/Fm have fallen.

DECLARATIONS

Funding: The authors thank PRO CIENCIA and CONCYTEC (Contract No.: PE501078996-2022-PROCIENCIA) for financing this study within the framework of the project: "Estudio de la variabilidad genética y caracterización de la roya del café (*Hemileia vastatrix*) en el Perú como base para el desarrollo de un programa de manejo integrado de la enfermedad en un contexto de cambio climático".

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data will be available at request.

Author's Contribution: D. V-P, F. L-R, R. B-V: carried out the experiment. L. A-H, V. C-C, supervised the experiment. S. B-A, C. C-S and A. J-O: wrote the manuscript and supported D. V-P, F. L-R, R. B-V.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Ajigboye, O., Bousquet, L., Murchie, E., & Ray, R. (2016). Chlorophyll fluorescence parameters allow the rapid detection and differentiation of plant responses in three different wheat pathosystems. *Functional Plant Biology*, 43, 356-369.

Alvarado-Huamán, L., Borjas-Ventura, R., Castro-Cepero, V., García-Nieves, L., Jimenéz-Dávalos, J., Julca-Otiniano, A., & Gómez-Pando, L. (2020). Dynamics of severity of coffee leaf rust (*Hemileia vastatrix*) on coffee, in Chanchamayo (Junin-Peru). *Agronomía Mesoamericana*, 31(3), 517-529.

Avelino, J., & Rivas, G. (2013). La roya anaranjada del cafeto: mito y realidad: desafíos de la caficultura en Centroamérica. San José: IICA, 194-291.

Avelino, J., Cristancho, M., Georgiou, S., Imbach, P., Aguilar, L., Bornemann, G., Läderach, P., Anzueto, F., Hruska, A., & Morales, C. (2015). The coffee rust crises in Colombia and Central America (2008–2013): impacts, plausible causes and proposed solutions. *Food Security*, 7, 303–321. https://doi.org/10.1007/s12571-015-0446-9

Baghbani, F., Lotfi, R., Moharramnejad, S., Bandehagh, A., Roostaei, M., Rastogi, A., & Kalaji, H. (2019). Impact of Fusarium verticillioides on chlorophyll fluorescence parameters of two maize lines. *European Journal of Plant Pathology*, 154, 337–346. https://doi.org/10.1007/s10658-018-01659-x

Bandara, A., Weerasooriya, D., Tesso, T., & Little, C. (2019). Stalk rot resistant sorghum genotypes are resilient to pathogen-mediated photosystem II quantum yield retardation. *Crop Protection*, 124, 104852. https://doi.org/10.1016/j.cropro.2019.104852

Barboričová, M., Filaček, A., Mlynáriková Vysoká, D., Gašparovič, K., Živčák, M., & Brestič, M. (2022). Sensitivity of fast chlorophyll fluorescence parameters to combined heat and drought stress in wheat genotypes. *Plant Soil and Environment*, 68(7), 309-316. https://doi.org/10.17221/87/2022-PSE

Borjas-Ventura, R., Alvarado-Huaman, L., Castro-Cepero, V., Rebaza-Fernández, D., Gómez-Pando, L., & Julca-Otiniano, A. (2020). Behavior of ten coffee cultivars against Hemileia vastatrix in San Ramón (Chanchamayo, Peru). *Agronomy*, 10(12), 1867. https://doi.org/10.3390/agronomy10121867

Bracken, P., Burgess, P., & Girkin, N. (2023). Opportunities for enhancing the climate resilience of coffee production through improved crop, soil, and water management. *Agroecology and Sustainable Food Systems*, 47(8), 1125-1157. https://doi.org/10.1080/21683565.2023.2225438

Campos, L.J.M., de Almeida, R.E.M., da Silva, D., Cota, L., Naoe, A., Peluzio, J., Bernardes, F., & da Costa, R. (2021). Physiological and biophysical alterations in maize plants caused by *Colletotrichum graminicola* infection verified by OJIP study. *Tropical Plant Pathology*, 46, 674–683. https://doi.org/10.1007/s40858-021-00465-x

Carrasco, L., & Escobar, H. (2002). Cambios en los contenidos de clorofila, proteínas y niveles de fluorescencia de clorofila en plantas de café (*Coffea arabica* L.) cultivadas en zonas áridas en diferentes condiciones de luminosidad. *Idesia*, 20(2), 111-118.

