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Selection of coffee genotypes resistant to rooster's eye [*Mycena citricolor* (Berk. & M.A. Curtis) Sacc.]

Selección de genotipos resistentes de café al ojo de gallo [*Mycena citricolor* (Berk. & M.A.Curtis) Sacc.]

Julio Gabriel-Ortega
Ashley Macías Salazar
Javier Salvatierra Quinto
Gema Burgos López
Jessica Morán Morán

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Corresponding author
j.gabriel@proinpa.org

Ph.D. in Science, PROINPA Foundation, Cochabamba, Bolivia, j.gabriel@proinpa.org http://orcid.org/0000-0001-9776-9235

Agricultural Engineer, Faculty of Natural Sciences and Agriculture, Southern Manabí State University. macias-ashley7150@unesum.edu.ec, https://orcid.org/0009-0005-5449-379X

Agricultural Engineer, Faculty of Natural Sciences and Agriculture, Southern Manabí State University. salvatierra-javier7146@unesum.edu.ec, https://orcid.org/0009-0003-9738-4740

Master of Science in Agriculture and Animal Science, independent consultant, Portoviejo, Manabí, Ecuador. burgosgema38@gmail.com https://orcid.org/0000-0002-0025-3679

Lecturer in the Master's Program in Agriculture and Animal Science, Graduate Institute of the Southern Manabí State University, Jipijapa, Ecuador.jessica.moran@unesum.edu.ec , https://orcid.org/0000-0002-6487-1038

Abstract: To evaluate the use of oxalic acid in selecting genotypes resistant to coffee leaf rust (*Mycena citricolor* (Berk. & M.A.Curtis) Sacc.), an experiment was conducted using a completely randomized design (CRD) with an unbalanced layout and 55 treatments at the UNESUM Biotechnology Laboratory. Ten genotypes were placed per tray, with two individual leaflets per genotype, for a total of 11 trays. Each leaflet was inoculated with three drops on each side of the midrib at a concentration of 2.25 g/150 mL of oxalic acid. Lesion size (LS) was measured in millimeters using a Vernier caliper starting on the third day, with daily readings continuing for six days. Using the LS data, the area under the relative lesion progression curve (AULRP) was determined as a relative measure over time. Analysis of variance (ANOVA) and comparison of means were performed using Tukey's multiple range test ($P < 0.05$), after verifying that the assumptions of normality and homogeneity of variances were met. Additionally, the best-fit curve was determined using the regression coefficient ($^{R^2}$). The results showed that genotypes 021-101-4 and 021-101-3 exhibited high resistance to necrosis caused by oxalic acid. Genotype 021-106-4 was the most susceptible, as were the cultivars Gheisha and Típica; thus, different levels of resistance to oxalic acid were identified, ranging from the most resistant to the most susceptible. Both the progenies and the parent lines evaluated had a logarithmic regression fit.

Keywords: coffee cultivars, logarithmic regression, lesion size, area under the relative lesion progression curve.

Resumen: Con el objetivo de evaluar el uso del ácido oxálico para obtener genotipos resistentes al ojo de gallo del cafeto (*Mycena citricolor* (Berk. & M.A.Curtis) Sacc.), fue implementado un experimento en un diseño experimental completamente aleatorio (DCA) desbalanceado con 55 tratamientos en el laboratorio de Biotecnología de la UNESUM. Se ubicaron 10 genotipos/bandeja con dos foliolos sueltos/genotipo, totalizando 11 bandejas. Se inoculo cada foliolo con tres gotas a cada lado de la nervadura a una concentración de 2,25 g/150 mL de ácido oxálico. Se evaluó el tamaño de lesión (TL) con un vernier en milímetros a partir del tercer día, y se continuó con la lectura diaria, durante seis días. Con los datos de TL se determinó el área bajo la curva de progreso de la lesión relativa (ABCPLr), como una medida relativa en el tiempo. Se realizó el análisis de varianza y la comparación de medias mediante la prueba múltiple de Tukey ($P<0,05$), una vez cumplidas los supuestos de normalidad y homogeneidad de varianzas. Asimismo, se determinó la mejor curva de ajuste mediante el coeficiente de regresión (R^2). Los resultados determinaron que los genotipos 021-101-4 y 021-101-3, mostraron alta resistencia a la necrosis causada por el ácido oxálico. El genotipo 021-106-4 fue la más susceptible al igual que los cultivares Gheisha y Típica, por lo que se determinó diferentes niveles de resistencia al acido oxálico, variando desde los más resistentes hasta los más susceptibles. Tanto las progenies como progenitores evaluados tuvieron una curva de ajuste de regresión logarítmica.

