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Diagrammatic scale for blister spot in leaves of coffee tree

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Blister Spot is a disease transmitted through seeds. This characteristic of the disease reveals the studies of severity can offer more replies than the studies of incidence. Due to the inexistence of standardized for visual quantification of the Blister Spot's severity, the aim of this work was to build up and validate a diagrammatic scale for evaluating the severity of Blister Spot in coffee tree. Leaves in the field were collected with different intensities of symptoms disease, and electronically determined the real severity. Based on the frequency distribution of the severity values, and according to the law of visual stimulus of Weber-Fechner, the maximum and minimum limits, and the intermediate levels of the scale were defined. The validation was realized by eight evaluators that estimated the severity in 50 leaves with different intensities of symptoms. An evaluation without the aid of the diagrammatic scale was realized, and two others with its use, having intervals of seven days. The accuracy, precision, repeatability and reproducibility of the estimate were evaluated. The developed scale shows seven 0 (0%), 1 (0.1-3%), 2 (3.1-6%), 3 (6.1-12%), 4 (12.1-25%) and5 (≥25.1%). Using the scale proposed, the evaluators presented better levels of accurancy, precision, reproducibility and repeatability in the estimate, once compared to the evaluators who did not use the diagrammatic scale. The diagrammatic scale was adjusted to aid in the visual estimate of the severity of the Blister Spot in coffee leaves

Key words: *Coffea arabica*, *Colletotrichum gloeosporioides,* pathometry.

INTRODUCTION

The Blister Spot is a disease of the coffee tree, whose causal agent found in Brazil, is the fungus *Colletotrichum gloeosporioides* PENZ. (Miranda, 2003). Its symptoms are light green spots with less brightness in relation to asymptomatic areas, having oily looking, ranging from 2 to 10 mm diameter. With the progress of the disease, the spots show the necrotic, which can coalesce and result in necrosis of bigger areas and leaves fall. Besides these symptoms, the pathogenic can be associated with the mummifications and fruits' abscision and the wilt and dry of the branch (Pozza et al., 2010). The season of higher intensity of these symptoms is between October and February, period of higher rainfall index (Ferreira et al., 2009a).

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To quantify the intensity of the symptoms described, aiming to select resistant cultivars, evaluate the efficiency of fungicides, study epidemiology and the management of disease, the researchers have used different methods. Studying the resistance of coffee *Colletotrichum* spp., Ferreira et al. (2005) evaluated the incidence of infected fruits, instead of measuring the severity of the disease. In another work, Ferreira et al. (2009a) have already used the counting number of injury in the leaves in order to evaluate the progress of the disease in different treatments with fungicides, although this methodology is laborious and a little precise to measure the severity of the disease. Moreover, in many cases, two smaller injuries can occupy a smaller area of the leaf when comparing to a large one, generating errors (Madden et al., 2007). In another attempt, instead of measuring the severity of the disease, Ferreira et al. (2009b) evaluated the incidence of Blister Spot to estimate its progress in the field, although this variable, depending on the aims of the work, can not provide the necessary precision and accurancy to represent the extension of the injured foliar area (Campbell and Madden, 1990). The way to estimate the severity of the disease in a more accurate way, precise and fast, is through the use of diagrammatic scales (Nutter Jr et al., 2006). Some diagrammatic scales have been developed to assess disease in coffee (Belan et al., 2014; Capucho et al., 2011; Custódio et al., 2011; Salgado et al., 2009).

To elaborate the diagrammatic scales, important aspects should be considered, such as checking out if the upper limit of the scale corresponds to the maximum real of disease observed, determine if the real intensity of disease and its representation in the scale are precise and if the subdivisions of the scale follow through on the law of Weber-Fechner. According to this law, the human visual acuity is proportional to the logarithm of the stimulus intensity (Horsfall and Barratt, 1945). Furthermore, in order to verify the quality of the estimate obtained from the diagrammatic scale, the levels of accuracy, precision and reproducibility have to be validated (Campbell and Madden, 1990, Nutter Jr et al., 1993; Nutter Jr and Schultz, 1995).

Likewise, the objective of this work was to develop and validate a diagrammatic scale to evaluate Blister Spot's severity in coffee tree.

