Reaction of coffee cultivars to *Hemileia vastatrix* Berk. & Broome strains in Venezuela, under controlled conditions

Reacción de cultivares de café a cepas de *Hemileia vastatrix* Berk. & Broome en Venezuela bajo condiciones controladas

Reação de cultivares de café a cepas de *Hemileia vastatrix* Berk. & Broome na Venezuela sob condições controladas

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Abstract

Coffee rust resistance is of great importance in worldwide coffee crops, since its biotrophic pathogen shows, currently, more than 55 identified races with diverse virulent genes. This work was based on the determination of the reaction of five commercial coffee cultivars inoculated, under controlled conditions, with three strains of *Hemileia vastatrix* (race XXXIX and pathotypes Hv01ve and Hv02ve) previously identified in Venezuela. Three velvet leaves taken from six selected plants of five cultivars (Caturra, Catuai amarillo, Monteclaro, Colombia 27 y Castillo) were inoculated with 80 µL of a uredospore dilution (5 x 10^5 uredospores.mL^{-1}), and incubated at 22 °C +/- 2 °C and 12 h light photoperiod. Incubation period, latency period, infection grade, number of initial lesions, number of sporulated lesions, area of lesion, and area of sporulated lesion were the variables used for evaluation. Results showed that number of initial lesion, number of sporulated lesion and area of sporulated lesion allowed to determine the presence of incomplete resistance in Castillo to race XXXIX, Castillo, Catuai and Colombia 27 to Hv01ve, and Monteclaro to Hv02ve, trials also showed possible complete resistance in Monteclaro to race XXXIX and Colombia 27 to Hv02ve. It is suggested that these results should be taken into account when selecting the coffee cultivars to be planted.

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Resumen

La resistencia del café a la roya es un tema de gran importancia en la caficultura a nivel mundial, motivado a que su agente causal, un hongo biotrofo, presenta actualmente más de 55 razas identificadas con diversos genes de virulencia en su composición génica. Este trabajo se basó en la determinación de la reacción de cinco cultivares comerciales de café ante la inoculación, bajo condiciones controladas, de tres cepas de *Hemileia vastatrix* (raza XXXIX, patotipos Hv01ve y Hv02ve) identificadas previamente en Venezuela. Se seleccionaron tres hojas terciopelos de cada una de seis plantas seleccionadas de los cinco cultivares (Caturra, Catuaí amarillo, Monteclaro, Colombia 27 y Castillo). Las hojas se inocularon con 80 µL de una dilución de uredosporas (5 x 10^5 uredosporas.mL^{-1}) del hongo, se incubaron a 22 °C +/- 2 °C y fotoperiodo de 12 h luz. Las variables evaluadas fueron Periodo de incubación, Periodo de Latencia, Grado de infección, Número de lesiones iniciales, Número de lesiones esporuladas, Área de lesión y Área de lesión esporulada. Los resultados obtenidos evidenciaron que las variables Número de Lesiones iniciales, Número de Lesiones esporuladas y Área de Lesión esporulada permitieron determinar la presencia de resistencia incompleta en los cultivares Castillo a la raza XXXIX, Castillo, Catuaí y Colombia 27 a Hv01ve y Monteclaro a Hv02ve. Además, las pruebas mostraron la posibilidad de resistencia completa en Monteclaro para la raza XXXIX y Colombia 27 para Hv02ve. Se sugiere considerar estos resultados para la selección de los cultivares de café a sembrar.