Palabras clave: cultivares de café, regresión logarítmica, tamaño de lesión, área bajo la curva de progreso de la lesión relativa.

Introduction

Coffee diseases are caused by microorganisms such as fungi, bacteria, viruses, and nematodes; coffee rust (*Hemileia vastatrix* Berk. & Broome) is the most significant, followed by other diseases such as anthracnose (*Colletotrichum* coffeanum), gall eye (*Mycena citricolor* Berk. & M.A. Curtis), iron spot (*Cercospora coffeicolla* Berkeley & Curtis), stringy rot (*Corticium koleroga*), root and stem rot (*Rosellinia* sp.), leaf scorch or wilt (*Phoma costarricenses*), pink rot (*Corticium salmonicolor*), and nematodes (*Meloidogyne* sp., *Pratylenchus* sp., and *Rotylenchulus* sp.) (Agrios, 2005; López, 2020)

Coffee leaf spot, caused by the basidiomycete fungus *Mycena citricolor* (Berk. & M.A. Curtis) Sacc. (Agrios, 2005; Granados et al., 2020), can result in significant economic losses in coffee production due to defoliation and eventual fruit drop. A maximum infection rate of 54% was reported following periods of heavy rainfall in coffee plantations located in an area prone to the disease, such as northern Guatemala; it was estimated that this level of infection resulted in a 56% loss in production (Avelino et al., 2018). In Costa Rica, coffee leaf spot has been reported since 1876 (Granados et al., 2020). Cyclical outbreaks occur approximately every fourteen years, linked to increased rainfall and the presence of the pathogen (Borbón, 1999).

The last major coffee leaf spot epidemic occurred in 2010; on that occasion, a 12% decrease (approximately 71,400,000 kg) in the estimated harvest for the production year (2010–2011) was recorded, resulting in a loss of approximately $60 million USD (Barquero, 2010a; 2010b). The most affected areas were the Central Valley and the Tarrazú region, also known as the Los Santos region. Between 1995 and 1998, coffee leaf spot affected approximately 3,000 ha of coffee plantations, of which 800 ha were located in the Los Santos region (Borbón, 1999). According to the National Technical Advisory Committee on ENSO Phenomena of the National Meteorological Institute (COENOS, 2010), the climatic conditions in 1995, 1998, and 2010 were similar and corresponded to years of transition from El Niño to La Niña.

The most significant effect of the "eye of the rooster" is premature leaf drop (Guerra, 2004; Barquero, 2007); this produces a layer of fresh leaf litter consisting of detached diseased foliage. There is a highly significant correlation (76%) between the infection index and the defoliation index ( ) (Granados et al., 2020). Both the asexual phase (geminiferous bodies) and the sexual phase (basidiocarps) of *M. citricolor* have been observed on leaves from different plant species that have fallen and are decomposing on the ground, both in the forest and in coffee plantations.

Furthermore, Ecuador has significant coffee-producing capacity, making it one of the few countries in the world that exports all types of coffee: washed Arabica, natural Arabica, and Robusta. The National Autonomous Institute for Agricultural and Livestock Research (INIAP) has developed a strategic plan for genetic improvement aimed at evaluating available germplasm and creating and developing new genetic material with high yields and adaptability to the country's diverse ecosystems (INIAP, 2020).

