

Severity of Leaf Rust and Brown Eyespot in Genotypes of *Coffea arabica* L. Cultivated with High Plant Density

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Abstract

This study investigated the severity of leaf rust and brown eyespot in genotypes of *Coffea arabica* L. cultivated with high plant density in the region of Caparaó-ES. The experiment was conducted in a competition field, cultivated with high plant density (8333 plants per hectare), following a randomized block design, with 16 genotypes and four replications. The plants were evaluated during consecutive harvests to study two complete reproductive cycles (from 2010 to 2012). Data were obtained for the phenological stages of flowering, graining, maturation and vegetative rest of each cycle. The severity of leaf rust (*Hemileia vastratrix*) and brown eyespot (*Cercospora coffeicola*) was evaluated using descriptive scales. It was observed that the genotypes are able to keep a considerable level of resistance to the leaf rust and brown eyespot when cultivated with increased density. The genotypes presented variability regarding the severity of the leaf rust and brown eyespot, indicating the existence of differential levels of resistance between them. For cultivation with high plant density, the genotypes Katipó, Paraíso MG H419-1, H419-3-3-7-16-4-1-1, Araponga MG1, Catuaí Amarelo 24/137, Catiguá MG2, Sacramento MG1, Pau-Brasil MG1, Catiguá MG3, Oeiras MG 6851 and Tupi present higher level of resistance for leaf rust. In addition, the genotypes Paraíso MG H419-1, Catiguá MG2, Pau-Brasil MG1, Catiguá MG3, Oeiras MG 6851, Tupi, Catuaí IAC 44, Catuaí IAC 81 and Catuaí IAC 144 present higher level of resistance for brown eyespot.

Keywords

Arabica Coffee, *Hemileia vastratrix*, *Cercospora coffeicola*, Genetic Control, Resistance

1. Introduction

Coffee is one of the most valuable commodities traded in the world and Brazil is the world's largest producer, cultivating both of the main species of coffee. Among the species of coffee, the Arabica coffee (*Coffea arabica* L.) is the most widely cultivated in the world. In the State of Espírito Santo, the production of arabica coffee is concentrated predominantly in the mountain regions.

The exploration of genetic resistance in improved cultivars is one of the main strategies to manage plant diseases in most agricultural species. The existence of genotypes with different levels of resistance allows the selection for resistant cultivars, improving the costs of production through the reduction of the need for others technologies, such as the chemical control of diseases [1].

The coffee leaf rust is caused by the biotrophic fungus *Hemileia vastratrix* (Uredinales: Pucciniaceae). This disease occurs in every region where coffee is cultivated worldwide. The disease is disseminated mainly by the action of rain, to short distances, and wind, to larger distances. From the 40 races of leaf rust already described in the world, 12 are found in Brazil, but with the expansion of the areas covered by resistant cultivars, there is always the possibility of development of new races of the pathogen [2].

Symptoms of the leaf rust are easily identifiable, manifesting mainly in the lower surface of the leaves, where spots appears, formatting pustules covered by masses of uredospores that present yellow to orange coloration; chlorotic spots appears in the upper surface, corresponding to the borders of the pustules. Severe attacks may cause loss of leaves; but, in susceptible genotypes, one spot of leaf rust is enough to cause the fall of the infected leaf [3]. The leaf spots and loss of leaves cause reduction of the active photosynthetic area, compromising the metabolism. Occasionally, the disease can also cause symptoms in branches, fruits young sprouts, but those cases are not normally observed in open environment [4].

The leaf rust is considered one of the main phytosanitary problems for species of coffee, the disease causes, overall, 35% of production loss. The main strategy used to manage this disease is the chemical control, with protector or systemic fungicides, and the cultivation of resistant cultivars [2]. The expression of genetic resistance is observed by the presence of chlorotic or yellow spots without sporulation. The presence of small chlorotic spots with poor sporulation is also a sign of intermediate resistance [3].

The brown eyespot is one of the oldest phytosanitary problems in coffee plantations; it is caused by the fungus *Cercospora coffeicola* (Montiliales, Dematiaceae). Water and wind are responsible for disseminating the pathogen, and it is problematic mainly for Arabica coffee, especially in nurseries or in early stages at field, but the disease can affect coffee plants in all development stages [2]. The estimated losses caused by this disease reached 30% of the production for past years in high regions of the Espírito Santo state, Brazil, where this research was developed [3].

