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Use of Detached Leaf Inoculation Method for the Early Selection of *Coffea arabica* L. for Resistance to *Hemileia vastatrix* Berk and Broome

Julio Quiroga-Cardona [1,*], Luisa Fernanda López-Monsalve [1], Vítor Manuel Pinto Várzea [2] and Claudia Patricia Flórez-Ramos [1]

[1] Plant Breeding, National Coffee Research Center, Cenicafé, Manizales 170009, Colombia; claudia.florez@cafedecolombia.com (C.P.F.-R.)
[2] LEAF—Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal
* Correspondence: julio.quiroga@cafedecolombia.com

Abstract: Three hybrid populations (F1) of Coffea arabica were evaluated under field and laboratory conditions, derived from sources carrying the SH1 coffee leaf rust (CLR) resistance gene and the CX.2385 line, obtained from the Caturra × Timor Hybrid CIFC-1343. The results obtained under controlled conditions and analyzed using survival curves allowed to estimate the probable times ($p < 0.05$) for the development of symptoms associated with CLR in the plants of populations evaluated. Phenotypic variation was observed as a defense response to *Hemileia vastatrix* infection, and plants with incomplete resistance to CLR were identified via an evaluation using the increasing lesions scale. The plants with incomplete resistance exhibited a delay in the development of the incubation period and an absence of the development of the dormancy period. Data suggest that when resistance genes in the sources are defeated by compatible strains, their recombination can give rise to new levels of resistance in the progeny. Additionally, the detached leaf methodology is recommended as an alternative to preselect genotypes with resistance to CLR, thus reducing the number of plants that are finally planted for field evaluations.

Keywords: survival curves; *Coffea arabica* genes; incubation period; latency period; incomplete resistance; coffee leaf rust

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1. Introduction

In *Coffea arabica*, the coffee leaf rust (CLR) resistance genes *SH1*, *SH2* and *SH4* were identified through reactions induced by different pathotypes of *Hemileia vastatrix* [1], and it was proposed that the interactions between these resistance mechanisms in the plant and the virulence mechanisms in CLR adapted to the gene-for-gene theory [2]. In coffee, this relationship is often applied to resistance factors in the plant and virulence factors in the pathogen in the homozygous state, as long as the resistance comes from dominant genes and the virulence from recessive genes [3]. However, resistance genes in *C. arabica* and virulence genes in *H. vastatrix* are not completely dominant [4].

Currently, the protection conferred by the genes encoding *C. arabica SH1*, *SH2*, and *SH4* is of little interest due to the speed with which variants of *H. vastatrix* can overcome it. However, plants harboring these genes may exhibit incomplete resistance when specific resistance is overcome by the pathogen [5,6]. In this way, incomplete resistance can become effective in the field for a longer time, providing protection to plants against pathogens that cause limiting diseases, compared to complete resistance, and its quantification can only be carried out through the study of races of the pathogen compatible with the host [7]. On the other hand, incomplete resistance is affected by various factors, including the environment [8], nutritional status [9–11], age, phenology, ontogenetic state of organs [8,12,13], and production potential of varieties [14,15].

In *C. arabica*, incomplete resistance to *H. vastatrix* is affected by the age of the leaves [16], the productivity of the plant [17], the interaction between the genotype and the environment [18–22], the physiological and nutritional status of the crop [9–11,23], and its genetic basis [24]. Studies on the latter factor have shown that incomplete resistance in *C. arabica* is related to interactions between genes with greater effects and genes with weaker effects that act in an additive manner [24]. The constitutive genetic background of the plant can have an effect, as can a homozygous state of the gene and its combination with other resistance mechanisms [24]. These mechanisms are probably similar to those of the genes with a greater effect, *SH6* through *SH9*, identified in the Timor Hybrid (HdT) [3], and their control is oligogenic but is determined by a variable number of genes [24].

In the search to shorten selection times for the development of coffee varieties, reduce resources, and make the evaluation and selection processes for disease resistance in the field more efficient, there are alternative methods that can be used, such as the use of detached leaves [25]. These methods require a short processing time evaluation and replicate the infection reactions caused by the pathogen under field conditions [25]. These methods have been adjusted for coffee [5,26]. The use of detached leaf inoculation of such alternative methods makes it possible to measure the components of incomplete resistance [26]. In coffee, these components quantified under laboratory conditions, present a high correlation (r = 0.83) with the severity of disease observed under natural conditions of the crop, which has allowed us to select coffee genotypes with an incomplete resistance to CLR. [24]. However, this process involves the collection of continuous records, which are essential for determining the times at which one or more specific symptoms occur. This large volume of information is highly useful when investigating the behavior of plants against the attack of pathogens and disease development [27].

The information obtained in this type of research, when adjusted to statistical assumptions, is generally obtained from parametric statistics. When this does not occur, it is necessary to resort to other statistical approaches for analysis, including time to event analysis [28]. This method is also known as survival analysis or reliability analysis [28,29]. This type of analysis has been implemented to study the interactions between different species [30–34]. Countless cases of plant-pathogen interactions can be cited, but the time until the occurrence of events is rarely used, and coffee is no exception.

Due to the importance of developing techniques in plant breeding for the evaluation and selection of CLR-resistant genotypes and the ability to establish a balance between the time required for the analysis and the efficiency of the selection, the present investigation aimed to select genotypes resistant to CLR using the detached leaf inoculation method. The data were analyzed using nonparametric statistics.

2. Materials and Methods

2.1. Location

The plants were established at the Naranjal Experimental Station of the National Coffee Research Center, Cenicafé, located in Chinchiná-Caldas, Colombia (04°59′ N, 75°39′ W, 1381 masl). This research center has an average temperature of 21.4 °C, 2782 mm of annual precipitation, and 77.5% relative humidity.