MATERIALS AND METHODS

In order to develop the diagrammatic scale, 120 coffee leaves with different severity levels of the disease were collected. These leaves were from coffee trees naturally infected by *C. gloeosporioides*, in farming of different cultivars. To onfirm the causal agent of the disease, the isolation in malt extraction MEA at 2% was realized, and the identity was verified through morphology analysis in optical microscope. The injured leaves were scanned in multifunctional printer. Having the help of software Assess® (American Phytopathological Society, St. Paul, MN, USA), the real severity of the disease was determined (percentage of injured foliar area).

Pale green spots were considered foliar injury, as well as necrotic scores inside these spots.

Considering the minimum and maximum levels of severity observed, the frequency distribution of data, in other words, how many leaves per interval of percentage of injured foliar area, to determine the most frequent intervals of the injures. Based on the law of visual acuity of Weber-Fechner (Horsfall and Barratt, 1945; Nutter Jr and Schultz, 1995), and in the intervals of class with higher frequency (Belan et al., 2014), the diagrammatic scale was developed. After establishing the intervals of severity to be represented, and considering the way and the distribution of the injuries, real images of leaves with injuries of Blister Spot were used to develop a scale.

In the validation test of the diagrammatic scale, 50 images of coffee leaves with symptoms of the disease, representing all the levels of the scale were used. These images were randomly inserted in individual slides for visualizing in Microsoft® PowerPoint® 2010, and presented to eight evaluators without experience in quantification of plant diseases, in three evaluations, with interval of seven days.

In the first evaluation, the evaluators scored the leaves presented without any help of the scale. After seven days, the same evaluators realized the second one, using the diagrammatic scale for the first time. To evaluate the repeatability of the estimates with the diagrammatic scale, seven days after the first evaluation, a new sequence of the same leaves was organized, and a second visual estimate with the aid of the scales was realized by the same evaluators.

The accuracy and precision of each evaluator were determined through the simple linear regression with real severity as independent variable and the estimate severity as dependent variable.

The accuracy of the estimate for each evaluator and the group f evaluators was determined by test t applied to the intercept of the linear regression (β_0), in order to verify Ho hypothesis: $\beta_0 = 0$, and to the angular coefficient of a line (β_1), to test Ho hypothesis: $\beta_1 = 1$, at level 5% of probability (p=0.05). Values of intercepts significantly different from 0 (zero) indicate overestimation (>0) or underestimation (<0) of the real severity at low levels of intensity of the disease, while values of angular coefficient of a line, which divert significantly from 1 (one) indicate systematic superestimation (>1) or underestimation (<1) of the real severity in all the intensities of the disease (Nutter Jr and Schultz, 1995).

The precision of the estimation was determined by coefficient of regression determination (R^2), variance of absolute errors (estimate severity minus real severity, and repeatability of the estimate, determined by regression analysis of the second evaluation in relation to the first one of the same sample unit, that is, the same set of leaves presented randomly (Nutter Jr et al., 1993).

The reproducibility of the estimate was evaluated by analyzing R^2 values obtained from linear regressions between the estimated severity of the same sample unit by different evaluators in pairs (Campbell and Madden, 1990; Nutter Jr and Schultz, 1995).

The data were tabulated using the software Microsoft® Excel® 2010, and the statistical analyses realized in the program SAS® v 9.3.

RESULTS

The severity of the 120 leaves wih Blister Spot, obtained electronically, showed minimum and maximum values of 0.98 and 46.9%, respectively. The frequency distribution of the severity had 93.3% of the leaves analyzed with injured foliar area in lower intervals of 24% (Table 1).

Based on frequency distribution, and the fact that the higher frequency is located in intervals lower than 24% of the disease's severity, the scale was developed with a

Table 1. Frequency distribution, at unit intervals, of the severity values (%) for Blister Spot in coffee tree.