The brown eyespot can cause symptoms on leaves and fruits, being more common on the leaves. The symptoms on leaves are circular spots with coloration from gray to brown, frequently surrounded by a yellow halo. On fruits, the disease causes necrotic spots, of dark brown coloration, and may result in deformation of the grains. On both cases, the premature fall of leaves and fruits may occur [3].

The strategies to manage this disease are mainly based on cultural practices, since the higher severities are normally associated to nutritional deficiencies, excessive radiation and inadequate irrigation or cultivation density. The chemical treatment and the cultivation of resistant cultivars are also important strategies for the management of the brown eyespot [2].

Considering the importance of the mentioned phytosanitary problems for the cultivation of Arabica coffee, there is a need to identify improved cultivars that may be recommended for new scenarios of cultivation, considering the environmental changes caused by the addition of other technologies in the system and the interaction of these changes with the plant-pathogen relation.

The region of Caparaó, localized in the southern part of Espírito Santo State, southeast of Brazil, is traditionally producer of Arabica coffee and is economically dependent of this agricultural activity. Since the cultivation of coffee in this region is mainly done in the mountains and under familiar management, the cultivation with high density of plants per area is recommended to increase the crop yield and the efficiency of land use.

The objective of this study was to investigate the effect of high plant density on the severity of leaf rust and brown eyespot of genotypes of *Coffea arabica* L., in the region of Caparaó-ES.

2. Materials and Methods

2.1. Experimental Setup

The experiment was conducted in competition field, in a region where Arabica coffee is typically cultivated in

the district of Celina, municipality of Alegre, geographic coordinates 20°45'S and 41°33'W, in the region of Caparaó, southern state of Espírito Santo, southeast of Brazil. The area has an altitude of 690 m and presents an average annual temperature of 22°C and rainfall between 1300 - 1800 mm per year, with the rainy season from October to April and the dry season from May to September. The experiment followed a randomized block design with 16 genotypes, four replications and six plants per experimental plot.

The plants were spaced by 2.00 × 0.60 m, with a total of 8333 plants per hectare, configuring a cultivation with high plant density. The agricultural practices were established in accordance with those normally employed in the region according to their need and following the current recommendations for the cultivation of Arabica coffee in Brazil [5] [6].

2.2. Selection of Genotypes

Sixteen genotypes of *Coffea arabica* L. originated from the breeding programs of institutions that are references in the development of cultivars of Arabica coffee, such as Instituto Agronômico de Campinas (IAC), Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Fundação Pro-Café (MAPA/Pro-café) and Instituto Agronômico do Paraná (IAPAR), were used in the experiment.

The genotypes were selected according to their characteristics of high crop yield and quality, prioritizing genotypes with a short canopy and rust resistance, agronomic characteristics that are highly important for cultivation with increased plant density.

2.3. Evaluation

The plants were cultivated until the stabilization of their reproductive phenological cycle, being evaluated during consecutive harvests to study two complete cycles of the culture (from 2010 to 2012).

The periodic evaluations of the severity of the diseases were performed in each phenological stage of the plants. Data were obtained for the stages of flowering (F), graining (G), maturation (M) and vegetative rest (R) of two cycles (F1, G1, M1, R1 for the first cycle and F2, G2, M2, R2 for the second cycle). The evaluations started on the stage of flower induction from the previous year (I0) and ended at the stage of flowering of the sequential cycle (F3).

The severity of each disease was evaluated using descriptive scales, establishing different levels of severity between score 1, corresponding to the absence of injury, and score 9, indicating a large number of affected leaves or diseased branches, with significant leaf fall. The severity in each period were used to generate a graphic of evolution of each disease and the area under the disease progress curve (AUDPC) were calculated and used to compare the level of resistance of the genotypes [7].

2.4. Data Analysis

The collected data were subjected to an analysis of variance using the F-test at 1% and 5% probabilities in order to identify the characteristics that can be used to differentiate between groups of genotypes with different behaviors. For those characteristics, the genetic parameters were estimated based on the model $Y_{ij} = \mu + g_i + b_j + \epsilon_{ij}$, considering the effect of genotypes as fixed. Each plant were subject to evaluation as a whole and the data for the experimental plot were calculated as the overall mean of the six plants. The means were studied using the Scott-Knott criteria at 5% of probability. The analyses were performed using the statistical software GENES [8].