2.2. Genotypes

Twenty plants from three populations derived from complex crosses between Ethiopian introductions, carriers of the *SH1* gene (CCC.32 and CCC.66), and varieties Caturra and Catuaí, which are susceptible to CLR, were evaluated: Population 1: [[(Caturra × CCC.32) × (Caturra × CCC.66)] × CX.2385], Population 2: [CX.2385 × [(Caturra × CCC.32) × (Caturra × CCC.66)]], and Population 3: [Catuaí × [(Caturra × CCC.66)] × CX.2385]. In previous works developed at the Centro de Investigação das Ferrugens do Cafeeiro (CIFC for its name in Portuguese), it was determined that these crosses segregate for the physiological resistance Groups C and EC (*SH1.5*, *SH5*), respectively. These populations

were crossed with the CX.2385 line, a carrier of incomplete resistance to the disease, which was obtained from a cross between the Caturra variety and the HdT CIFC-1343.

2.3. Field Evaluations

The incidence of CLR was quantified under field conditions using the scale of incidence in the field [35]. This scale takes the entire plant as the unit of observation. Between 2017 and 2019, in the months with the highest incidence of the disease in Colombia (April and August), two evaluations of incidence were carried out per year. All the plants were evaluated for their incomplete resistance to CLR using the detached leaf inoculation method. Fully extended young leaves were taken from branches of the second third of the tree, located in the first two nodes, from the outside to the inside. The leaves were collected with the petiole, washed, and disinfected by being immersed in a 3% hypochlorite solution under constant agitation for 30 to 40 s. Immediately after, they were washed with distilled water and then immersed in alcohol at a concentration of 70% for 1 min. The alcohol was removed by rinsing them with distilled water, followed by rinsing with sterile distilled water. Four leaves were deposited per genotype, arranged with the underside facing up, in transparent plastic boxes with a lid (37 cm wide by 26 cm high by 40 cm long) acting as a humid chamber. The leaves were then kept for 12 h in complete darkness.

2.4. Laboratory Evaluations

The inoculum was obtained from isolated plants the CX.2385 line (Figure 1A), with the scraping technique on a Petri dish, and prepared in a solution with sterile distilled water and 0.7 mg of urediniospores being added to each ml of water. The dispersion of the urediniospores and homogenization of the solution were carried out by shaking under ultrasound for 20 s. Finally, the solution was subjected to constant magnetic stirring, and, with a micropipette, 8 drops of 5 µL were deposited on each leaf and then incubated in complete darkness for 48 h (Figure 1B). The inoculated leaves were subjected to alternating periods of illumination, 9 h of light and 15 h of darkness, for 60 days with LED light lamps; the configuration was 70% intensity, red color 100%, blue color 100%, green color 0% and 30 µmol m^2s^{-1}, and it was set up 75 cm above the leaves (Figure 1C). The quantification of luminosity was carried out with the TARGAS-1 portable photosynthesis system device; the precision was ±1 µmol m^2s^{-1}.

Figure 1. (**A**) CX.2385 plants isolated to obtain the CLR inoculum. (**B**) Inoculation boxes containing detached leaves inoculated with CLR. (**C**) Lighting conditions of the detached leaf evaluation room.

It was guaranteed that the interior humidity of each chamber stayed close to the saturation point, the temperature was between 22 and 24 °C, and the progression of the disease was recorded from day 10 after inoculation (DAI) (Figure 2) on a scale comprising increasing severity of lesions for the evaluation of incomplete resistance [5].

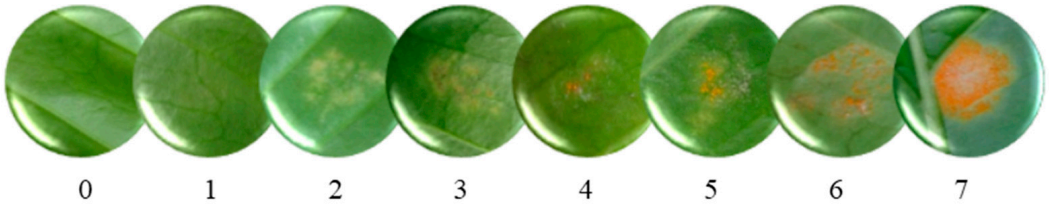

Figure 2. Scale of increasing lesions to quantify severity to CLR. (**0**) Absence of visible injury. (**1**) Appearance of small, discolored lesions. (**2**) Increased lesion surface and deeper discoloration. (**3**) Intensification of lesions and discoloration. (**4**) The appearance of the first spores. (**5**) Sporulation in less than 25% of the lesion surface. (**6**) Sporulation between 25% and 50% of the lesion surface. (**7**) Sporulation in more than 50% of the lesion surface.

2.5. Experimental Design and Analysis

The experiment was replicated four times in a completely randomized design. The data were analyzed by survival analysis, taking the leaf as an experimental unit and each inoculated site as an observation unit. The survival estimators that correspond to the appearance of each symptom were obtained by the Kaplan–Meier method [34] with 95% confidence intervals ($p < 0.05$) using Equation (1). The data were processed using R and the *survival* and *survminer* packages.

$$S(t) = P(T \geq t) \quad (1)$$

where

- S (t) = survival function.
- T = the most likely time at which symptoms develop in each genotype.
- P (t) = the conditional probability function that describes the instantaneous risk for the symptom to develop at time t, from day 10 DAI to day 60 DAI.

3. Results

3.1. Incidence of CLR under Field Conditions

Six evaluations were performed under field conditions, with a maximum value of 3 and a minimum value of 1, according to the incidence scale of Eskes and Toma-Braghini [35] (Table 1). Although grade 3 can be considered a degree of apparent susceptibility, it was classified as a resistance reaction due to the non-effect of the disease on production.

