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Modeling the brown eye spot sampling in Arabica coffee

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***Abstract*— *Coffee production has a great socioeconomic importance for Brazil. It generates direct and indirect jobs, and foreign exchange, with Brazilian Arabica coffee production estimated between 42 - 46 million bags (60 kg) in 2020. It is the main agribusiness activity in the State of Espírito Santo, Brazil with expected production between 13 - 15 million bags, and around 30% of this production is Arabica coffee. Technologies are recommended to coffee growers to increase yield, and production of specialty coffees on sustainable properties. Among the principles of integrated management is the monitoring of pests and diseases to determine the level of pest control. The estimate of the number of leaves to be sampled in the monitoring becomes an important tool to increase the accuracy of the obtained information. This research was carried out aiming to determine the minimum number of leaves necessary to evaluate the infestation of brown eye spot (BES) of coffee in Arabica coffee (Coffea arabica L.) without affecting the accuracy of the collection method. It was observed that the estimate of the minimum number for sampling was 46 leaves for the characteristics of incidence, and severity of BES in Arabica coffee. The modeling applied in this study allows to conclude that it is possible to recommend an optimum number of Arabica coffee leaves for these edaphoclimatic conditions, and variety, and it can* *serve as a basis for monitoring in an integrated pest and disease management program in Arabica coffee.*

I. INTRODUCTION

Coffee growing has great socioeconomic importance for Brazil, generating direct and indirect jobs, and foreign exchange. Between 57.15 - 62.02 million bags of processed coffee (60 kg) will be harvested, and it is estimated that between 42.20 - 45.98 million bags are Arabica coffee. Coffee is the main agribusiness activity in the State of Espírito Santo, Brazil with production expected to be between 13.02 - 15.44 million bags of processed coffee, with around 30.84% of Arabica coffee (CONAB, 2020).

A set of technologies is recommended for the production of specialty coffees on sustainable properties, aiming at the increasing of coffee growing in this Brazilian state. The choice of the variety with the production of inspected seedlings, correct implantation of crops, adequate nutrition, use of techniques for the integrated management of pests, diseases and soil conservation, good harvesting and post-harvesting practices is essential for obtaining special coffees. Biotic and abiotic factors can lead to reductions in coffee production with significant losses in beverage, productivity, and particularly in quality. Among these factor we may find the brown eye spot (BES) of coffee caused by *Mycosphaerella* (or *Cercospora*) *coffeicola* (Cooke) J.A.Stev. & Wellman (1944) that can attack coffee leaves, and fruits (Santos et al., 2008; Souza et al., 2015).

Integrated pest and disease management is one of the tools to be used to reduce the use of pesticides, minimize their impact on the environment, and increase the efficiency of pest and disease control. Monitoring of pests and diseases is one of the principles of integrated pest management. However, it is observed that there are several recommendations regarding the ideal number of leaves to be collected in different processes in conducting the coffee culture. Malavolta (1980) recommends sampling 100 leaves for the assessment of nutritional status, while Silva and Miranda (2016) recommend the collection of 100 to 200 leaves for monitoring diseases.

Several similar methodologies can be used to determine the optimal number of data to evaluate characteristics in order to leave the empirical method aside, such as Guarçoni et al. (2020) who determined the optimal size of plants per experimental plot to evaluate agronomic, and sensory characteristics of arabica coffee. Also, Guarçoni et al. (2017) determined the experimental plot size to evaluate agronomic characteristics of cabbage 'F1 Shinsei Hybrid'. Both surveys used simulation, and the linear plateau response model method, meanwhile Guarçoni et al. (2017) also used the maximum curvature method. Other works have also used this estimation, and simulation methods to determine the optimal number of experimental plot plants, such as for the pineapple 'Vitória' using the linear plateau response model and maximum curvature (LEONARDO et al., 2014). Guimarães et al. (2019) determined the ideal experimental plot size for the cactus 'Pera Gigante', and Pereira et al. (2018) determined the minimum number of Q-Graders, and R-Graders for sensory evaluation of Arabica and Conilon coffees.

These methods use blank or uniformity tests, where only one variety is planted, and receiving the same cultivation practices. Through the visual inspection method of the maximum curvature, the coefficients of variation CV (X) are calculated for each plot size X where V (X) is the variance of the plots with X basic units (UB) and X´ is the average. The set of points obtained from the pairs [X, CV (X)] are joined forming a curve where the point of maximum curvature is determined by visual inspection, and considering the value of the point's abscissa as the optimal size of the plot (Le Clerg, 1967).