3. Results and Discussion

The severity of leaf rust in the genotypes of Arabica coffee is presented in **Figure 1**, where, in general, low values of severity were observed, which is attributed to the level of resistance that those genotypes were able to express, even under conditions of high plant population per area.

Despite the low levels of severity for leaf rust, some similarity exists between most of the genotypes regarding the period when the infection increases. In many genotypes, the severity increases by the end of the first reproductive cycle, after the last phenological stage of the first cycle (R1), presenting higher values in the start of the second cycle. This fact occurred because the production of fruits and harvest of the previous cycle weakened the plants, since the fruits are strong drains for water and nutrients; it is natural that the vigor and resistance of the plants is negatively affected. Furthermore, this period also coincides with the period of year when the lower

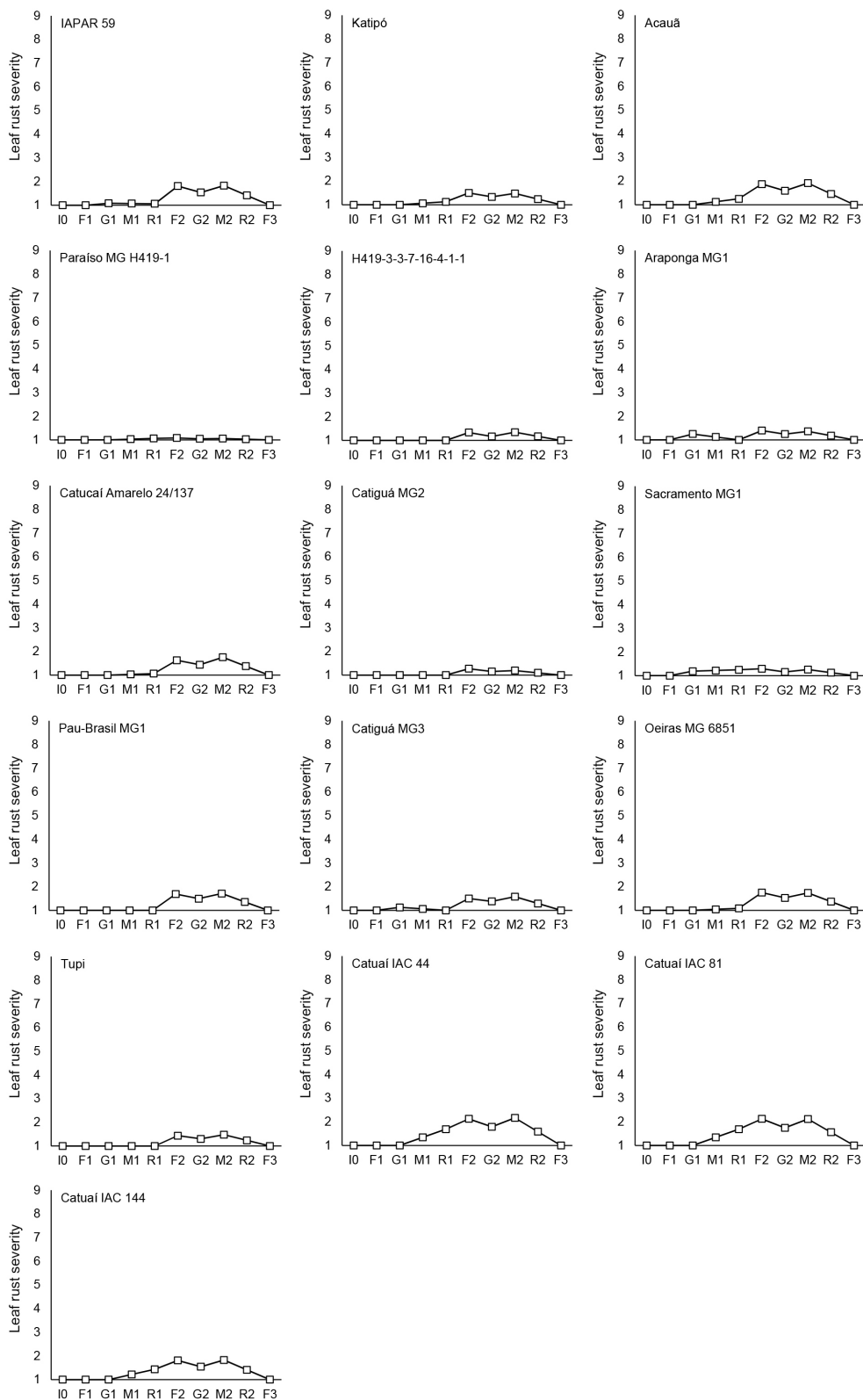


Figure 1. Severity of leaf rust (*Hemileia vastatrix*) in 16 genotypes of *Coffea arabica* L. along two harvests (I0: floral induction of previous cycle; F: flowering; G: granation; M: maturation; R: vegetative rest; and 1, 2 and 3 for first, second and third reproductive cycles).