Table 1. CLR incidence in the field in the three populations evaluated.

Population	CLR Incidence in Field										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total Plants**
1. [(Caturra × CCC.32) × (Caturra × CCC.66)] × CX.2385											5
2. CX.2385 × [(Caturra × CCC.32) × (Caturra × CCC.66)]			1	9							10
3. [Catuaí × (Caturra × CCC.66)] × CX.2385		1		4							5
Total Plants		**1**	**1**	**18**							**20**

3.2. Probable Times of Symptom Onset under Controlled Conditions

The average DAI at the appearance of small, discolored lesions (grade 1) and the percentage of inoculated sites that developed grade 1 lesions was evaluated. Variation between genotypes was seen (Figures 3 and 4 and Table 2). The Caturra variety and the CX.2385 line exhibited the shortest incubation periods, with 14.34 and 14.94 DAI, respectively (Figure 4).

Figure 3. Probability of developing CLR symptoms in the coffee population evaluated according to survival estimators ($p < 0.05$).

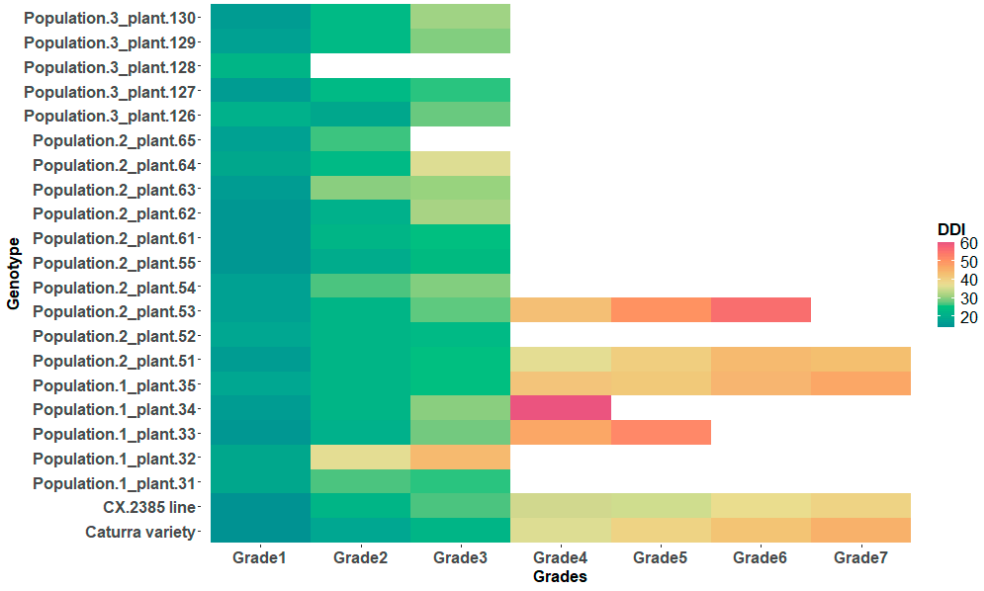

Figure 4. Probable days of developing CLR symptoms in the coffee population evaluated according to survival estimators ($p < 0.05$).

In all the plants evaluated, some tissue was affected by CLR. The survival estimators ($p < 0.05$) showed that the most likely times for the development of the fungus during the incubation period were not absolute but rather highly variable (Figure 4). The incubation period was 14.34 DAI in the Caturra variety, 14.94 DAI in the CX.2385 line, and 22.50 DAI in the plant 128 (population 3).

The symptom values recorded in the incubation period were variable, ranging from 32.8% to 100%. The lowest percentage (32.8%) was presented by plant 128, which did not develop more advanced symptoms. In the other plants, symptoms were detected in 87.5% to 100% of the lesions (Table 2). Lesions of genotypes 33, 34, 35, 51, and 53, Caturra variety, and CX.2385 line showed sporulation, with visible differences in density, indicating different levels of resistance for each plant (Table 2). In all cases, the disease developed gradually, with intense chlorotic zones, followed by the formation of uredosporic sori.

Table 2. CLR observed symptoms and percentages of injuries evaluated in coffee population according to the scale of increasing injuries.

Population	Plant	Percentages of Involvement in the Scale of Increasing Injuries						
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6	Grade 7
1	31	93.80	87.50	6.30				
	32	100.00	27.30	9.40				
	33	93.80	62.50	37.50	3.90	0.80		
	34	100.00	87.50	43.80	0.80			
	35	100.00	75.00	62.50	6.30	5.50	5.50	5.50
2	51	100.00	100.00	75.00	46.10	44.50	40.60	29.70
	52	87,50	62.50	37.50				
	53	100.00	81.30	63.30	18.00	6.30	0.80	
	54	100.00	81.30	37.50				
	55	100.00	100.00	100.00				
	61	100.00	100.00	62.50				
	62	100.00	87.50	56.30				
	63	100.00	43.80	12.50				
	64	93.80	50.00	21.90				
	65	100.00	93.80					
3	126	87.50	31.30	18.80				
	127	100.00	68.80	25.00				
	128	32.80						
	129	87.50	43.80	18.80				
	130	100.00	62.50	37.50				
Caturra variety		100.00	100.00	100.00	78.10	78.10	78.10	77.30
CX.2385 line		100.00	100.00	87.50	26.60	25.80	24.20	24.20

The times to reach the most advanced stages of the disease within the incubation period varied from plant to plant. The earliest expression of grade 2 was observed in the Caturra variety (18.2 DAI), the latest in plant 32 (36.4 DAI). The CX.2385 line developed symptoms 4.3 days later than the Caturra variety and 13.9 days earlier than the most recent genotype (plant 32) (Figure 4).