However, in order to select material resistant to coffee leaf spot, it is possible to inoculate plants with the pathogen, which is not always advisable; therefore, the advantage of using the toxin produced by the pathogen on individual leaflets has been recognized. Rao and Tewari (1987) observed that necrotic lesions similar to those caused by *M. citricolor* developed when drops of oxalic acid solutions were placed on coffee leaves. In liquid culture, increases in oxalic acid levels followed the fungus’s growth curve. These results suggest a key role for oxalic acid in the pathogenesis of *M. citricolor*, and the demonstration of calcium oxalate formation in this study provides concrete evidence for the hypothesis of calcium sequestration by the host. Oxalic acid, by lowering the pH, stimulates the indoleacetic acid oxidase and macerating enzyme systems, leading to tissue disintegration and leaf drop (Rao & Tewari, 1987).

Additionally, *Mycena citricolor* is known to synthesize toxins such as oxalic acid and oxalates, which are secondary metabolites secreted into the environment by fungi, bacteria, and plants. Oxalates are associated with various processes occurring in the soil, such as nutrient availability, mineral weathering, or the precipitation of metal oxalates. Oxalates are also listed among the low-molecular-weight compounds that indirectly participate in the degradation of the lignocellulosic complex by fungi, which are considered the most effective wood degraders (Gadd, 1999; Graz, 2024).

Active regulation of oxalic acid concentration is related to enzymatic activities; therefore, the biochemistry of microbial biosynthesis and degradation of oxalic acid has also been presented (Gadd, 1999; Graz, 2024).

Based on the above, the objective of this study, as outlined in the preceding paragraphs, was to evaluate the use of oxalic acid to select genotypes resistant to coffee leaf spot (*Mycena citricolor* (Berk. & M.A. Curtis) Sacc.).

Methodology

Location

The research was conducted at the Biotechnology Laboratory of the Southern Manabí State University (UNESUM) in Los Ángeles, Jipijapa, Manabí. It is located at 1°21'10.14" south latitude and

80°33'50.40" west longitude, at an altitude ranging from 230 to 313 meters above sea level (Gabriel et al., 2024).

Treatments

The treatments consisted of 55 coffee genotypes from the coffee breeding program at the Southern Manabí State University (Table 1).

Table1 .

Coffee accessions used in the study.

Treatment	Genotypes
T1	Catimor CIFC-P1
T2	Yellow Bourbon
T3	021-100-1
T4	021-100-2
T5	021-100-3
T6	021-100-4
T7	021-100-5
T8	021-100-6
	Catimor CIFC-P1
T9	Red Caturra
T10	021-101-1
T11	021-101-2
T12	021-101-3
T13	021-101-4
T14	021-101-5
T15	021-101-6
	Yellow Bourbon
T16	Acawa
T17	021-104-1
T18	021-104-2
T19	021-104-3
T20	Yellow Bourbon
T21	Typical
T22	21-105-1
T23	21-105-2
T24	21-105-3
T25	21-105-4
T26	21-105-5
T27	21-105-6
T28	21-105-7
T29	Arara
T30	Catucai 785-15

T31	021-106-1
T32	021-106-2
T33	021-106-3
T34	021-106-4
T35	021-106-5
T36	021-106-6
T37	021-106-7
T38	021-106-8
T39	021-106-9
T40	021-107-1
T41	021-107-2
T42	Arara Gheisha
T43	021-108-1
T44	021-108-2
T45	021-108-3
T46	021-108-4
T47	021-108-5
T48	Arara Catucai-2 SL
T49	021-109-1
T50	021-109-2
T51	021-109-3
T52	021-109-4
T53	021-109-5
T54	021-109-6
T55	021-109-7

Experimental Procedure

The appropriate concentration of oxalic acid was determined according to Rao’s (1987) recommendations, resulting in a concentration of 2.25 g/150 mL of distilled water. This dose was applied to individual leaves, which were placed in 30 x 40 cm humidity chambers lined with paper towels moistened with distilled water. Ten leaves were placed in each humidity chamber, and three drops of oxalic acid were applied to each side of the midrib. Eight humidity chambers were used. Eleven humidity chambers were used.