Palabras clave: resistencia, roya del cafeto, raza, patotipos

Resumo

A resistência do café à ferrugem é um tema de grande importância na cafeicultura mundial, motivada porque seu agente causal, um fungo biotrófico, possui atualmente mais de 55 raças identificadas com diferentes genes de virulência em sua composição genética. Este trabalho baseou-se na determinação da reação de cinco cultivares comerciais de café à inoculação, sob condições controladas, de três cepas de *Hemileia vastatrix* (raça XXXIX, patótipos Hv01ve e Hv02ve) previamente identificadas na Venezuela. Foram selecionadas três folhas aveludadas de cada uma das seis plantas selecionadas das cinco cultivares (Caturra, Catuaí Amarelo, Monteclaro, Colombia 27 e Castillo). As folhas foram inoculadas com 80 µL de uma diluição de uredinosporos (5 x 10^5 uredosporas.mL^{-1}) do fungo e incubadas a 22 ºC) +/- 2 °C e fotoperíodo de 12 h luz. As variáveis avaliadas foram Período de Incubação, Período de Latência, Grau de infecção, Número de lesões iniciais, Número de lesões esporulantes, Área da lesão e Área da lesão esporulante. Os resultados obtidos mostraram que as variáveis Número de lesões iniciais Número de lesões esporulantes e Área de lesão esporulante permitiram determinar a presença de resistência incompleta nas cultivares Castillo à raça XXXIX Castillo Catuaí e Colombia 27 ao patótipo Hv01ve e Monteclaro ao Hv02ve. Além disso, os testes mostraram a possibilidade de resistência completa em Monteclaro para a raça XXXIX e Colômbia 27 para Hv02ve. Sugere-se considerar esses resultados na seleção das cultivares de café a serem plantadas.

Palavras-chave: resistência, ferrugem do cafeiro, raças, patótipos.

Introduction

The apparent loss or broken down of coffee rust resistance in materials previously free of the disease is a frequent fact in coffee producer countries worldwide (Avelino & Rivas, 2013). These changes are related to the high adaptive potential of the fungus and to variations and relative frequency of the races of *Hemileia vastatrix* Berk. & Br. (Pires *et al*., 2020), limiting *C. arabica* production in the world. Its genetic resistance is the main control measure of the pathogen which has been transferred to *C. arabica* commercial cultivars through Timor hybrid (natural crossing of *C. arabica* x *C. canephora*) (Avelino & Rivas, 2013; Talhinhas *et al.,* 2017).

In the development of the pathogenesis cicle of the rust disease, three processes can be recognized: infection, sporulation, and dissemination. Infection can be subdivided into spore germination, penetration and colonization. In the coffee-rust pathosystem, this process is highly influenced by environmental conditions which should be favorable to the pathogen. *H. vastratix* developed and maintained only one reproductive structure, the uredospore, with thick walls, which is adapted for survival, dispersion, infection, and also for sexual reproduction, by means of cryptosexuality, contributing to the epidemic development (Avelino *et al*., 2018).

In the interaction coffee-*H. vastratix*, the plant has developed effective mechanisms for recognition and response to infection. Biotrophic parasites, such as mildews and rusts, have developed specific mechanisms to maintain host cells alive during infection (Schulze-Lerfert & Panstruga, 2003). In its interaction, the fungus uses various strategies to infect its host, among which the extended and effective suppression of the immunological system, and, simultaneously, induction of specific genes for biotrophic establishment (Lima *et al*., 2022; Schulze-Lerfert & Panstruga, 2003).

Silva *et al*. (2022) indicated that induced resistance to pathogens in plants is associated to a set of defense responses; activation of these responses depends on the efficiency of the host to recognize the presence of pathogens by its mechanisms of perception and sign transduction, which implies, among others, formation of oxygen reactive species (ROS), transitory changes in the ions flow through the plasmatic membrane and changes in phosphorilation state (phosphorilation of kinase protein (MAPK) and other diverse proteins).

Plants have the ability to recognize a potential invasion by a pathogen and thus develop various defense mechanisms; in turn, pathogens develop strategies to overcome them during evolutive mechanisms (Ríos & Debona, 2018). The pathogen, in general, employs several strategies to infect the host; one of these is the secretion of effector proteins capable of suppressing defense responses of the plant and allowing colonization of host tissue. In response to infection, plants have developed an innate immunological system consisting of two lines of defense that limit pathogen proliferation in the tissue. Pattern triggered Immunity (PTI) is activated by pathogen associated molecular pattern (PAMP), and the second line of defense, effector triggered immunity (ETI) which fits to the gene by gene theory proposed by Flor in 1971 (Pires *et al*., 2020). This theory explains the interaction between coffee and *H. vastratix*, by which resistance in coffee plants is conditioned by at least nine dominant genes with main effects (SH1-SH9). Genes SH1, SH2, SH4 and SH5 are found in *C. arabica*, while SH6, SH7, SH8 and SH9 are present in *C. canephora*, and SH3 is located in *C. liberica* (Pires *et al*., 2021).