The maximum grade reached by plant 65 was 2, which was visible in 93.8% of the lesions (Table 2) at 26 DAI. This time was 8 days later than that of the Caturra variety and 4.2 days later than that of CX.2385 line. In the other plants, symptoms arose between 19.0 and 23.0 DAI. Plants 35 and 51 developed symptoms at the same time (Figure 4).

In Caturra variety, grade 3 was observed 3.6 days after presenting grade 2. The progression of the disease was slower in plant 32, at 17.1 days after CX.2385 line and 22.2 days after the Caturra variety (Figure 4). Plants 31 and 127 presented equal times for the development of grade 3 symptoms. Grade 3 was present to different degrees, with values ranging from 6.3% to 100% (Table 2).

The shortest latency periods (grade 4) were recorded for the CX.2385 line (34.5 DAI) and the Caturra variety (36 DAI). This symptom developed in only 25% of the evaluated plants (33, 34, 35, 51, and 53). The highest percentages of sporulating sites were observed in plants 51 and 53 (Table 2). Plant 34 was the latest to develop sporulating sites, 24 days later than the Caturra variety did and 25.5 days after the CX.2385 line did.

The stages of disease development greater than the latency period (grades 5, 6, and 7) were detected only in plants 33, 35, 51, and 53 and in the Caturra variety and CX.2385 line. Grade 6 was detected in CX.2385 line at 37.4 DAI, affecting 24% of the inoculated sites, and at 42.7 DAI in the Caturra variety, affecting 78.1%. With a difference of a few days, the same degree was recorded in plants 35, 51, and 53, which presented widely dissimilar infection values, ranging from 0.8% to 40.6% (Figures 3 and 4 and Table 2).

Grade 7 was observed in the Caturra variety and CX.2385 line. Genotypes 35 and 51 developed the same symptoms at 48 and 44 DAI, respectively (Figure 4). The lesions appeared in variable percentages, between 5% and 30% (Figure 3 and Table 2). The percentage affecting genotype 35 was 18.6% less than that of CX.2385 line and 71.8% less than that of the Caturra variety. The effect of plant 51 was 5.5% greater than that of CX.2385 line but 47.6% lower than that of the Caturra variety. The percentages of grade 7 lesions in the Caturra variety and CX.2385 line were widely dissimilar, with values greater than 75% in the Caturra variety and less than 25% in the CX.2385 line (Table 2).

3.3. Probability of Survival to the Expression of CLR Symptoms in Coffee Population

Population 1, made up of plants 31, 32, 33, 34, and 35, presented heterogeneity in resistance reactions from day 10 to day 60 DAI (Figure 5). Under the exposure conditions, evaluation time, and inoculum used, the development of grade 4 fungi was not present in plants 31 and 32. In plants 31, 32, and 35, the development of the different symptoms did not occur quickly. In contrast, in plants 33 and 34, the symptoms were most common during the first 35 days of observation.