The trays were arranged on benches in the Biotechnology laboratory, with each treatment properly labeled. Lesion size (LS) measurements began 36 hours after inoculation and continued daily for six days. A Vernier caliper was used to measure LS in millimeters. LS measurements were recorded in an Excel database and then analyzed using Infostat software.

Experimental Design

The experiment was conducted using a completely randomized unbalanced design (CRUD) with 55 genotypes (treatments) (Gabriel et al., 2022), which were placed in 11 humidity chambers.

The response variables evaluated were: relative lesion progress ( ) lesion size (mm) (TL), and area under the relative lesion progress curve (%) (ABCPLr). This variable was determined based on LS, as a measure relating LS to time, and was expressed as a percentage (Gabriel et al., 2017). A regression analysis was performed between LS and time to determine the regression coefficient ($^{R^2}$), thereby estimating the best-fit curve.

Statistical Analysis

After verifying that the variables met the assumptions of normality and homogeneity of variances, and based on the defined “ ” model, an analysis of variance (ANOVA) was performed to test hypotheses regarding fixed effects, as well as comparisons of treatment means using Duncan’s test ($P < 0.05$). The analysis of variance was also used to estimate the variance components for the random effects. The analyses were performed using Infostat software (Infostat, 2020).

Normality Test

The normality test for the variables using the Shapiro-Wilk test ($P < 0.05$) was not significant, suggesting that the variables were normally distributed. Likewise, the Levene test ($P < 0.05$) determined that there were no significant differences among the variances, indicating homogeneity of variances. This allowed for the continuation of ANOVA and mean comparisons using Tukey’s multiple comparison test ($P < 0.05$).

Results

s Analysis of ABCPLr

Table 2 shows the analysis of variance for ABCPLr, which revealed significant differences ($P < 0.05$) among the evaluated progenies and parental lines, and the coefficient of variation (CV) was 22.12%, which is appropriate for this type of research.

Table 2.

Analysis of variance for ABCPLr.

F.V.	Gl	SC	CM	F	p-value
Model	54	3,505.17	66.91	13.94	<0.0001
Genotype	54	3,505.17	66.91	13.94	<0.0001
Error	606	2,909.15	4.80		
Total	659	6,414.32			
CV (%)	22.12				

Table 3 shows the analysis of means using Tukey's multiple range test ($P < 0.05$) for the treatments, revealing significant differences for the ABCPLr variable, where the best treatment was genotype 021-101-4 with an ABCPLr of 4.64%. Genotype 021-106-4 was the most susceptible, with an ABCPLr of 14.36%

Different levels of resistance to oxalic acid (synthesized by the coffee cock's-eye fungus (*Mycena citricolor*)) were observed. The genotypes tolerant to infection caused by oxalic acid were genotypes 021-101-3, 021-104-2, 021-100-6, Catimor CIFC-P1, 021-101-5, 021-100-5, 021-101-2, Yellow Bourbon, 021-101-1, 021-101-6, 021-100-2, 021-109-7, and 021-104-1, with ABCPLr percentages ranging from 4.77% to 8.35%, respectively (Table 3). The remaining genotypes were susceptible, with ABCPLr ranging from 8.56% to 14.33%.

Table 3.

Analysis of means for the ABCPLr of each genotype.

Genotype	ABCPLr (%)	
021-101-4	4.64	a
021-101-3	4.77	b
021-104-2	6.10	b

021-100-6	6.50	b
Catimor CIFC-P1	6.94	b
021-101-5	7.04	b
021-100-5	7.07	b
021-101-2	7.51	b
Yellow Bourbon	7.54	b
021-101-1	7.67	b
021-101-6	7.77	b
021-100-2	8.21	b
021-109-7	8.25	b
021-104-1	8.35	b
Catucai-2 SL	8.56	c
021-100-3	8.74	c
Acawa	8.87	c
021-108-4	8.88	c
021-104-3	9.11	c
021-109-3	9.18	c
021-100-4	9.37	c
021-109-5	9.39	c
021-109-4	9.57	c
021-100-1	9.62	c
021-109-6	9.90	d
Red Caturra	9.92	d
021-107-2	10.13	d
021-109-2	10.56	e
Arara	10.96	f
021-105-5	10.99	f
021-106-5	11.10	f
021-105-3	11.10	f
021-108-2	11.12	f
021-109-1	11.28	g
021-106-7	11.31	g
021-105-4	11.47	h
021-106-9	11.59	i
021-106-3	11.59	i
021-105-6	11.61	i
021-105-1	11.65	i
021-108-3	11.74	i
021-108-1	11.79	i
021-106-1	11.82	i
Catucai 785-15	11.89	i
021-107-1	12.11	j