There are two types of resistance: vertical and horizontal. Vertical resistance, also known as qualitative, specific, total, or resistance due to major effect genes, is specific against some pathogenic races, slowing down its reproduction, which is equivalent to an immunity reaction.

The main effect associated to vertical resistance is reduction of initial inoculum and, consequently, delay on the start of the epidemic. This initial delay is considered determinant because reduces the damage in a critical phase of crop production (Ríos & Debona, 2018; Dallangnol & Vieira de Araujo, 2018).

In horizontal resistance, also called partial or incomplete, the host shows a susceptible reaction, but with a lower rate of disease development; its genetic nature implies that many genetic changes in pathogen population are required to be able to overcome resistance (Dallangnol & Vieira de Araujo, 2018).

The objective of the present study was to evaluate, under controlled conditions, the reaction of commercial coffee cultivars to three strains of *Hemileia vastratix* previously identified in Venezuela, in order to know the level of resistance of these materials to the pathogen.

Materials and methods

The trial was conducted in the Virology and Molecular Biology laboratory of the Universidad Centroccidental Lisandro Alvarado. Uredospores of three *H. vastratix* strains previously identified as race XXXIX, Hv01ve and Hv02ve (Venezuelan pathotypes) (Ramirez-Poletto *et al*., 2024), which are maintained on plants located at the Trompillo experimental field of the Instituto Nacional de Investigaciones Agrícolas of Táchira state (INIA-Tachira). Spores were collected from the lesions on the medium high leaves, using 3 cm long plastic straw sections. Uredospore viability was evaluated by germination test on potato broth (200 g potato.L^{-1} H_2O + 2 g glucose). Inoculum was prepared with a suspension of 5.7 x 10^5 uredospores. mL^{-1}, treated with a 40 °C water bath for 10 minutes to increase germination rate (Deepak *et al*., 2012).

Seed of cultivars Caturra (susceptible control), Catuai Amarillo, Monteclaro, from INIA Táchira; Colombia 27 and Castillo, from grower farms of Sanare (Lara state) and Campo Elias (Yaracuy state), respectively, were placed in water for 22 days (changing the water every 3 days) to speed germination process. Seeds were then sowed in organic soil until plant development. When plants had six leaves were transplanted to new polyethylene bags with similar organic soil and kept under plant nursery conditions for six months. They were then taken to the laboratory for the trial.

Following Lizardo-*Chávez et al.* (2022) procedure, in the laboratory, temperature was set up to 22 °C using an air conditioner, light was supplied by fluorescent lamps (1770 µmol.m^{-2}.s^{-1}) connected to a timer (Exceline Mastertime 120 V, República Bolivariana de Venezuela) for a 12/12 h photoperiod; to keep humidity near 80 %, mini-humidifiers (Shenzhen, China), were used, in addition, water was maintained in the dishes under the planting pots for watering, and plastic curtains for block separators were sprayed with water. Temperature and humidity were measured with a KTJ® TA218C manual thermo-hygrometer, both in the laboratory environment and in the blocks.

Six plants per cultivar were randomly placed in each of three blocks separated with plastic curtains (to avoid cross contamination), corresponding to the three *H. vastratix* strains. Three velvet leaves (young and suave texture) were selected per plant and cultivar and inoculated with one drop of 10 µl of uredospore suspension on each of eight spots on the underside of the leaves. Leaves were then sprayed with a small amount (to avoid wash off) of distilled water, plants covered with transparent plastic bags and kept under complete darkness for 72 h. After incubation time, plastic bags were removed and the inoculated leaves were cleaned with moistened cotton to remove non-germinated spores (Capucho *et al*., 2009).