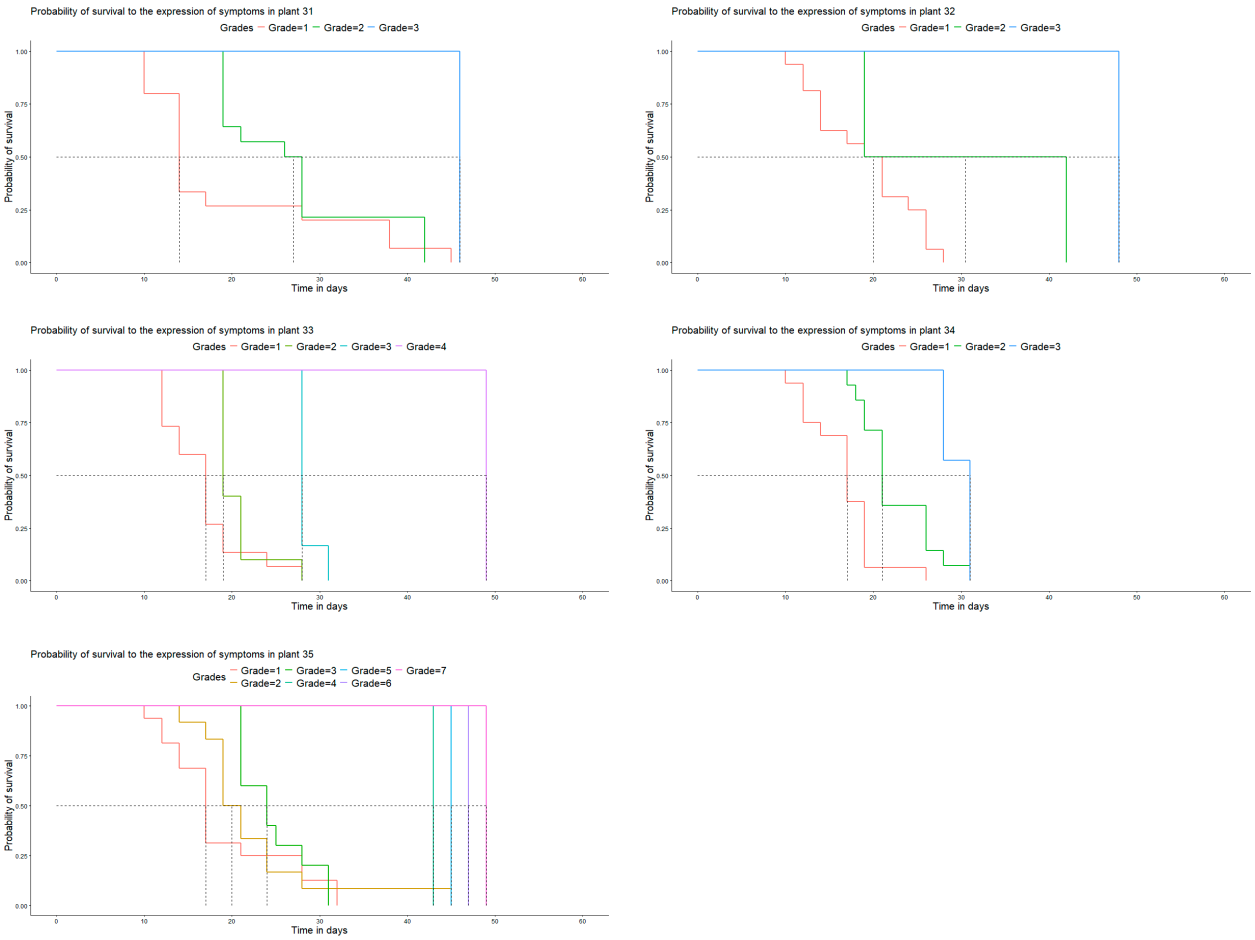

Figure 5. Days vs. probability of survival to disease development in the genotypes of population 1 under laboratory conditions. Dashed lines: median number of days to reach each degree and median probability of survival.

In population 2, there was also a variation in the reactions of resistance to CLR. Plants 52, 54, 55, 61, 62, 63, and 64 showed no development of a latency period (grade 4), similar to what we observed in plants 31 and 32 of population 1. Plants 51 and 53 had the highest incidence, which was similar to that of the CX.2385 line. Plant 65, which did not develop to a degree higher than 2, stood out (Figure 6).

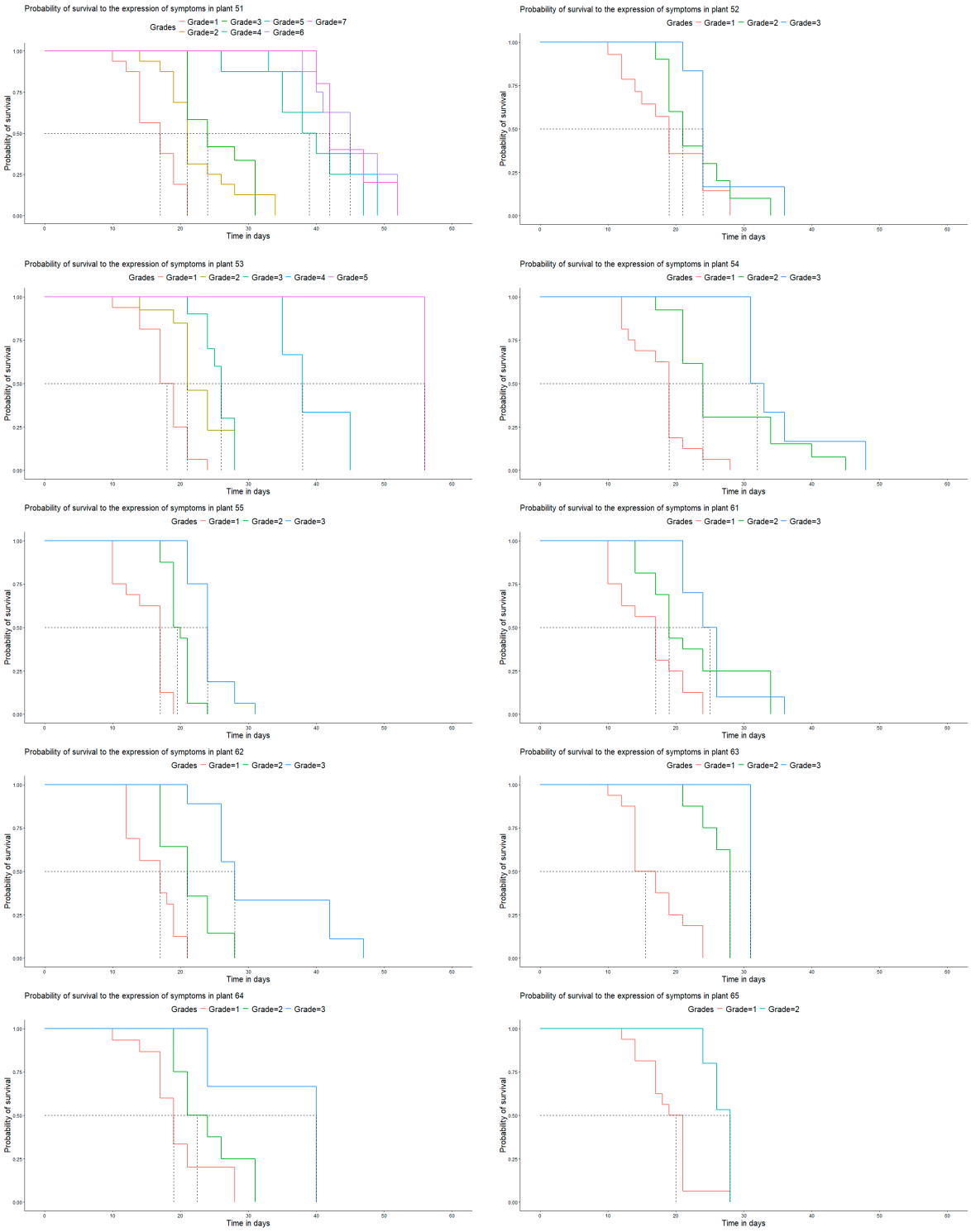

Figure 6. Days vs. probability of survival to CLR disease development in the genotypes of population 2 under laboratory conditions. Dashed lines: median number of days to reach each degree and median probability of survival.