temperature combined with the density of cultivation may form a favorable microclimate for the disease (**Figure 1**). The means for severity of brown eyespot also did not exceed the severity level 3 (**Figure 2**), which means that

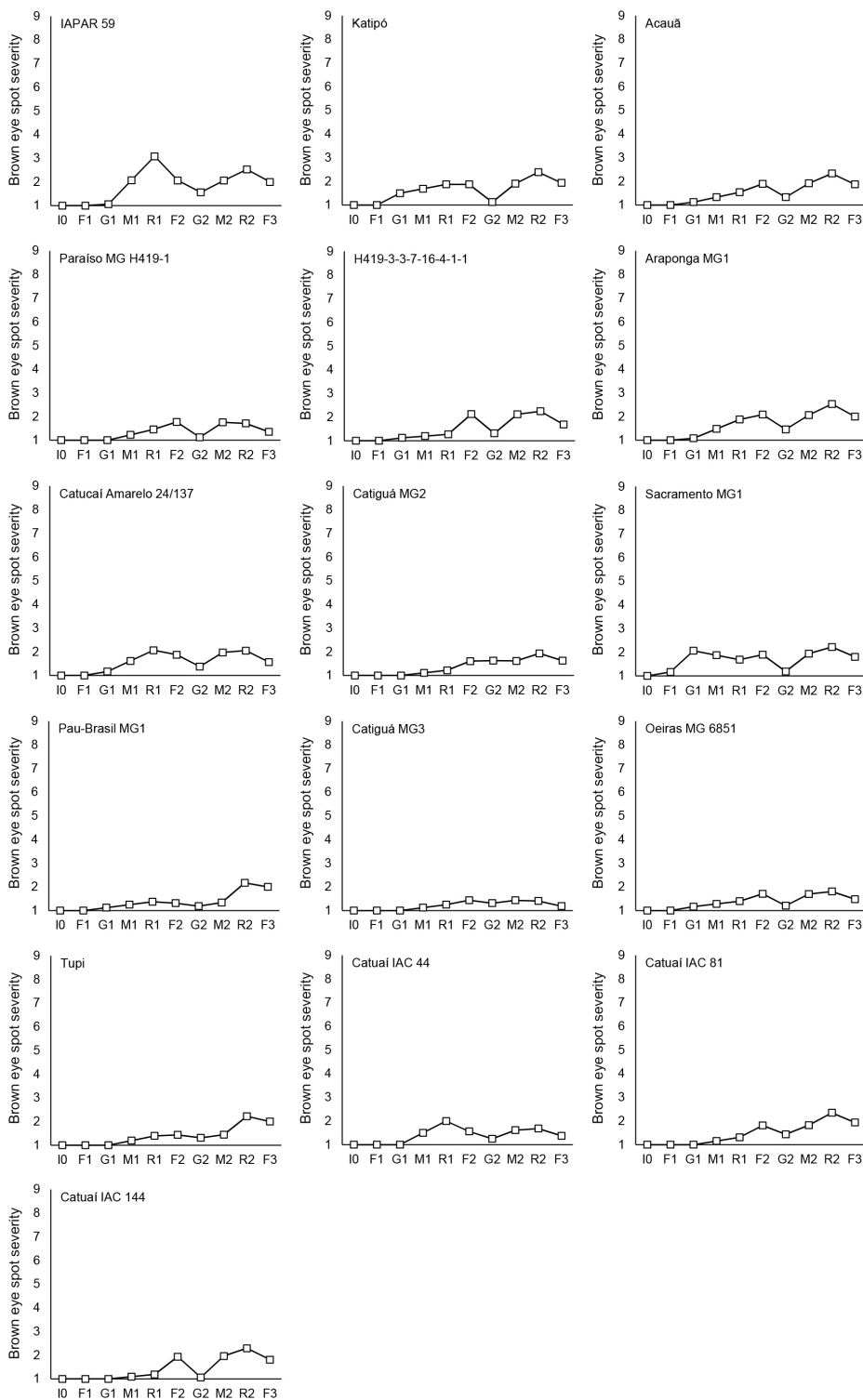


Figure 2. Severity of brown eyespot (*Cercospora coffeicola*) in 16 genotypes of *Coffea arabica* L. along two harvests (I0: floral induction of previous cycle; F: flowering; G: granation; M: maturation; R: vegetative rest; and 1, 2 and 3 for first, second and third reproductive cycles).