Ceacero, C., Díaz-Hernández, J., Del Campo, A., & Navarro-Cerrillo, R. (2012). Evaluación temprana de técnicas de restauración forestal mediante

fluorescencia de la clorofila y diagnóstico de vitalidad de brinzales de encina (*Quercus ilex* sub. ballota). *Bosque*, 33(2), 191-202. https://doi.org/10.4067/S0717-92002012000200009

Cerda, R., Avelino, J., Harvey, C., Gary, C., Tixier, P., & Allinne, C. (2020). Coffee agroforestry systems capable of reducing disease-induced yield and economic losses while providing multiple ecosystem services. *Crop Protection*, 134, 105149. https://doi.org/10.1016/j.cropro.2020.105149

ComexPeru (2023). Café Peruano: Un sector con potencial, pero…. Available at https://www.comexperu.org.pe/articulo/cafe-peruano-un-sector-con-potencial-pero%E2%80%A6. Revised on 30th March 2025

Costa, M., Zambolim, L., Caixeta, E., & Pereira, A. (2007). Resistência de progênies de café Catimor à ferrugem. *Fitopatologia Brasileira*, 32, 121-130. https://doi.org/10.1590/S0100-41582007000200005

Ebrahimi, P., Shokramraji, Z., Tavakkoli, S., Mihaylova, D., & Lante, A. (2023). Chlorophylls as natural bioactive compounds existing in food by-products: a critical review. *Plants*, 12(7), 1533. https://doi.org/10.3390/plants12071533

Eskes, A. B. (1983). Incomplete resistance to coffee leaf rust. In: Lamberti, F., Wallen, J., Van der Graaff, N. (Eds) Durable resistance in crops. NATO Advanced Science Institute Series, 55. https://doi.org/10.1007/978-1-4615-9305-8_26

Fromm, I. (2023). Reducing inequalities in the coffee value chain: threats and opportunities for small-scale farmers in Central America and East Africa. In J. Stanton & R. Caiazza (Eds.), Agricultural value chains - some selected issues (pp. 1-15). London: IntechOpen, *Sustainable Development*. https://dx.doi.org/10.5772/intechopen.110191

Gallego-Sánchez, L.M., Canales, F., Montilla-Bascón, G., & Prats, E. (2020). RUST: a robust, user-friendly script tool for rapid measurement of rust disease on cereal leaves. *Plants*, 9(9), 1182. https://doi.org/10.3390/plants9091182

Gortari, F., Guiamet, J., & Graciano, C. (2018). Plant–pathogen interactions: leaf physiology alterations in poplars infected with rust (*Melampsora medusae*). *Tree Physiology*, 38(6), 925–935. https://doi.org/10.1093/treephys/tpx174

Gull, A., Lone, A., & Wani, N. (2019). Biotic and abiotic stresses in plants. In A.B. Oliveira (Ed.), Abiotic and biotic stress in plants (Vol. 174, pp. 1-15). London: IntechOpen. https://doi.org/10.5772/intechopen.85832

Honorato Júnior, J., Zambolim, L., Duarte, H., Aucique-Pérez, C., & Rodrigues, F. (2015a). Effects of Epoxiconazole and Pyraclostrobin Fungicides in the Infection Process of *Hemileia vastatrix* on Coffee Leaves as Determined by Chlorophyll a Fluorescence Imaging. *Journal of Phytopathology*, 163. https://doi.org/10.1111/jph.12399

Honorato Júnior, J., Zambolim, L., Aucique-Pérez, C., Resende, R., & Rodrigues, F. (2015b). Photosynthetic and antioxidative alterations in coffee leaves caused by epoxiconazole and pyraclostrobin sprays and Hemileia vastatrix infection. https://doi.org/10.1016/j.pestbp.2015.01

ICO (2013). Report on the outbreak of coffee leaf rust in Central America and Action Plan to combat the pest. Available at https://www.ico.org/documents/cy2012-13/ed-2157e-report-clr.pdf. Revised on 30th March 2025

Jezeer, R., Santos, M., Verweij, P., RBoot, R., & Clough, Y. (2019). Benefits for multiple ecosystem services in Peruvian coffee agroforestry systems without reducing yield. *Ecosystem Services*, 40, 101033. https://doi.org/10.1016/j.ecoser.2019.101033