Population 3 presented resistance similar to that described for population 2 (Figure 5). Plants that did not develop grade 3 resistance symptoms were found. Plant 128 was of great interest and stood out among all the genotypes analyzed. This plant was characterized by late development of the infection period and a delayed symptom effect. Symptoms were occasionally observed at all the inoculated sites (Figure 7), reaching only grade 1, and no more advanced symptoms developed.

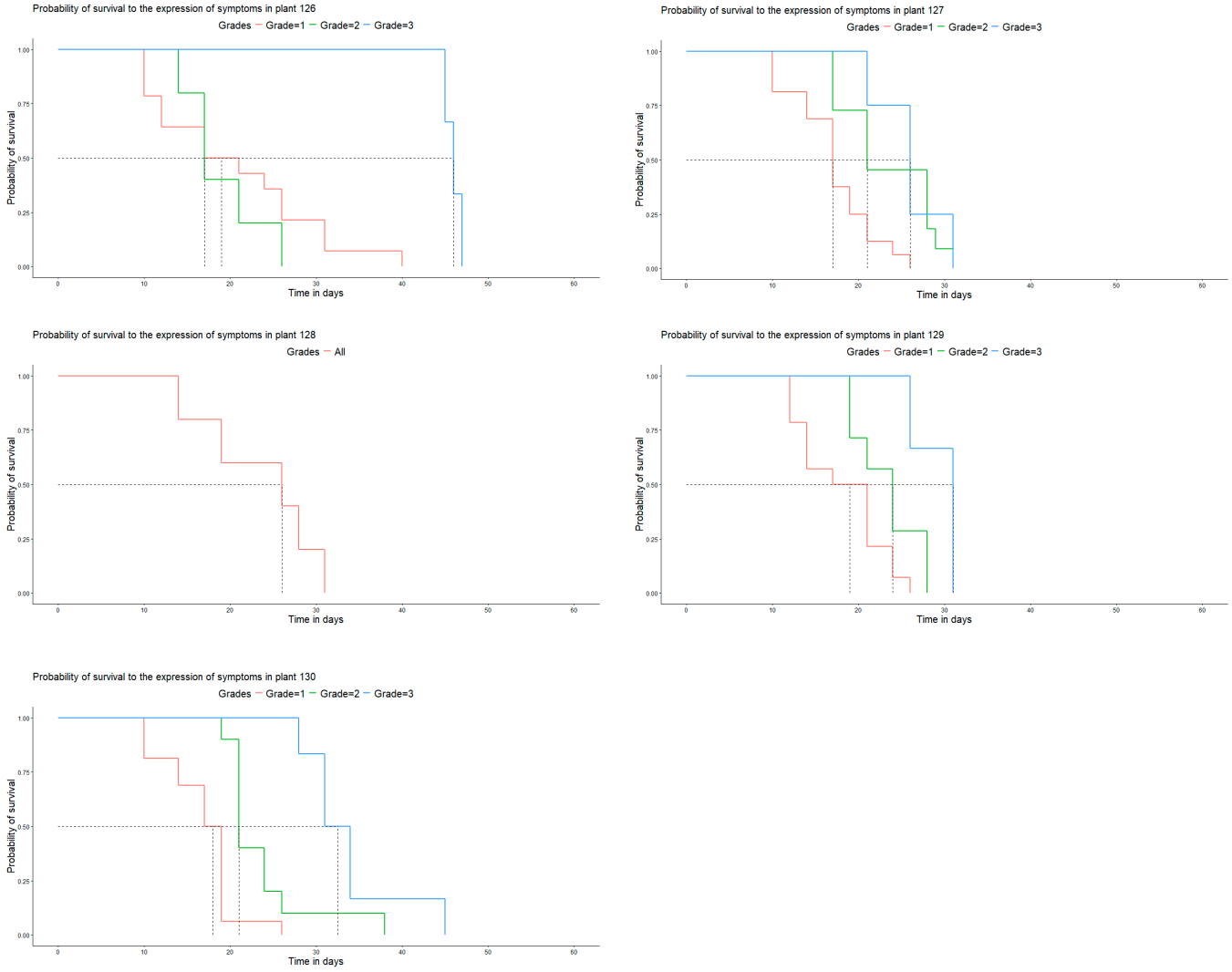

Figure 7. Days vs. probability of survival to CLR disease development in the genotypes of population 3 under laboratory conditions. Dashed lines: median number of days to reach each degree and median probability of survival.

No plants of the Caturra variety or the CX.2385 line survived until the development of the maximum degree of disease. In these genotypes, the disease developed and presented all its stages of development. Short durations (compared with those of the other genotypes) were the most likely to be present, indicating a greater risk of disease development in the first days after contact between the rust and the plant. The genotypes expressed all the symptoms of the disease in less than 60 days (Figure 8).

The controlled conditions in which the research was conducted guaranteed that all the evaluated genotypes presented an equal opportunity to be infected by the fungus. However, as the observation time increased, the probability that the disease did not develop on each genotype decreased. In the case of plants 65 and 128, a low probability of CLR developing during the infection period was observed ($p < 0.05$), without symptoms developing beyond

grade 2, an effect attributed to the genetic resistance that the plants presented the used inoculum (Figure 9). Likewise, the formation of chlorotic halos of variable size was observed, and in some cases swollen tissues were observed.