Interval (severity %)	Frequency	Percentage	Cumulative frequency	Cumulative percentage (%)
0-1	9	7.5	9	7.5
1-2	3	2.49	12	9.99
2-3	2	1.66	14	11.65
3-4	6	4.98	20	16.63
4-5	7	5.81	27	22.44
5-6	2	1.66	29	24.1
6-7	7	5.81	36	29.91
7-8	5	4.15	41	34.06
8-9	3	2.49	44	36.55
9-10	7	5.81	51	42.36
10-11	4	3.32	55	45.68
11-12	5	4.15	60	49.83
12-13	4	3.32	64	53.15
13-14	8	6.64	72	59.79
14-15	4	3.32	76	63.11
15-16	11	9.13	87	72.24
16-17	6	4.98	93	77.22
17-18	0	0	93	77.22
18-19	4	3.32	97	80.54
19-20	4	3.32	101	83.86
20-21	4	3.32	105	87.18
21-22	2	1.66	107	88.84
22-23	4	3.32	111	92.53
23-24	1	0.83	112	93.36
>24	8	6.64	120	100

bigger number of classes below this value, considering the following scores of percentage intervals; Score 0 – 0%, 1 – 3%, 2 – 3 to 6%, 3 – 6 to 12%, 4 – 12 to 24%, and 5 - 24 to 50 % of severity (Figure 1), following through on “Weber-Fechner” law. The lower and upper limit of the disease’s severity represented in the scale were 0 (zero) and 46.9%, respectively. Images of leaves with value of severity higher than 46.9% were not added to the diagrammatic scale due to the fact of not being found leaves with severity over this value.

Evaluation with the scale provided a higher precision and accuracy in relation to the evaluation without using the scale (Table 2). All the evaluators superestimated the severity of the diasease when it was not being used. Among the evaluators, at least one of the hypotheses $\beta_0 = 0$ e $\beta_1 = 1$, of the linear regression between real and estimated severity, was rejected, while for the evaluators A and E the two hypotheses were rejected (Table 2), indicating superestimation of the disease’s severity.

Through the use of the diagrammatic scale, the evaluators were more accurate to evaluate the disease’s severity. In the first evaluation using the scale, 62% of the evaluators showed intercept and angular coefficient of a line of linear regression equal to 0 (zero) and 1 (one), respectively. However, in the second one, no evaluator had angular coefficient different from 0 and 1 (Table 2).

All the evaluators presented low precision without using the scale. Nevertheless, using it, both in the first and in the second evaluation, there was a significant increase in R^2 values, obtaining then higher precision (Table 2).

There was reduction in the absolute errors when the scale was used, occurring lesser extent of the values (Figure 2). In fact, the minimum and maximum values observed for the residues of all evaluators without using the scale were, respectively, -36.09 and 93.98, while using the scale, the average between the two evaluations, had the interval reduced -39.76 and 23.85.

The values of the absolute errors were also reduced with the use of the scale. In the evaluation without the scale, 52% of the residues were out of the interval between -10 and +10. Between the two evaluations, using the scale, the average of 98% of the absolute errors were into this interval, that is, there was reduction of the errors.

The evaluators showed a good repeatability at the the estimate of severity for Blister Spot in coffee leaves with the use of the scale proposed. The increase in the precision was confirmed by the repeatability of the estimate. The average of variation in the first evaluation explained in comparation with the second one was of