021-106-8	12.46	k
021-105-2	12.89	j
021-108-5	12.95	j
021-106-6	13.02	j
021-106-2	13.24	m
Geisha	13.32	n
021-105-7	13.44	o
Typical	13.90	p
021-106-4	14.33	q
DSH	3.70	

Means with the same letters are not significantly different (P < 0.05

Regression Analysis

Table 4 and Figure 1 show a distinct pattern in the regression curve for the leaves due to the effect of oxalic acid on the evaluated genotypes.

In family 021-100, it was determined that the progeny followed a logarithmic fit with a regression coefficient R^2= 0.84. The female parent, Catimor CIFC-P1, had an R^2 coefficient of 0.84, and the male parent, Yellow Bourbon, had an R^2 coefficient of 0.80; both showed a better fit to a logarithmic curve.

Table 4.

Regression coefficient (R^2) for the best fit of the TL development curve over time.

Cultivar	R^2 coefficient of a linear curve	R^2 coefficient of a logarithmic curve
Catimor CIFC-P1	0.63	0.84
Yellow Bourbon	0.58	0.80
021-100	0.53	0.84
Catimor CIFC-P1	0.63	0.98
Red Caturra	0.69	0.88
021-101	0.87	0.96
Yellow Bourbon	0.63	0.89
Acawa	0.48	0.50
021-104	0.70	0.84
Yellow Bourbon	0.51	0.74
Tipica	0.57	0.80
021-105	0.48	0.80
Arara	0.63	0.85
Catucaí 785-15	0.46	0.70
021-106	0.57	0.76
021-107	0.48	0.71
Arara	0.56	0.59
Geisha	0.37	0.77
021-108	0.32	0.59
Arara	0.57	0.86
Catucaí-2L SL	0.57	0.72
021-109	0.57	0.77

In family 021-101, it was determined that the progeny had a logarithmic fit with a regression coefficient of R^2= 0.84. The female Catimor parent CIFC-P1 had an $R^2$of 0.84, and the male red Caturra parent had an $R^2$of 0.80, indicating a better fit to a logarithmic curve.

In family 021-104, it was determined that the progeny followed a logarithmic fit with a regression coefficient of R^2= 0.84. The female Bourbon Yellow parent (R^2= 0.89) and the male Acawa parent (R^2= 0.50) also showed a better fit to a logarithmic curve.

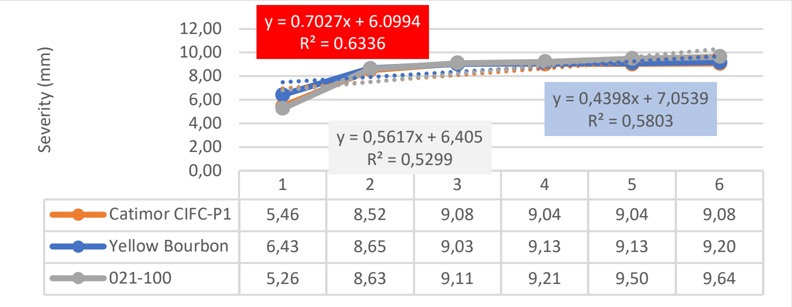
In family 021-105, it was determined that the progeny followed a logarithmic fit with a regression coefficient of R^2= 0.80. The female Bourbon yellow parent (R^2= 0.74) and the male Típica parent (R^2= 0.80) also showed a better fit to a logarithmic curve.