Jin, Y., Li, D., Liu, M., Cui, Z., Sun, D., Li, C., Zhang, A., Cao, H., & Ruan, Y. (2023). Genome-Wide association study identified novel SNPs associated with chlorophyll content in maize. *Genes*, 14(5), 1010. https://doi.org/10.3390/genes14051010

Julca, A., Borjas, R., Alvarado, L., Castro, V., & Belloa, S. (2019). Relationship between the incidence and severity of coffee rust (*Hemileia vastatrix*) in San Ramón, Chanchamayo, Peru. *Journal of Science and Research*, 4(4), 1-9.

Julca-Otiniano, A., Echeverría Anyosa, C., Ladera Manyari, Y., Borjas Ventura, R., Cruz Janampa, R., Bello Amez, S., & Crespo Costa, R. (2013). Una revisión sobre la roya del café (*Hemileia vastatrix*), algunas experiencias y recomendaciones para el Perú. *AgroEnfoque*, 28(189), 29-31.

Julca-Otiniano, A., Alvarado-Huaman, L., Borjas-Ventura, R., Castro-Cepero, V., León Rojas, F., Valderrama Palacios, D., & Bello Amez, S. (2023). Variedades de café (*Coffea arabica*), una revisión y algunas experiencias en el Perú. *Revista de Investigación e Innovación Agropecuaria y de Recursos Naturales,* 10(2), 134-155. https://doi.org/10.53287/ruyx4519vm15b

Julca-Otiniano, A., Alvarado-Huamán, L., Castro-Cepero, V., Borjas-Ventura, R., Gómez-Pando, L., Pereira, A. P., Nielen, S., Ingelbrecht, I., Silva, M. d. C., & Várzea, V. (2024). New races of *Hemileia vastatrix* detected in Peruvian coffee fields. *Agronomy*, 14, 1811. https://doi.org/10.3390/agronomy14081811

Khan, N., Essemine, J., Hamdani, S., Qu, M., Amy, M., Perveen, S., Stirbet, A., Govindjee, G., & Zhu, X. (2021). Natural variation in the fast phase of chlorophyll a fluorescence induction curve (OJIP) in a global rice minicore panel. *Photosynthesis Research*, 150, 137–158. https://doi.org/10.1007/s11120-020-00794-z

Kopacki, M., Wagner, A., & Michałek, W. (2016). Pathogenicity of Fusarium oxysporum, Fusarium avenaceum, and Sclerotinia sclerotiorum and their effect on photosynthetic activity of chrysanthemum plants. *Acta Scientiarum Polonorum. Hortorum Cultus*, 15(3), 59–70.

Koutouleas, A. & Collinge, D. (2022). Coffee Leaf Rust: Back with a Vengeance. *British Society for Plant Pthology*. https://doi.org/10.13140/RG.2.2.28685.41444

Kushalappa, A.C., & Martins, C.P. (1980). Incubation and generation periods for *Hemileia vastatrix* on coffee in Viçosa, Minas Gerais. *Fitopatologia Brasileira*, 5, 177-183.

Leguizamón, J., Orozco, L., & Gómez, L. (1998). Períodos de incubación (pi) y de latencia (pl) de la roya del cafeto en la zona cafetera central de Colombia. *Cenicafé*, 49(56), 325–339.

León-Burgos, A.F., Unigarro, C.A., & Balaguera-López, H.E. (2022). Soil waterlogging conditions affect growth, water status, and Chlorophyll "a" Fluorescence in Coffee Plants (*Coffea arabica* L.). *Agronomy*, 12(6), 1270. http://doi.org/10.3390/agronomy12061270

Li, Y., Liu, J., Lv, P., Mi, J., & Zhao, B. (2022). Silicon improves the photosynthetic performance of oat leaves infected with *Puccinia graminis* f. sp. avenae. *Frontiers in Plant Science*, 13, 1037136. https://doi.org/10.3389/fpls.2022.1037136

Liu, F., Zhang, M., Hu, J., Pan, M., Shen, L., Ye, J., & Tan, J. (2023). Early Diagnosis of Pine Wilt Disease in *Pinus thunbergii* Based on Chlorophyll Fluorescence Parameters. *Forests*, 14, 154. https://doi.org/10.3390/f14010154

Lovelace, A., Dorhmi, S., Hulin, M., Li, Y., Mansfield, J., & Ma, W. (2023). Effector Identification in Plant Pathogens. *Phytopathology*, 113(4), 637-650.