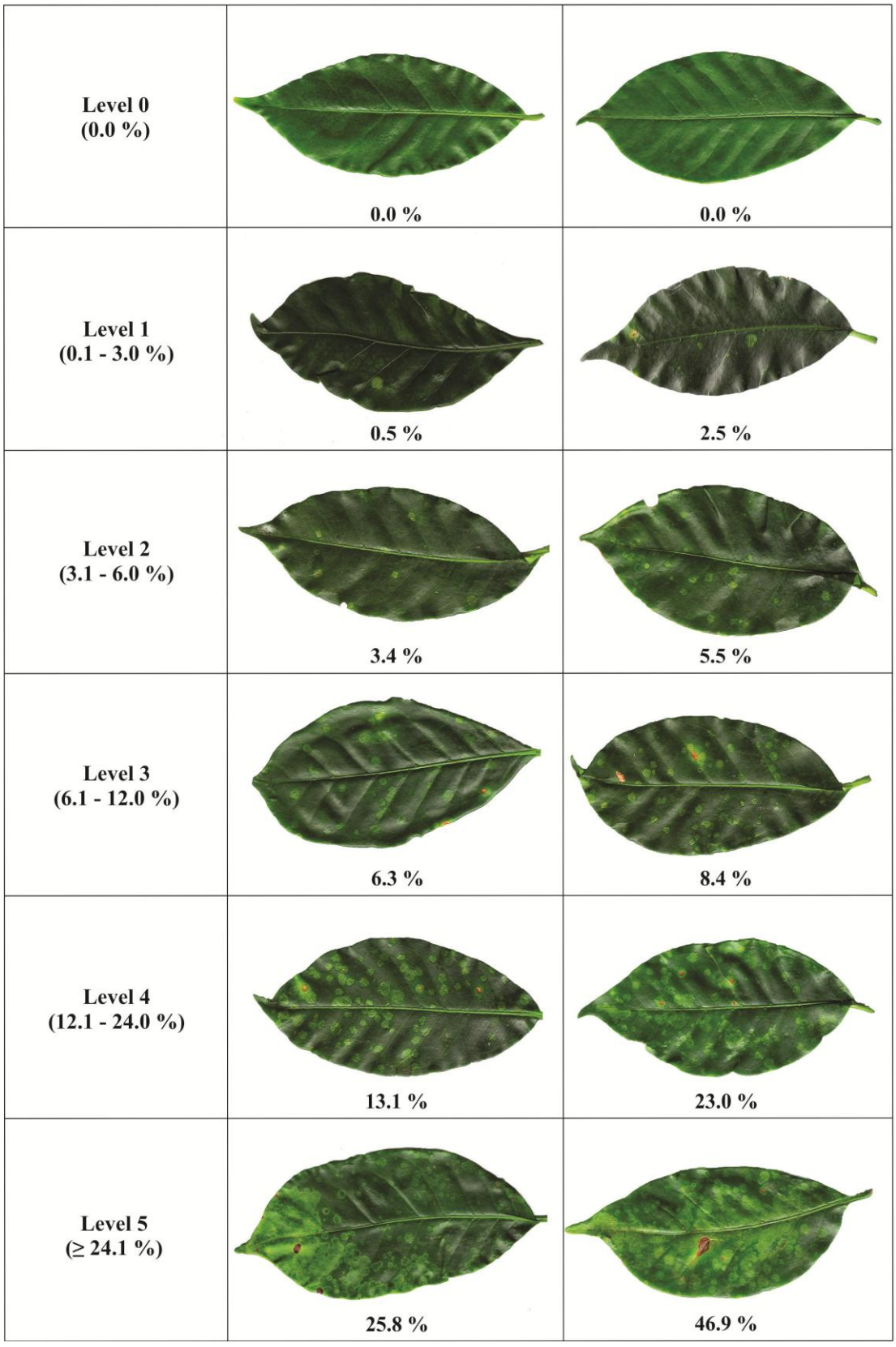

Figure 1. Diagrammatic scale to evaluate the severity for Blister Spot (*Colletotrichum gloeosporioides* PENZ) in coffee leaves (*Coffea arabica* L.). Numbers represent the percentage of foliar area affected by the disease.

Table 2. Intercept (β_0), angular coefficient of a line (β_1) and coefficient of determination (R^2) of equations for linear regression relating visual estimations of severity for Blister Spot in coffee leaves, realized by evaluators with and without the aid of the diagrammatic scale, for real severity determined electronically.

Evaluators	Without scale			First evaluation with scale			Second evaluation with scale		
	β_0	β_1	R^{2**}	β_0	β_1	R^{2**}	β_0	β_1	R^{2**}
A	5.59*	1.57*	0.74	1.92^{ns}	0.79^{ns}	0.79	1.01^{ns}	0.94^{ns}	0.88
B	6.96^{ns}	2.57*	0.64	2.1^{ns}	0.88^{ns}	0.73	1.44^{ns}	0.97^{ns}	0.76
C	8.17*	0.43^{ns}	0.25	0.76^{ns}	0.97^{ns}	0.79	0.3^{ns}	1.02^{ns}	0.85
D	0.72^{ns}	1.73*	0.66	0.3^{ns}	1.08^{ns}	0.86	0.14^{ns}	1.06^{ns}	0.86
E	7.99*	1.56*	0.62	0.84^{ns}	0.98^{ns}	0.82	0.35^{ns}	1^{ns}	0.83
F	1.84^{ns}	1.56*	0.69	0.1^{ns}	0.98^{ns}	0.80	0.4^{ns}	0.99^{ns}	0.83
G	26.13*	0.08^{ns}	0.00	0.35^{ns}	1.03^{ns}	0.83	0.21^{ns}	1.02^{ns}	0.84
H	36.83*	0^{ns}	0.00	0.34^{ns}	1^{ns}	0.81	0.11^{ns}	1.02^{ns}	0.83