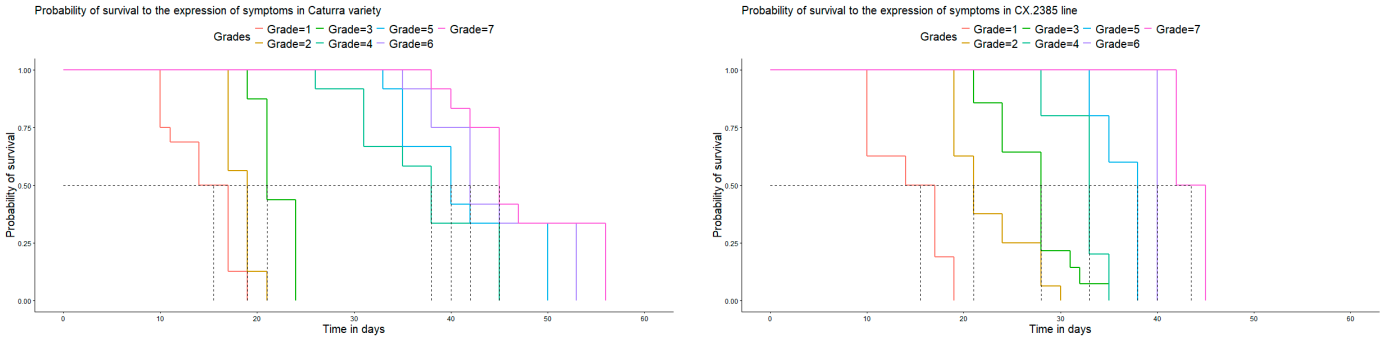

Figure 8. Days vs. probability of survival to the development of the CLR disease in the Caturra variety and CX.2385 line under laboratory conditions. Dashed lines: median number of days to reach each degree and median probability of survival.

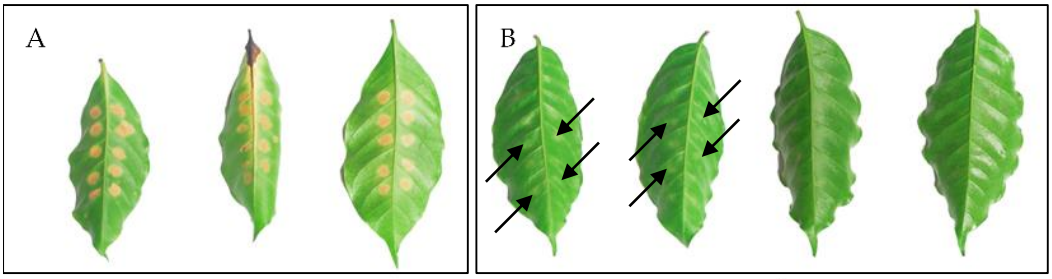

Figure 9. (**A**) Symptoms of Caturra variety susceptible to CLR and, (**B**) plants resistant to CLR selected at 60 DDI. The arrows indicate grade 2 lesions in resistant plants.

Regarding the controls, in the Caturra variety and CX.2385 line, the fungus developed spores, reaching the maximum degree according to the evaluation scale. The greatest effect was observed in the Caturra variety (Table 2).

4. Discussion

When the objective of a breeding program is to find resistant plants for limiting and potential diseases, it is necessary to evaluate large plant populations, such as the plants with favorable resistance alleles, which are are more likely to emerge. The size of the population will be a function of the number of loci that the breeders want to introgress into the same genotype, and the variation in the expression of the resistance components will be the result of the level of homozygosity of the resistance genes in the parents. In F1 plants, the absence of segregating patterns is expected when the resistance mechanisms are in a homozygous state. In contrast, the results obtained in this research suggest that CLR resistance segregates within the evaluated populations, a behavior that has been reported in hybrid populations developed from sources of *C. arabica* [4]. In the plants evaluated in this work, an apparent segregation by resistance was observed, expressed by longer periods required to develop a latency period, a lower density of sporulating lesions, and, in some cases, early necrosis of the lesions, which is consistent with other reports [4].

In *C. arabica*, the percentages of urediniospores that germinate and infect tissues are similar in resistant and susceptible plants [36]. However, in genotypes considered resistant, the plant responses occur a few days after the fungus begins the infection process. These reactions in the plant against the attack of pathogens are modulated by the expression of proteins that regulate defense responses, blockages in the formation of the appressorium,

mycelial growth, and modifications of the cell wall, affecting the synthesis of lignin in the cells that surround stomatal tissue and sometimes cytoplasmic tissue [37–40].

All this leads to the development of chlorosis, swelling of the tissues, and a reduction in the penetration of the fungus, making it difficult to establish, preventing colonization and reducing the reproduction of the pathogen on the tissue [36,41]. These resistance reactions generally occur because the defense mechanisms that plants deploy to attack pathogens involve quantitative and polygenic inheritance [40].

In this study, no plants immune to the pathogen were identified, that is, all of them presented some degree of disease. However, highly resistant plants with variable phenotypic responses, including the development of chlorotic zones of different intensities and swelling, were identified (plants 65 and 128). These symptoms are frequently observed in the *Rubiaceae* family, where tissue deformation occurs at the site of infection, accompanied by chlorotic regions, and these symptoms are considered reactions that occur in incomplete resistance responses of coffee to *H. vastatrix* [42]. In resistant genotypes of *Coffea*, during infection by *H. vastatrix*, the cells of the mesophyll develop hypertrophy as an effect of the activation of secondary metabolites, accumulation of phenolic components, and thickening of the cell wall [36]. These responses are the product of the activation of the metabolic pathway of shikimic acid, which is the same pathway that yields the biochemical compounds that lignin is made from. Lignin is responsible for the thickening of cell walls, the first barrier of plants in defense against the attack of phytopathogens.