Once the first symptoms appeared (10 d), inoculated leaves were observed daily, herein starting evaluation of the variables, following Lizardo-Chávez *et al*. (2022) methodology, which used leaf discs. Variables taken were as follows: infection period (PI) was determined by counting the days from inoculation to the appearance of the chlorotic lesions; latency period (PL), the days from inoculation to pustule formation (26 d). For infection grade (GI), at the moment of the first pustules shown, a six class scale used by Cohelo de Sousa *et al*. (2020) was utilized; the number of initial lesions (NLi), and number of sporulating lesions (NLe), were taken from 26 to 60 days after inoculation, counting the number of lesions individually for each leaf; in the same period of time, length and width of the lesions were measured and used to calculate lesion area (AL) and sporulated lesion area (Ale).

A complete random design was used to analyze data for each separate strain. Leaf was the experimental unit; three leaves per plant, six plants per cultivar and five cultivars were used in the analysis for each of the three *H. vastratix* strains. Analysis of variance and the Tukey media test were run using Infostat (2020) program.

Results and discussion

The separate analysis of cultivar reaction to each *H. vastratix* strain showed that race XXXIX induced significant difference between cultivars for most of the variables (table 1). Monteclaro cultivar did not show any reaction to this race. With regard to the number of initial lesions (NLi) and the number of sporulated lesions (NLe), in Castillo cultivar values were significantly lower. No significant difference was observed with respect to lesion area (AL) or to area of sporulated lesion, although the latter was reduced in Castillo, Catuai and Colombia 27 compared to the control Caturra. With respect to incubation period (PI) and latency period (PL), cultivar Colombia 27 had a later reaction to the race than the other cultivars; however, no significant difference was observed for infection grade (GI). In summary, Monteclaro and Castillo were the cultivars with better resistance or tolerance response against race XXXIX.

With regard to the reaction of materials to pathotype Hv01ve, all the cultivars showed to be susceptible (table 2). All the materials had similar PI, except Catuai which had a longer period (33 d), as well as for PL. However, for GI there was little difference among cultivars. With respect to NLi, the lowest value was shown by Catuai, followed by Colombia 27 and Castillo, whereas for NLe, Catuai and Colombia 27 had the lowest values. As to AL, cultivars Catuai, Colombia 27, Caturra and Monteclaro were statistically similar, but Castillo had higher value. Regarding to ALe, Castillo, Catuai and Colombia 27 showed lower values than the control Caturra, which indicate the presence of certain resistance in Castillo, Catuai, Colombia 27 and Monteclaro to Hv01ve.

When cultivar reaction was evaluated against pathotype Hv02ve, it was found also differences among them in the variables (table 3). Colombia 27 cultivar did not show infection during the trial. Again, the most important variables to observe significant differences were NLi, NLe and Ale, in which Monteclaro stood out from the other materials, suggesting possible gene resistance in those cultivars. In the combined analysis of the interaction strain x cultivar, variables NLi, NLe, and Ale showed significant difference among cultivars.

Caturra was the most susceptible to the three fungal strains for the three variables (figure 1).The other cultivars showed variable reaction depending on the strain; Catuai and Castillo had higher values for these variables against strain Hv02ve than against the other two strains. On the other hand, Colombia 27 and Monteclaro demonstrated, in general, to be more resistant to this strain, followed by the reaction to Hv01ve, for the three variables (figure 1).

Table 1. Coffee cultivars reaction to *Hemileia vastatrix* race XXXIX under controlled conditions.