* and ns represent situations the null hypothesis ($\beta_0 = 0$ ou $\beta_1 = 1$) was, respectively rejected and not, by test t (P= 0.05); ** represents significant situations with probability of 5% for test t (P <0.05).

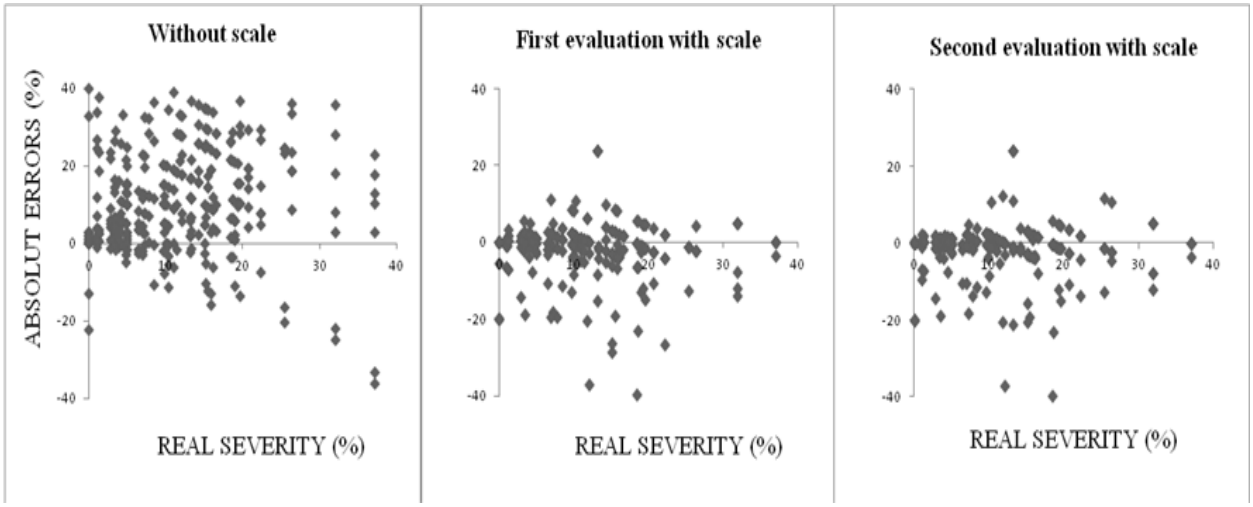

Figure 2. Distribution of residues (estimate severity – real severity) of the estimations of Blister Spot in coffee leaves estimate without and with the aid of the diagrammatic scale in two evaluations.

91%, and the value of the angular coefficient of a line was significantly similar, showing precision of the estimate in 100% of the evaluators (Table 3).

The scale showed high reproducibility. Without its usage, the value of R^2 of the regressions between the pair of evaluators varied from 0 to 0.76, with the average of 0.31 (Table 4). Using the diagrammatic scale, R^2 values varied from 0.79 to 0.94% and 0.82 to 0.98% in the first and second evaluations, respectively, being ≥0.75 in 87% of the evaluators' combinations.

DISCUSSION

The diagrammatic scale proposed in this article like others, were constructed defining the intervals according to the law of "Weber-Fechner" (Custódio et al., 2011; Salgado et al., 2009), however, without following the same intervals of severity proposed by Horsfall and Barratt (1945) due to the disease's peculiarity. Among these characteristics, the maximum severity found in the field and the frequency intervals of higher concentration for the severity of the disease must be observed. (Belan et al., 2014; Capucho et al., 2011). Thus, the scale developed was divided into the intervals in logarithm scale in order to reduce the erros in the estimate of the disease.