The maximum curvature is a simple and easy method to be used. However, the fact of the visually determining the optimal size of the experimental plot may constitute a source of error because there is no criterion to identify the maximum curvature point on the curve (Paranaiba et al., 2009). The method of visual inspection of the maximum curvature was improved, and the optimal size of the X_OP plot was determined algebraically. This method was used to estimate the optimal size of experimental plots of single, double, and triple corn hybrids, where a function like $CV_X = \frac{A}{X^B}$was established to explain the relationship between the variation coefficient ($CV_{(X)}$), and optimal size of the experimental plot (X); and then the size of the experimental plot was determined algebraically (Cargnelutti Filho et al., 2011). The linear plateau response model method was used to estimate the parameters, with the optimal size of the experimental plot obtained when the linear model turns into a plateau (Guarçoni et al., 2020).

Determination of the minimum number of leaves necessary to evaluate pests and diseases in Arabica coffee is important because if the number of leaves is less than necessary, less accurate estimates will be obtained. On the other hand, if excessive number of leaves is used, more time and resources will be spent than necessary. Thus, this determination is an important tool to increase the accuracy of the information obtained, and to optimize the cost/benefit ratio of the labor used. The objective of this work was to determine the minimum number of leaves that can be collected for assessing the BESin Arabica coffee, without reducing the sampling reliability.

II. MATERIAL AND METHODS

This research was carried out at the Experimental Farm of Venda Nova (FEVN) (20°222’59”S; 41°11’08”W, 736 m altitude), located in the municipality of Venda Nova do Imigrante, and atthe Experimental Farm Mendes da Fonseca (FEMF) (20°22’04”S; 41°03’42”W, 941 m altitude), located in the municipality of Domingos Martins, State of Espírito Santo, Brazil in a competition experiment for selection of Arabica coffee cultivars with 6-years-old, and spaced 2,5 x 1,0 m. The coffee crop has been conducted in a traditional cultivation system using Good Agricultural Practices. All cultural treats were carried out based on sustainable production techniques for Arabica coffee (Alixandre et al., 2020). Fertilization, and soil correction have been carried out according to the results of soil analysis; liming was carried out in June, and fertilization from October to March (Prezotti, 2016).

Sample Preparation

Each sample consisted of one hundred leaves collected at random from the 3rd and 4th pairs of leaves (Huerta 1963), in the median part of the canopy of the five central plants of the experimental plot of the Arabica coffee cultivar Obatã, and 20 leaves were collected from each plant (Malavolta, 1980; Barbosa Junior et al., 2019). Sampling was conducted in the beginning of September. The leaves were taken to the Entomology/Phytopathology laboratory of the Capixaba Institute for Research, Technical Assistance and Rural Extension (Incaper), and were individually analyzed for the incidence, and severity of the BES. The incidence was evaluated observing the presence of the symptoms of the BES.

Incidence (I) of BES was calculated according to the following equation:

I (%) = (NFD/NFT)∗100, where:

I = incidence (%);

NFD = number of diseased leaves;

NFT = total number of leaves.

For severity, the incidence was estimated by the number of symptoms per leaf, dividing this value by the number of infected leaves, and expressed in average of symptoms per infected leaf (Ribeiro et al., 1981; Cardoso et al., 2016; Lima et al., 2018).

Statistical analysis

Bootstrap method (Mammen and Sandi, 2012) was used aiming at a greater consistency of the regression methods in obtaining the optimal size of the experimental plot. This method consists of a statistical resampling technique that established a new framework for statistical analysis based on simulation. The linear plateau response model was used to determine the optimal number of leaves to assess the incidence, and severity of the BESin Arabica coffee. The bootstrap method was used to group the different number of leaves, and their respective variation coefficients, and 1,000 sample simulations were performed with 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 leaves (Leonardo et al., 2014; Guarçoni et al., 2020).