Lu, Y., & Yao, J. (2018). Chloroplasts at the Crossroad of Photosynthesis, Pathogen Infection and Plant Defense. *International Journal of Molecular Sciences*, 19, 3900. https://doi.org/10.3390/ijms19123900

Maia, T., Badel, J.L., Fernandes, M.B., Bragança, C.A.D., Mizubuti, E.S.G., & Brommonschenkel, S.H. (2017). Variation in Aggressiveness Components in the *Hemileia vastatrix* Population in Brazil. *Journal of Phytopathology*, 165, 174-188. https://doi.org/10.1111/jph.12548

Mariño, Y. (2014). Respuesta fotosintética de *Coffea arabica* L. a diferentes niveles de luz y disponibilidad hídrica. *Acta Agronómica*, 63(2), 128-135. http://doi.org/10.15446/acag.v63n2.38454

Marques, M., Vitorino, L., Rosa, M., Mendes, M., Guimarães, F., & Bessa, L. (2024). Leaf physiology and histopathology of the interaction between the opportunistic phytopathogen *Fusarium equiseti* and *Gossypium hirsutum* plants. *European Journal of Plant Pathology*, 168, 329-349.

Maspul, K. (2023). A harmonious symphony of fourth-wave coffee culture: how the relationship between coffee and local wisdom contributes to sustainability and ethical practices in community. *Ulil Albal: Jurnal Ilmiah Multidisiplin*, 2(5), 1896-1922. http://doi.org/10.56799/jim.v2i5.1519

MIDAGRI (2013). Plan Nacional contra la roya amarilla. Available at https://www.midagri.gob.pe/portal/plan-nacional-contra-la-roya-amarilla/notas/9232-declaran-en-emergencia-zonas-cafetaleras. Revised on 30th March 2025

MIDAGRI (2025). Situación actual del café en el país. Available at https://www.midagri.gob.pe/portal/485-feria-scaa/10775-el-cafe-peruano#:~:text=El%20Per%C3%BA%20posee%20425%2C416%20hect%C3%A1reas,67%20provincias%20y%20338%20distritos. Revised on 30th March 2025.

Morales, Y., & Grajea, R. (2021). Virulence genes of new population of coffee rust (*Hemileia vastatrix*) affecting coffee variety 'Lempira', in Honduras; resistant and susceptible varieties. In S. Sivasankar, N. Ellis, L. Jankuloski, & I. Ingelbrecht (Eds.), Mutation breeding, genetic diversity and crop adaptation to climate change. CABI, Wallingford.

Moreno, S., Perales-Vela, H., & Álvarez, M. (2008). La fluorescencia de la clorofila a como herramienta en la investigación de efectos tóxicos en el aparato fotosintético de plantas y algas. *Revista de Educación Bioquímica*, 27(4), 119-129.

Motyka, O., Štrbová, K., & Zinicovscaia, I. (2020). Chlorophyll content in two medicinal plant species following Nano-TiO2 exposure. *Bulletin of Environmental Contamination and Toxicology*, 104(3), 373-379. http://doi.org/10.1007/s00128-020-02787-z. PMid:31955229

Palacios, D., León-Rojas, F., Borjas-Ventura, R., Alvarado-Huamán, L., Castro-Cepero, V., Bello-Amez, S., Cedano-Saavedra, C., & Julca-Otiniano, A. (2025). Photosynthetic efficiency affected by different isolates of

coffee leaf rust from Cajamarca, Peru. In Press. International Journal of Agriculture and Biosciences. https://doi.org/10.47278/journal.ijab/2025.031

Pires, M., Alves, M., & Pozza, E. (2020). Multispectral radiometric characterization of coffee rust epidemic in different irrigation management systems. *International Journal of Applied Earth Observation and Geoinformation*, 86, 102016. https://doi.org/10.1016/j.jag.2019.102016

Pozza, E.A., Santos, É.R., Gaspar, N.A., Vilela, X.M.S., Alves, M.C., & Colares, M.R.N. (2021). Coffee rust forecast systems: development of a warning platform in Minas Gerais State, Brazil. *Agronomy*, 11(11), 2284. http://doi.org/10.3390/agronomy11112284