In family 021-106, it was determined that the progeny followed a logarithmic curve with a regression coefficient of R^2= 0.80. The female parent Arara (R^2= 0.85) and the male parent Catucaí 785-15 (R^2= 0.70) also showed a better fit to a logarithmic curve.

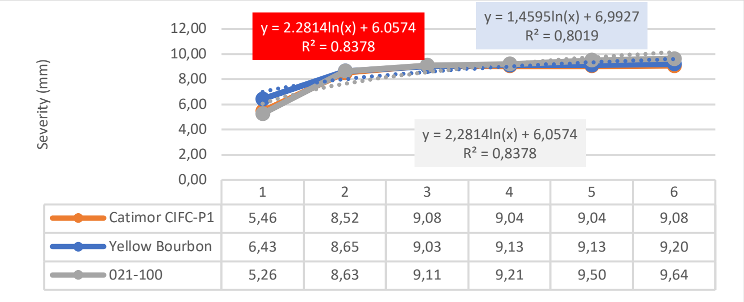
In family 021-107, it was determined that the progeny followed a logarithmic fit with a regression coefficient of R^2= 0.71.

In family 021-108, the progeny was found to follow a logarithmic curve with a regression coefficient of R^2= 0.59. The female parent Arara (R^2= 0.77) and the male parent Gheisha (R^2= 0.77) also showed a better fit to a logarithmic curve.

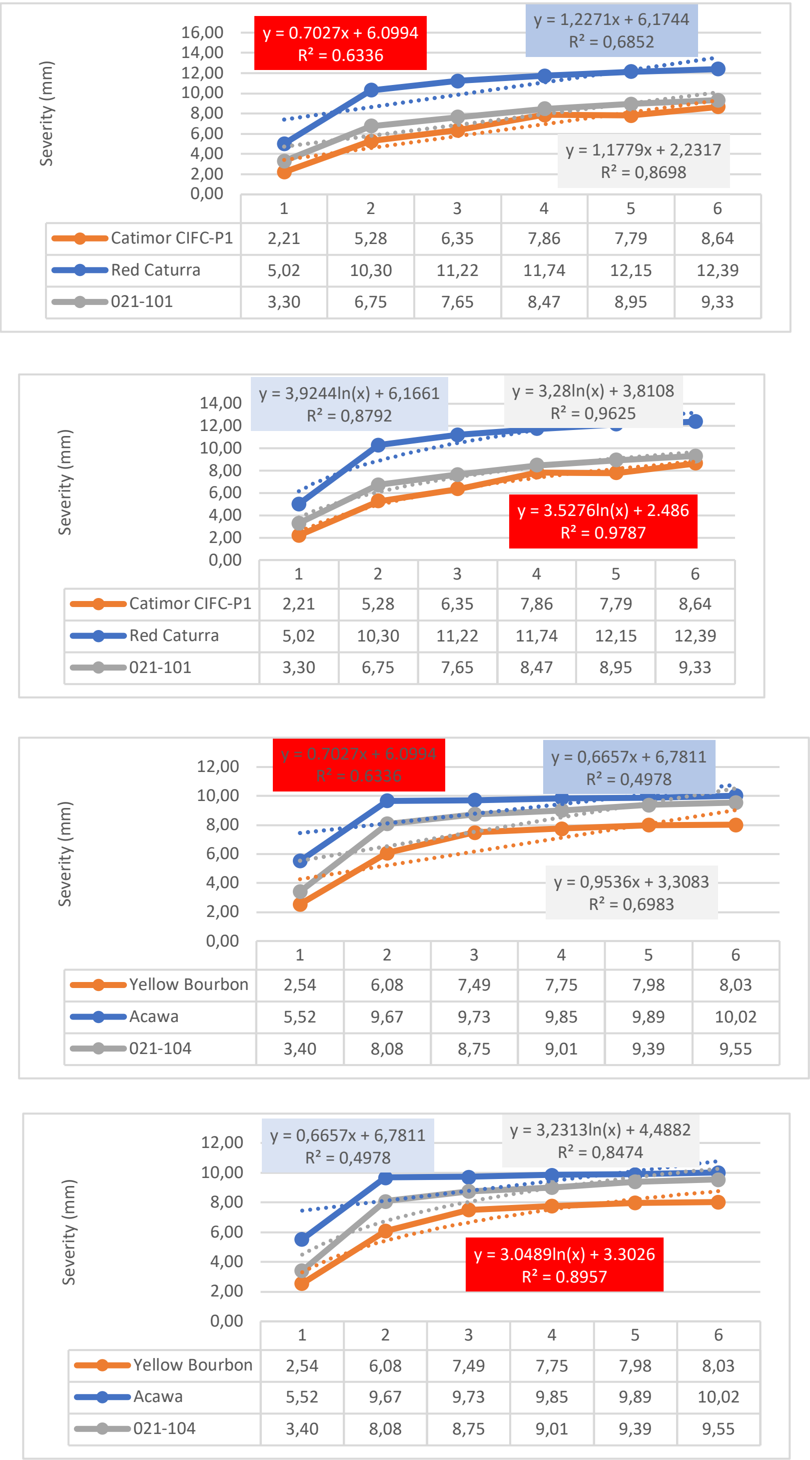
In family 021-109, it was determined that the progeny had a logarithmic fit with a regression coefficient of R^2= 0.59. The female parent Arara (R^2= 0.86) and the male parent Catucaí-2L SL (R^2= 0.77) also showed a better fit to a logarithmic curve.