The groupings of the pairs [X, CV (X)] were used to estimate the parameters of the linear plateau response model. For this method, the optimal size of the number of leaves occurs when the linear model becomes a plateau (equation 1):

$$Y_i = \begin{cases} \beta_0 + \beta_1 X_i + \varepsilon_i se X_i \leq X_0 \\ P + \varepsilon_i se X_i > X_0 \end{cases} (equation\ 1),$$

where, Yi is the response variable;β_0 is the linear coefficient of the linear model of the segment prior to the plateau; β_1 the slope of this same segment; $\varepsilon_i$is the error associated with ith observation; P is the plateau, and X_0 is the connection point of the two segments. P and X_0 are parameters to be estimated.

The regression models were tested by the F test, and the angular coefficients by the t test. The software 'R' was used to carry out the simulations of the bootstrap process (R CORE TEAM, 2021), and SAEG program was used to obtain the statistics of the methods for obtaining the optimal plot size (Ribeiro Júnior and Melo, 2008).

III. RESULTS AND DISCUSSION

BESseverities obtained were 0.38 (FEVN), and 0.66 (FEMF) lesions/leaf. The coefficient of variation of the characteristics depending on the number of leaves decreases up to 50 leaves. From this point on, the largest number of leaves sampled provides the least increase in sampling accuracy (Table 1).

The optimal number of leaves - Xof for incidence, and severity of the BESwere 46, and 45 leaves in the location of FEVN, and 46, and 43 leaves in FEMF(Figure 1), respectively, using the coefficient of variation of the infestation characteristics of the BES as a function of the number of plants, obtained from 1,000 sample simulations by the bootstrap method, with 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 leaves.

Methodologies for leaf diagnosis in coffee were developed to assess nutritional status, and there are differences between the authors on the number of leavesthatmust becollected for thisdiagnosis, and itcan varyfrom 2 to 144 leaves (Cintra, 2012).

Table 1. Grouping of different number of Arabica coffee leaves, and respective coefficients of variation to the brown eye spot incidence and severity in two locations (FEVN – 736m, and FEMF – 941 m), Brazil

Number of coffee leaves	Coefficient of variation– CV (%)			
	FEVN[1]		FEMF[2]	
	Incidence	Severity	Incidence	Severity
10	70.25558	79.97715	40.42603	50.86065
20	49.37637	56.57730	29.86538	34.10877
30	39.50008	44.18615	24.56249	29.24666
40	35.22965	40.30568	20.38460	22.72637
50	30.11190	34.37184	18.89769	21.92404
60	27.79280	32.25399	16.67411	19.42357
70	25.72629	29.51076	15.47767	19.02895
80	24.20590	27.07437	15.23577	17.48351
90	23.30275	26.43872	13.67379	16.59606
100	22.15214	25.29883	12.79388	15.39272

[1.]Experimental Farm Venda Nova; [2.]Experimental Farm Mendes da Fonseca.

Among these methodologies, the most used is the recommendation of 100 leaves sampled (Malavolta, 1980).Other diagnostic methodologies recommend sampling the 4th pair of leaves, from the apex of the branch of mature plants, and collecting 50 leaves at the beginning of flowering (Mills and Jones Junior, 1996), or collecting 200 leaves at the four cardinal points, in the 3rd pair from the apex of the branches, at the median plant height (Raij, 2011). This methodology was also recommended to evaluate the incidence of the rust in Conilon coffee (Ventura et al., 2017).

These diagnostic sampling methods have also been used as a basis for monitoring coffee diseases (Silva and Miranda, 2016). However, there are no studies on the number of leaves collected for diagnosis of the BESin Arabica coffee. Results obtained in this research suggest that intensity, and severity of this disease can be evaluated using circa 46 leaves in each experimental plot. This shows that the standard sampling of 100 to 200 leaves (Fornazier et al., 1995, 2017, 2019; Silva and Miranda, 2016) does not provide an increase in the accuracy of the results, but more time, human labor, and financial resources will be spent. However, a number less than 46 leaves per experimental plot may compromise the accuracy in the evaluation of this disease in Arabica coffee.

Other samples have been carried out in other Arabica coffee cultivars in order to establish scientific criteria for sampling of the BESin experimental units under conditions of low, and high incidence. Also, work has been carried out aiming at evaluating rust and the coffee leaf miner, important pests in coffee growing. These data will make it possible to determine the ideal number of leaves to be sampled for joint monitoring of coffee diseases, and pests.

IV. CONCLUSIONS

- The minimum number of leaves required to sample the incidence and severity of the brown eye spot in Arabica coffee is 46.
- The number of leaves sampled can be reduced by approximately 50%, reducing the effort, and the sampling cost.
- Works to determine the number of leaves needed for sampling may increase the efficiency of monitoring other diseases and pests in coffee crop.