These variations in resistance, to a large extent, are related to different factors, including environmental factors [9–11,16], genetic factors [8,24], and the product of interactions between them [18–22]. Therefore, to decrease the effects of interactions between genotype and the environment, this work was carried out under controlled conditions.

When any of the parents are not homozygous, a segregation of hybrid populations occurs, in which case resistance and susceptibility are not the absolute attributes of the plant. In contrast, they are characterized by compatible or incompatible specific interactions between the host, its resistance mechanisms, the pathogen, and its virulence mechanisms [43]. In the case of the resistance conferred by genes from *C. arabica SH1*, *SH2*, and *SH4*, its resistance capacity is regulated by its level of homozygosity and interaction with other resistance genes present in the plant [3,44].

The resistance reactions we observed show that the combination of the *SH1* gene with the resistance genes derived from HdT can have a genetic control effect on the pathotype used, allowing the expression of various phenotypic resistance reactions and the apparent additive action of genes. Therefore, it is possible to phenotypically select resistant plants, facilitating the combination of resistance alleles in a genotype and providing an efficient barrier against the pathogen [40]. Additionally, in the HdT and its derived varieties, resistance is controlled by genes with independent segregation [45], allowing the genetic configurations of the progeny to be diverse in their reactions to disease [8].

In the three populations evaluated, some plants presented intermediate reactions in terms of intensity (grade 3), with a progressive increase in chlorotic tissue. This type of resistance reaction is most common in plants when they are affected by the rust causing fungi [41]. In plants classified as susceptible, the resistance response is delayed, allowing the development, growth, and sporulation of fungus [36], which is consistent with what was observed within the plants of the evaluated populations. In this research, late phenotypic defense responses were observed, but these responses allow the pathogen to affect tissues in different proportions. The level of resistance of the evaluated plants and the infectivity rates of the pathogen were decisive in each genotype to restrict the development of the fungus, and this plant-pathogen interaction caused variations in each genotype's risk per se of developing symptoms associated with the disease.

The survival curves allowed us to identify the probability and model the level of resistance of the plant to the development of *H. vastatrix*. What was observed was adjusted to what was expected in this type of experiment [28,31–33]. The symptoms observed in resistant plants are often preceded by the cessation of growth and colonization of

the pathogen [3,36], making this characteristic an indicator of its high genetic value and agronomic interest. We must bear in mind that when breeding and selection are carried out to seek genetic resistance against pathogens that limits crop production, yield reductions may occur [14,15]. Additionally, the relationship between genetic resistance to a pathogen and the production capacity of the plant will always be closely related to the effects of the environment and the genetic configuration of the varieties [8].

All plants presented incomplete resistance under field conditions, but two presented the lowest incidence of the disease under conditions of pressure from virulent races (Table 1). Therefore, a strict selection was carried out based on the incidence values under controlled conditions, and it was complemented with the values obtained under field conditions. The latter parameter (field evaluations) indisputably reflects the level of genetic resistance of the plant under conditions of high pressure from the inoculum to specific races.

In the selection process within any plant breeding program for the resistance to diseases, the selection parameters must always be as high as possible to reduce the future risk that the pathogen will pass through introgressed resistance mechanisms, which is a natural and inevitable process. Given this risk, the ideal strategy to mitigate the impacts of a possible loss of resistance is to make use of genetic diversity. Its uses and benefits are documented for different crops [46–48], and coffee is no exception. Its genetic diversity has yielded a wide spectrum of benefits, including economic, environmental, and social benefits, as has been demonstrated with the varieties released by the National Federation of Coffee Growers and its research center Cenicafé in Colombia [49].

Although the CX.2385 line exhibited a high CLR incidence in the laboratory, with a grade of 7 on the scale of growing lesions and a grade of 6 under field conditions [5,12], the severity of these lesions under cultivation conditions does not seem to have negative effects on production, confirming its high incomplete resistance to CLR. Contrasting findings were observed for the Caturra variety, which was highly susceptible to normal cultivation conditions in Colombia and whose production can be harmed at any time of the year, at any altitude, and in the absence of chemical control for the disease. Therefore, from the identified and selected plants, we will continue to obtain segregating populations and to advance them to further generations to fix and select progenies for the various characteristics of agronomic value, which will benefit Colombian coffee growers.

In this research, the agronomic management of the plants and the research conditions, temperature, humidity, evaluation times, and light intensity were the same for all the genotypes. This ruled out environmental effects or effects attributed to the physiological state of the plant [9,16]; therefore, the variations in the resistance response observed are the expression of resistance characteristics of the genotype and are undoubtedly related to the genetic background of the parents and the combinations of resistance factors in the progeny.

5. Conclusions

This research allowed us to identify hybrid plants (F1), which are highly resistant to CLR, and they have the potential to be utilized when looking for new sources of genetic resistance to CLR in Colombia. New evidence is presented of the phenotypic response of the combination of resistance factors from *C. arabica* and HdT, which have different resistance spectra that are expressed when the genes are not combined or are acting individually. The results obtained allow us to recommend the plant preselection methodology under controlled conditions, to quickly identify the genetic potential of resistant plants to *H. vastatrix*. In the case of Colombia, the costs associated with the process of registering variables essential for the selection of disease resistance—such as CLR, in coffee cultivation—can represent around 30% of the costs per cycle in research processes. The methodology described here contributes to the existing body of literature by reducing the number of plants that reach evaluations under field conditions and is crucial in the efficient use of economic resources in the selection and development of coffee varieties.

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Data Availability Statement: Restrictions apply to the dataset. The data sets presented in this article are not easily available because they were obtained with resources from the National Federation of Coffee Growers of Colombia and are part of the development program for *C. arabica* varieties with genetic resistance to CLR. Requests to access the data sets should be directed to the National Coffee Research Center—Cenicafé.

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