	1	2	3	4	5	6
Catimor CIFC-P1	5,46	8,52	9,08	9,04	9,04	9,08
Yellow Bourbon	6,43	8,65	9,03	9,13	9,13	9,20
021-100	5,26	8,63	9,11	9,21	9,50	9,64



	1	2	3	4	5	6
Catimor CIFC-P1	5,46	8,52	9,08	9,04	9,04	9,08
Yellow Bourbon	6,43	8,65	9,03	9,13	9,13	9,20
021-100	5,26	8,63	9,11	9,21	9,50	9,64



	1	2	3	4	5	6
Catimor CIFC-P1	2,21	5,28	6,35	7,86	7,79	8,64
Red Caturra	5,02	10,30	11,22	11,74	12,15	12,39
021-101	3,30	6,75	7,65	8,47	8,95	9,33

	1	2	3	4	5	6
Catimor CIFC-P1	2,21	5,28	6,35	7,86	7,79	8,64
Red Caturra	5,02	10,30	11,22	11,74	12,15	12,39
021-101	3,30	6,75	7,65	8,47	8,95	9,33

	1	2	3	4	5	6
Yellow Bourbon	2,54	6,08	7,49	7,75	7,98	8,03
Acawa	5,52	9,67	9,73	9,85	9,89	10,02
021-104	3,40	8,08	8,75	9,01	9,39	9,55

	1	2	3	4	5	6
Yellow Bourbon	2,54	6,08	7,49	7,75	7,98	8,03
Acawa	5,52	9,67	9,73	9,85	9,89	10,02
021-104	3,40	8,08	8,75	9,01	9,39	9,55

Figure 1. Regression fit curves for coffee progenies and parents inoculated with oxalic acid

We observed a variety of responses to oxalic acid. The least affected (most resistant) genotype was 021-101-4, a cross between Catimor CIFC-P1 and Yellow Bourbon. Both parents were moderately resistant, indicating that genes conferring resistance to *Mycena citricolor* were transferred to some of their progeny. The parent cultivars—Acawa, Red Caturra, Arara, Catucai 785-15, Geisha, and Typica—were susceptible. In this regard, Castro et al. (2024) found moderate resistance in the parent cultivars Catimor CIFC-P1 and Catucai-2 SL and susceptibility in all other cultivars evaluated. These results are consistent with those observed in our study.