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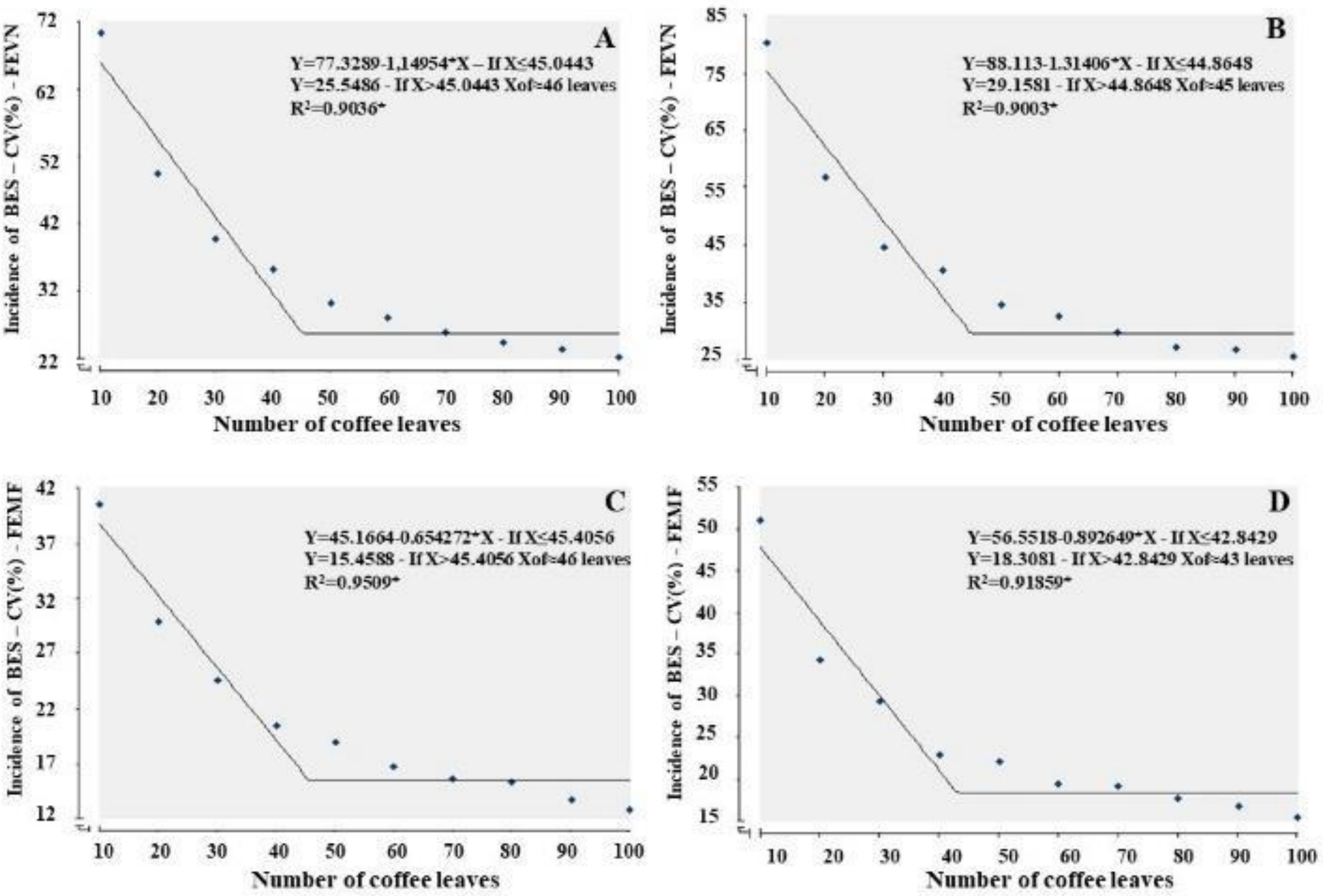

*Fig.1 – Relationship between the coefficient of variation and plot size using the linear plateau response model for the incidence (A) and severity (B)at FEVN, and incidence (C) and severity (D)at FEMF of the brown eye spot in Arabica coffee cv. Obatã. * and ** = significant, respectively, at 5%, and 1% of probability, by the F and t tests; ns = not significant.*

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