Cultivar	PI	PL	GI	NLi	NLe	AL	ALe
Castillo	26.67 b	44.00 b	3.67 a	6.44 c	2.67 b	26.25 a	0.04 ab
Catuai	26.00 b	44.67 b	4.00 a	17.43 ab	6.10 a	29.30 a	0.03 ab
Caturra	26.00 b	47.20 b	3.86 a	21.29 a	7.87 a	31.05 a	0.08 a
Colombia 27	36.67 a	58.00 a	4.00 a	12.50 b	6.00 a	33.08 a	0.04 ab
Monteclaro	0.00 c	0.00 c	0.00 b	0.00 d	0.00 c	0.00 b	0.00 b
R^2	0.97**	0.97**	0.98**	0.94**	0.92**	0.94**	0.62**
C.V. (%)	9.42	10.05	8.53	17.85	10.57	11.14	1.14

Means with same letter in the columns are not significantly different (p > 0,05) (Tukey test). PI = Incubation period (d), (PL) Latency period (d), GI = Infection grade or type of reaction (scale class), NLi = Number of initial lesions, NLe = Number of sporulated lesions, AL = Lesion area (cm^2), ALe = sporulated lesion area (cm^2).

Table 2. Coffee cultivars reaction to *Hemileia vastatrix* pathotype Hv01ve under controlled conditions.

Cultivar	PI	PL	GI	NLi	NLe	AL	ALe
Castillo	26.00 b	44.50 bc	4.00 a	6.42 bc	2.21 bc	40.31 a	0.02 c
Catuai	33.00 a	48.00 a	3.00 a	3.33 c	1.67 c	28.00 b	0.02 c
Caturra	26.00 b	46.67 ab	4.00 a	24.87 a	9.33 a	27.30 b	0.09 a
Colombia 27	26.00 b	44.00 c	3.75 a	4.5 bc	1.25 c	28.00 b	0.01 c
Monteclaro	26.00 b	46.22abc	3.61 a	9.61 b	3.78 b	26.83 b	0.04 b
R^2	0.70**	0.63**	0.36ns	0.92**	0.94**	0.67**	0.94**
C.V. (%)	6.80	2.55	13.35	24.51	20.76	13.43	20.88

Means with same letter in the columns are not significantly different (p > 0,05) Tukey test. PI = Incubation period (d), (PL) Latency period (d), GI = Infection grade or type of reaction (scale class), NLi = Number of initial lesions, NLe = Number of sporulated lesions, AL = Lesion area (cm^2), ALe = sporulated lesion area (cm^2).

Table 3. Coffee cultivars reaction to *Hemileia vastatrix* pathotype Hv02ve under controlled conditions.

Cultivar	PI	PL	GI	NLi	NLe	AL	ALe
Castillo	26.00 a	46.40 ab	3.90 a	12.80 c	6.60 a	38.70 a	0.07 a
Catuai	26.67 a	44.67 b	3.95 a	22.22 b	9.78 a	27.95 b	0.10 a
Caturra	26.17 a	46.67 ab	3.95 a	28.11 a	9.72 a	26.22 c	0.10 a
Colombia 27	0.00 b	0.00 c	0.00 b	0.00 e	0.00 b	0.00 d	0.00 b
Monteclaro	26.00 a	48.00 a	3.93 a	6.33 d	1.83 b	26.26 c	0.02 b
R2	0.99**	0.99**	0.99**	0.93**	0.83**	0.99**	0.83**
C.V. (%)	3.69	4.07	3.36	22.16	36.7	0.22	37.07

Means with same letter in the columns are not significantly different (p > 0,05) Tukey test. PI = Incubation period (d), (PL) Latency period (d), GI = Infection grade or type of reaction (scale class), NLi = Number of initial lesions, NLe = Number of sporulated lesions, AL = Lesion area (cm^2), ALe = sporulated lesion area (cm^2).

These results suggest that when pathotype Hv01ve prevails, the most indicated cultivars to crop would be Castillo, Catuai or Colombia 27, whereas with the Hv02ve pathotype present, the recommended cultivars would be Colombia 27 and Monteclaro. Finally, if the dominant strain in a region is race XXXIX, the indicated cultivars would be Castillo and Monteclaro. All this leads to the need to study the geographic distribution of the *H. vastratix* strains found in this study. In the absence of this information, in endemic areas of coffee rust, it is recommended to combine cultivars Castillo, Colombia 27 and Monteclaro.