Quiroga-Cardona, J. (2021). La resistencia incompleta del café a la roya: una revisión. Revista Cenicafé, 72(2), e72208. https://doi.org/10.38141/10778/72208

Quispe-Apaza, C. S., Mansilla-Samaniego, R. C., López-Bonilla, C. F., Espejo-Joya, R., Villanueva-Caceda, J., & Monzón, C. (2017). Genetic diversity of *Hemileia vastatrix* in two coffee-producing areas in Peru. *Revista Mexicana de Fitopatología*, 35(3), 418-436.

Quispe-Apaza, C., Mansilla-Samaniego, R., Espejo-Joya, R., Bernacchia, G., Yabar-Larios, M., & López-Bonilla, C. (2021). Spatial and temporal genetic diversity and population structure of *Hemileia vastatrix* from Peruvian coffee plantations. The *Plant Pathology Journal*, 37(3), 280-290. https://doi.org/10.5423/PPJ.OA.10.2020.0192

Rodrigues Junior, C.J., Bettencourt, A.J., & Rijo, L. (1975). Races of the pathogen and resistance to coffee rust. *Annual Review of Phytopathology*, 13(1), 49-70. http://doi.org/10.1146/annurev.py.13.090175.000405

Rodriguez, A., Yonny, M., Nazareno, M., Galmarini, C., & Bouzo, C. (2014). Eficiencia fotoquímica máxima e índice de potencial fotosintético en plantas de melón (*Cucumis melo*) tratadas con bajas temperaturas. *FAVE. Sección Ciencias Agrarias*, 13(2), 8-16.

Rozo, Y., Escobar, C., Gaitán, Á., & Cristancho, M. (2012). Aggressiveness and genetic diversity of Hemileia vastatrix during an epidemic in Colombia. *Journal of Phytopathology*, 160(11-12), 732-740. http://doi.org/10.1111/jph.12024

Salcedo-Sarmiento, S., Aucique-Pérez, C., Silveira, P., Colmán, A., Silva, A., Corrêa, P., Rodrigues, F., Evans, H., & Barreto, R. (2021). Elucidating the interactions between the rust *Hemileia vastatrix* and a Calonectria mycoparasite and the coffee plant. *iScience*, 24(5), 102352. https://doi.org/10.1016/j.isci.2021.102352

Salim Akhter, M., Noreen, S., Mahmood, S., Athar, H., Ashraf, M., Alsahli, A., & Ahmad, P. (2021). Influence of salinity stress on PSII in barley (*Hordeum vulgare* L.) genotypes, probed by chlorophyll-a fluorescence. *Journal of King Saud University - Science*, 33(1), 101239. https://doi.org/10.1016/j.jksus.2020.101239

Santacreo, R. (1989). Evaluación del nivel de resistencia horizontal a *Hemileia vastatrix* Berk et Br. en Germoplasma de *Coffea arabica* L. y Catimor. *Turrialba*, 39(3), 377-386.

Saravia-Castillo, G., Castro-Cepero, V., Julca, A., Alvarado-Huamán, L., & Borjas-Ventura, R. (2022). Effect of flooding stress on cocoa (*Theobroma cacao* L.). *Journal of Selva Andina Biosphere*, 10(2), 78-85.

Silva, C., Várzea, V., Guerra-Guimarães, L., Azinheira, H., Fernandez, D., Petitot, A., Bertrand, B., Lashermes, P., & Nicole, M. (2006). Coffee resistance to the main diseases: leaf rust and coffee berry disease. *Brazilian Journal of Plant Physiology*, 18(1), 119-147. http://doi.org/10.1590/S1677-04202006000100010

Silva, M. C., Guerra-Guimarães, L., Diniz, I., Loureiro, A., Azinheira, H., Pereira, A. P., Tavares, S., Batista, D., & Várzea, V. (2022). An Overview of the Mechanisms Involved in Coffee-*Hemileia vastatrix* Interactions: Plant and Pathogen Perspectives. *Agronomy*, 12(2), 326. https://doi.org/10.3390/agronomy12020326

Spanic, V., Viljevac Vuletic, M., Drezner, G., Zdunic, Z., & Horvat, D. (2017). Performance Indices in Wheat Chlorophyll a Fluorescence and Protein Quality Influenced by FHB. Pathogens (Basel, Switzerland), 6(4), 59. https://doi.org/10.3390/pathogens6040059

Stirbet, A., & Govindjee (2011). On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem II: Basics and applications of the OJIP fluorescence transient. https://doi.org/10.1016/j.jphotobiol.2010.12.010.