Castro et al. (2024) determined that the yellow Bourbon parent was the most susceptible to *Mycena citricolor* under field conditions. In our study, we determined that this parent exhibited moderate resistance. This contradiction is possibly due to the absence of environmental effects in the laboratory setting. It is known that *Mycena citricolor* thrives in environments with high humidity and cool temperatures, primarily affecting leaves and fruits, which causes them to drop (Avelino et al., 2018).

The economic impact of this disease varies across different coffee-growing regions in Latin America. In Puerto Rico, losses of up to 75% have been estimated; in Costa Rica, up to 15% of the total hectares planted with coffee have been affected; and in Guatemala, an incidence of 49% of this disease has been reported (Vidal *et al*., 2021). In Honduras, the Arabica coffee cultivars most tolerant to “ojo de gallo” were Típica, Bourbon, Catuai, Pacas, and Caturra; and the most susceptible were IHCAFE 90, Lempira, Parainema, other Catimores, and Sarchimores (Yizard, 2018).

Oxalic acid and oxalates are secondary metabolites secreted into the surrounding environment by fungi, bacteria, and plants. Oxalates are linked to a variety of soil processes, such as nutrient availability, mineral weathering, and the precipitation of metal oxalates (Graz, 2024).

The exact mechanism by which *Mycena citricolor* infection develops is unclear [National Service for Health, Safety, and Food Quality (SENASICA, 2014)]; however, it is believed that the fungus’s dispersal structures (spores) release oxalic acid onto the leaf blade, which alters the pH and induces the production of enzymes that degrade cell walls. Once the fungus has established itself within the plant, it likely uses the plant’s metabolism to feed, degrading the metabolic energy contained in reserve carbohydrates, as is the case with other fungi (Vargas, 2003; Foster *et al.*, 2003; Barquero, 2011). In our study, we applied oxalic acid, a secondary metabolite secreted by *Mycena citricolor,* to destroy the cellular tissue of the leaves (Rao & Tewari, 1987).

Regarding the genotypes' response to infection and lesion development on coffee leaves caused by oxalic acid, we observed distinct responses; in all cases analyzed—including both progenies and parents—the genotypes exhibited a logarithmic fit curve. Similar results were found when studying UNESUM's coffee germplasm, where the Catimor CIFC-P1, Yellow Bourbon, and Acawa cultivars showed a logarithmic fit (Gabriel et al., 2026). In this regard, it is known that coffee leaf rust is more dependent on the amount of primary inoculum than on the infection rate (*r*), which implies that implementing management strategies that reduce the initial inoculum level would significantly delay the development of the epidemic (Wang and Arauz, 1999), thereby reducing production and economic losses. This value (*r*) represents an increase in the amount of inoculum or disease (incidence or severity) and can be calculated in days, weeks, or years; in general, the value of r for multi-cycle diseases is higher than the *r* (*rm*) of monocyclic diseases (Agrios, 2005).

This value allows for comparisons between epidemics—for example, those occurring over several years or under different conditions—and also enables comparisons and correlations among the various elements and stages of an epidemic, such as primary inoculum, spore release, latent period, infectious period, and others (Achicanoy, 2000). It can be considered a measure of disease risk and is one of the three key epidemiological parameters for developing management strategies; the others are the amount of initial inoculum (y_0) and the time (t) during which the pathogen and host interact. The value of r in diseases with multiple cycles is the most important factor for establishing management guidelines that reduce disease in an agroecologically and economically sustainable manner (Nutter, 2007). If the apparent infection rate is low, reducing the initial disease level slows the progression of the disease (Arauz, 2011). Granados et al. (2020) determined that the disease followed a logistic growth curve for epidemics, which provided the best fit. This indicates that the progression of the disease depends on several factors (primary inoculum, spore release, latent period, infectious period, and others) and that it is a polycyclic disease.

Conclusions

Genotypes 021-101-4 and 021-101-3 showed high resistance to necrosis caused by oxalic acid. Genotype 021-106-4 was the most susceptible, as were the cultivars Gheisha and Típica; thus, different levels of resistance to oxalic acid were determined, ranging from the most resistant to the most susceptible.

Both the progenies and the parent lines evaluated had a logarithmic regression fit curve.

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