Quiroga –Cardona (2021) indicated that environmental conditions can affect significantly resistance modulation; the effect of shade and its relation with rust epidemic has been well documented and diverse effects have been suggested, probably due to highly complex interactions between crops, environment, pathogen biological factors and physiological state of the plant. According to Várzea *et al.* (2023), as the leaf surface is wet, temperature is one of the most important factors to determine the amount of germinating and penetrating spores. Optimum temperatures for uredospore germination are from 20 to 25 °C. In the present research, temperature was kept constant at 22 °C, which allowed optimal conditions for fungal spore germination and penetration.

In this work, with controlled conditions, temperature and light intensity could have influenced in the infection process, coinciding the results with those obtained by Eskes (1983), who conducted his studies in the field, in greenhouse and in laboratory, and concluded that incomplete resistance, at different levels, apparently, can be specific of a race and is affected by leaf age and light intensity. Therefore, it is necessary to confirm results of the present work under natural temperature and light.

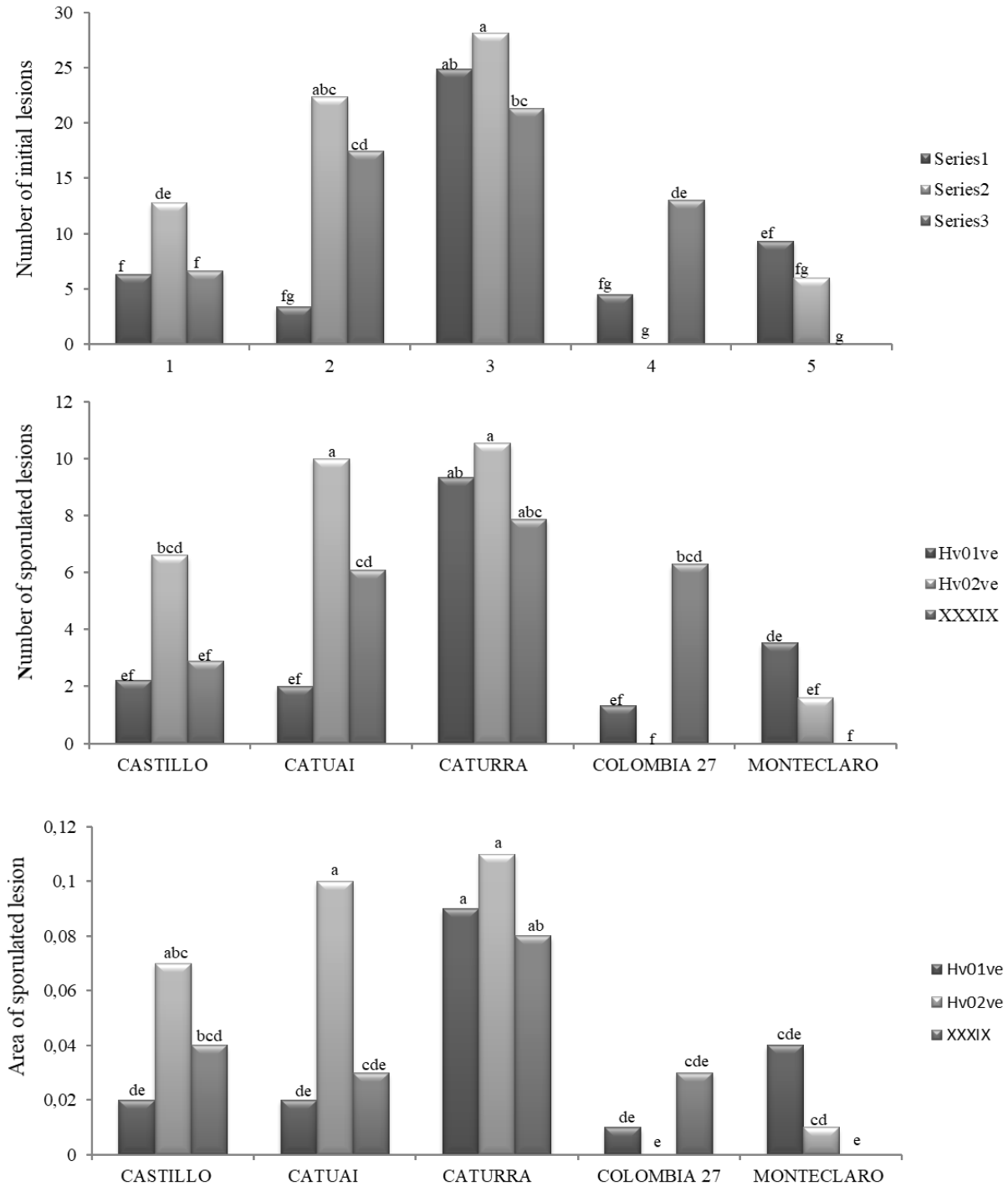

Figure 1. Combined analysis of reaction of coffee cultivars against Hemileia vastratix strains, with regard to the variables. (A) Number of initial lesions, (B) Number of sporulated lesions, (C) Area of sporulated lesion (cm^2). Bars: dark gray, pathotype Hv01ve; light gray, pathotype Hv02ve; medium gray, race XXXIX. Same letters on bars are not significantly different ($p>0.05$), Tukey test.

All the studied variables gave important information for the trial and the interaction strain x cultivar, coinciding, partially, with what was said by Várzea *et al*. (2023), who indicated that the components of incomplete resistance that generate more information in the field are the number of lesion per leaf, or foliar area unit, or percentage of leaf discs with lesions, latency period, incubation period and size of the lesion. In the present study, the variables that better indicated the grade of resistance were the number of initial lesions, number of sporulated lesions and area of sporulated lesion.