those genotypes had low levels of severity in conditions of high density of cultivation. The moment of higher severity also occurred in the phenological stages where the plant were recovering from the higher nutrient and hydric demands, after the periods of flowering and grain formation, when there is the presence of stronger metabolic drains in the plant of coffee.

For both plant diseases, even under conditions of high plant population per area, the genotypes still maintained a high level of resistance (Figure 1 and Figure 2).

Others authors have also observed variability regarding the severity of coffee leaf rust in genotypes of *Coffea arabica*, even when cultivated with the same conditions, indicating the possibility of classifying genotypes by the level of severity as resistant or susceptible [9]. The resistance to leaf rust in coffee is controlled by S_H genes of major effect, with the possible involvement of genes of smaller effect as well [10] [11]. The study of the action of such genes and the expression of the level of resistance that they confer are valuable tools for the sustainable management of plant diseases.

It is being reported that, in competition field of genotypes of Arabica coffee, some cultivars showed complete resistance to leaf rust, while others lost their resistance and became susceptible depending on the environmental conditions [12]. In addition, for other plant diseases that cause leaf spots, there are reports showing lower severity in genotypes that carry genes that confer tolerance to foliar diseases. Physiological characteristics could play an important role in the determination of resistance for leaf spots, allowing the selection for resistance by choosing plants with smaller number of lesions and less defoliation [13].

The severity of the brown eyespot is correlated to the nutritional status, where higher levels of damage caused by the disease are likely to be found in plants with nutritional deficiency. It was observed that increasing levels of potassium and calcium had positive effects to manage the severity of brown eyespot in coffee plants [14].

The mean square related to the genotypes was statistically significant for both variables. The mean for the AUDPC for the brown eyespot was higher than the mean for the leaf spot, showing that the first disease may have caused damage for the plants, resulting in higher levels of severity along the cycles. The AUDPC for leaf rust ranged from 832.79 to 1433.03, while the AUDPC for brown eyespot ranged from 946.83 to 1668.92 (Table 1).

The estimate values of genotypic variances ($\hat{\sigma}_g^2$) were higher than the values of environmental variance ($\hat{\sigma}_e^2$) for both AUDPC, therefore, the phenotypic variance ($\hat{\sigma}_p^2$) observed between the genotypes can be associated, in the most part, to the effect of their genetic differences, with a relatively smaller influence of environmental factors over the variance (Table 1).

Table 1. Genotypic mean squares (MS_g); means; minimums; maximums; estimate of phenotypic ($\hat{\sigma}_p$), genotypic ($\hat{\sigma}_g$) and environmental ($\hat{\sigma}_e$) variances; coefficients of genotypic determination (H^2), coefficients of genetic variation (CV_g) and variation index (CV_g/CV_e) of the areas under the disease progress curve for leaf rust and brown eyespot of 16 genotypes of *Coffea arabica* L.

Parameter	Leaf rust	Brown eyespot
MS_g	50636.81*	65839.90**
Mean	1053.10	1244.03
Minimum	832.79	946.83
Maximum	1433.03	1668.92
$\hat{\sigma}_p^2$	12659.20	16459.97
$\hat{\sigma}_g^2$	7457.33	11968.51
$\hat{\sigma}_e^2$	5201.86	4491.45
H^2	58.90	72.71
$\hat{\rho}$	26.38	39.98
CV_g (%)	8.20	8.79
CV_g/CV_e	0.59	0.81

*Significant at 5% and **Significant at 1% of probability, by the F test.

Considering the coefficient of genotypic determination (H^2), the AUDPC for brown eyespot presented high values (72.71%) than the AUDPC for leaf rust (58.90%). The magnitude of these estimates indicates that the resistance for brown eyespot may be less influenced by environmental factors than the resistance for leaf rust [15].

The coefficients of genetic variation (CV_g) were relatively low when compared to experimental CV, resulting in variation indexes (CV_g/CV_e) of 0.59 for leaf rust and 0.81 for brown eyespot. This fact indicates favorability to selection of genotypes to improve the resistance for brown eyespot in the conditions of this experiment [15].