Strasser, R., Srivastava, A., & Tsimilli-Michael, M. (2000). The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U. & Mohanty, P. Probing photosynthesis: Mechanism, regulation and adaptation. Taylor & Francis Group, pp: 445-483.

Subit, D., Sierra, P.M., & Casanovas, E. (2021). El cultivo del café (Coffea arabica L) y su susceptibilidad a la roya (*Hemileia vastatrix* Berkeley and Broome) en la provincia Cienfuegos. *Revista Científica Agroecosistemas*, 8(3), 109-114.

Suffert, F., Goyeau, H., Sache, I., Carpentier, F., Gelisse, S., Morais, D., & Delestre, G. (2018). Epidemiological trade-off between intra- and interannual scales in the evolution of aggressiveness in a local plant pathogen population. *Evolutionary Applications*, 11(5), 768-780. http://doi.org/10.1111/eva.12588. PMid:29875818

Talhinhas, P., Batista, D., Diniz, I., Vieira, A., Silva, D., Loureiro, A., Tavares, S., Pereira, A., Azinheira, H., Guerra-Guimarães, L., Várzea, V., & Silva, M. (2017). The coffee leaf rust pathogen Hemileia vastatrix: one and a half centuries around the tropics. *Molecular Plant Pathology*, 18(8), 1039-1051. https://doi.org/10.1111/mpp.12512. Epub 2017 Jan 25. PMid:27885775; PMCID: PMC6638270

Tomar, R., & Jajoo, A. (2013). A quick investigation of the detrimental effects of environmental pollutant polycyclic aromatic hydrocarbon fluoranthene on the photosynthetic efficiency of wheat (*Triticum aestivum*). *Environmental Toxicology*, 22(8), 1313-1318. https://doi.org/10.1007/s10646-013-1118-1

Toniutti, L., Breitler, J.-C., Etienne, H., Campa, C., Doulbeau, S., Urban, L., Lambot, C., Pinilla, J.-C. H., & Bertrand, B. (2017). Influence of environmental conditions and genetic background of Arabica coffee (*C. arabica* L) on leaf rust (*Hemileia vastatrix*) pathogenesis. *Frontiers in Plant Science*, 8, 2025. https://doi.org/10.3389/fpls.2017.02025

Várzea, V. (2016). Protocol to send samples of coffee leaf rust to CIFC. Lisboa: Instituto Superior de Agronomia. Portugal.

Várzea, V., Pereira, A., & Silva (2023). Screening for Resistance to Coffee Leaf Rust vastatrix in San Ramón (Chanchamayo, Peru). In Ingelbrecht, I., Silva, M., & Jankowicz-Cieslak, L. (Eds). Mutation Breeding in Coffee with Special Reference to Leaf Rust. Austria.

Vitória, E., Krohling, C., Borges, F., Ribeiro, L., Ribeiro, M., Chen, P., Lan, Y., Wang, S., Moraes, H., & Furtado, M. (2023). Efficiency of fungicide application using an unmanned aerial vehicle and pneumatic sprayer for control of *Hemileia vastatrix* and Cercospora coffeicola in mountain coffee crops. *Agronomy*, 13, 340. https://doi.org/10.3390/agronomy13020340

Yan, K., Han, G., Ren, C., Zhao, S., Wu, X., & Bian, T. (2018). Fusarium solani infection depressed photosystem performance by inducing foliage wilting in apple seedlings. *Frontiers in Plant Science*, 9, 479. https://doi.org/10.3389/fpls.2018.00479

Zulkarnaini, Z., Sakimin, S., Mohamed, M., & Jaafar, H. (2019). Relationship between chlorophyll content and soil plant analytical development values in two cultivars of fig (*Ficus carica* L.) as brassinolide effect at an open field. *IOP Conference Series: Earth and Environmental Science,* 250, 012025. https://doi.org/10.1088/1755-1315/250/1/012025