Cultivars, in general, showed differences ($p>0.05$) for incubation period, the start of the first symptoms occurred 26 and 40 days after inoculation, agreeing with Bustamante *et al*. (2001), although some of the lesions progressed, with the exception of Monteclaro and Colombia 27, which did not show symptoms against race XXXIX and Hv02ve, respectively, thus they are considered resistant. In this case, in these two cultivars, probably, immunity activated by effectors (ETI) was in place, which leads to complete resistance, without producing any symptom to the disease (Burbano-Figueroa, 2020); this author

also indicates that ETI adjusts to the gene for gen theory present in rust disease. In the coffee-*H. vastratix* pathosystem, resistance in the coffee plants is conditioned by at least nine dominant genes with main effects (SH1-SH9) (Pires *et al*., 2020).

Results of the trial did not show a direct relationship between latency period (PL) and infection grade (GI), some low values of PL and high of GI were observed for some materials. Várzea *et al*. (2023) named this intermediate compatibility and suggested that this might be due to an incomplete resistance of the coffee plants or to a less aggressiveness of *H. vastratix*. Most of the components of incomplete resistance are a quantitative extension of the scale used for the type of reaction or infection grade (GI). These components, as well as the GI, are related with the same basic criteria, such as the size of the lesion, sporulation intensity, and appearance of chlorosis or necrosis. Latency period is related to the size of the lesion; when fungal growth is slow, sporulation is, generally, delayed and lesions will be smaller. The types of reaction "0" (chlorosis without sporulation) or necrotic spots will reduce sporulation intensity and duration (Várzea *et al.,* 2023).

Conclusion

The present study showed differences in the reaction of the tested coffee cultivars to the three strains of *H. vastratix* used, based on the number of rust lesions produced on the leaves and on the sporulated area. In addition, it demonstrated difference among the strains on the severity of the disease induced.

Recommendation

Lack of information about races and pathotypes present in the coffee producer states in the country, leads to recommend planting the three cultivars Castillo, Colombia 27 y Monteclaro together, in rust endemic areas, since they were the ones with better outcome against different strains in this study. Also, it is recommended to continue research to identify other races and pathotypes present in the country

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