The means of AUCPD of leaf rust and brown eyespot for each genotype is presented in **Table 2**. The criteria of Scott-Knott allowed to identify two groups of homogeneous behavior for each disease.

The AUDPC for each disease led to the identification of two groups of genotypes. For leaf rust, the genotypes Katipó, Paraíso MG H419-1, H419-3-3-7-16-4-1-1, Araponga MG1, Catucaí Amarelo 24/137, Catiguá MG2, Sacramento MG1, Pau-Brasil MG1, Catiguá MG3, Oeiras MG 6851 and Tupi showed smaller means, indicating that the level of resistance of these genotypes is higher in comparison with others. For the brown eyespot, the genotypes Paraíso MG H419-1, Catiguá MG2, Pau-Brasil MG1, Catiguá MG3, Oeiras MG 6851, Tupi, Catuaí IAC 44, Catuaí IAC 81 and Catuaí IAC 144 presented smaller AUDPC, which also indicates greater resistance. The other genotypes had lower level of resistance for each disease (**Table 2**).

The cultivation with higher density promotes the formation of a microclimate in the coffee crop, which can be favorable to the development of fungal diseases, since the relative humidity tends to be higher in the system, the light inside the canopy tends to be less intense and the temperature tends to be lower. However, even under such conditions, the severity of the leaf rust and brown eyespot in those genotypes of Arabica coffee were not high enough to reach control levels. In addition, it is possible to observe different levels of resistance among the genotypes, as shown in **Table 2**, allowing the selection of those with higher resistance to be recommended for cultivation with high density in regions where those coffee diseases are more problematic.

Others studies have reported that cultivation with higher density have been favoring the leaf rust, causing higher severity than in spaced cultivations. These studies also alerts that a careful selection is required to recommend cultivars for the dense systems, reaffirming the importance of the results obtained in the present study [16]. As for the brown eyespot, there are studies reporting that the increase of cultivation density reduced the incidence and severity of this disease in susceptible genotypes of Arabica coffee [17].

Table 2. Means of areas under the disease progress curve for leaf rust (*Hemileia vastratrix*) and brown eyespot (*Cercospora coffeicola*) of 16 genotypes of *Coffea arabica* L.

Genotype	Area under the disease progress curve	
	Leaf rust	Brown eyespot
IAPAR 59	1107.09 a	1534.05 a
Katipó	1008.40 b	1348.17 a
Acauã	1142.48 a	1279.23 a
Paraíso MG H419-1	877.77 b	1132.80 b
H419-3-3-7-16-4-1-1	943.61 b	1267.71 a
Araponga MG1	988.81 b	1379.83 a
Catucaí Amarelo 24/137	1059.88 b	1318.83 a
Catiguá MG2	915.61 b	1155.63 b
Sacramento MG1	980.08 b	1405.64 a
Pau-Brasil MG1	1057.14 b	1129.66 b
Catiguá MG3	1026.21 b	1033.64 b
Oeiras MG 6851	1080.16 b	1155.59 b
Tupi	984.91 b	1155.10 b
Catuaí IAC 44	1272.51 a	1178.44 b
Catuaí IAC 81	1261.39 a	1234.43 b
Catuaí IAC 144	1143.62 a	1195.73 b

Means followed by the same letter in each column do not differ by the Scott-Knott test at 5% of probability.

4. Conclusions

The genotypes used in this experiment are able to keep a considerable level of resistance to the leaf rust and brown eyespot when cultivated with increased density.

The genotypes of *Coffea arabica* L. studied in this experiment present variability regarding the severity of the leaf rust and brown eyespot, indicating the existence of differential levels of resistance between those genotypes.

For cultivation with high plant density, the genotypes Katipó, Paraíso MG H419-1, H419-3-3-7-16-4-1-1, Araponga MG1, Catucaí Amarelo 24/137, Catiguá MG2, Sacramento MG1, Pau-Brasil MG1, Catiguá MG3, Oeiras MG 6851 and Tupi present higher level of resistance for leaf rust.

For cultivation with high plant density, the genotypes Paraíso MG H419-1, Catiguá MG2, Pau-Brasil MG1, Catiguá MG3, Oeiras MG 6851, Tupi, Catucaí IAC 44, Catucaí IAC 81 and Catucaí IAC 144 present higher level of resistance for brown eyespot.

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