Images of leaves with severity value higher than 46.9% were not included in the diagrammatic scale because leaves with higher value of intensity than this one were not found. To develop diagrammatic scales for brown wye spot (Custódio et al., 2011) and bacterial bight in

Table 3. Intercept (β_0), angular coefficient of a line (β_1) and coefficient of determination (R^2) of equations for linear regression related with the second to the first estimate of severity for Blister Spot in coffee leaves, realized by the same evaluator with the diagrammatic scale.

Evaluators	Coefficients		
	β0	β1	R^{2}**
A	2.25^{ns}	0.85^{ns}	0.67
B	0.21^{ns}	1.01^{ns}	0.88
C	0.37^{ns}	0.98^{ns}	0.92
D	-0.26^{ns}	0.96^{ns}	0.97
E	-0.17^{ns}	0.99^{ns}	0.96
F	0.70^{ns}	0.99^{ns}	0.96
G	-0.01^{ns}	0.98^{ns}	0.98
H	0.04^{ns}	0.99^{ns}	0.97

* and ns represent situations the null hypothesis ($\beta_0 = 0$ ou $\beta_1 = 1$) was, respectively, rejected and not by test t (P= 0.05); ** represents significant situations with probabilities of 5% for test t (P <0.05).

Table 4. Coefficient of determination (R^2) of equations of linear regression related to visual estimate of Blister Spot's severity in coffee leaves between evaluators in pairs without the aid of the diagrammatic scale in the first and second evaluations.

Without scale							
Evaluators	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	0.76	0.31	0.73	0.74	0.73	0.01	0.02
B		0.28	0.67	0.55	0.61	0.00	0.00
C			0.21	0.18	0.35	0.12	0.02
D				0.56	0.72	0.01	0.04
E					0.54	0.00	0.01
F						0.00	0.00
G							0.61

1° Evaluation with scale							
Evaluators	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	0.76	0.84	0.77	0.81	0.76	0.79	0.82
B		0.83	0.8	0.85	0.84	0.86	0.79
C			0.91	0.91	0.89	0.9	0.88
D				0.91	0.9	0.91	0.91
E					0.94	0.94	0.89
F						0.91	0.93
G							0.92

2° Evaluation with scale							
Evaluation	**B**	**C**	**D**	**E**	**F**	**G**	**H**
A	0.78	0.85	0.85	0.82	0.83	0.84	0.84
B		0.82	0.85	0.85	0.85	0.83	0.82
C			0.98	0.95	0.94	0.95	0.93
D				0.95	0.94	0.95	0.94
E					0.97	0.95	0.93
F						0.96	0.95
G							0.94

coffee leaves (Belan et al., 2014), the authors also used the upper limits of the scales next to the maximum limit found in the fields, 49.0 and 45.1% respectively.

The diagrammatic scales have the function of helping in the determination of the leaf injured area, approximating the evaluation from the real. In general, the diagrammatic scales that have already been built up provide more precision and accuracy in relation to the evaluations without its use (Capucho et al., 2011; Salgado et al., 2009). Without using the scale, the evaluators tended to overestimate the disease (Belan et al., 2014, Custódio et al., 2011) and in relation to the hypotheses $\beta_0 = 0$ e $\beta_1 = 1$, were less accepted when scales in the evaluation of the disease severity were not used (Andrade et al., 2005).

According to Amorim (1995), the evaluator's visual ability in the quantification of the disease is connected with factors, such as training, experience and individual perception, resulting in different answers to different visual stimulus. According to Vale et al. (2004), realizing several evalutions, the accurate evaluators follow standards of minimum deviation between the estimated value and the real severity.

In the evaluation with the scale, 98% of the deviation ranges were between -10 and +10, being these acceptable according to criteria adopted by training programs in the quantification of diseases, such as Disease Pro (Nutter Jr and Worawitlikit, 1989). However, the presence of some level of absolute error in the measurements using the diagrammatic scale in order to quantify the severity of the disease can be compensated if the evaluators keep the level of error realizing the other evaluations (Micheref et al., 2006).

The repeatability refers to the evaluator's capacity, using the scale, for repeating the estimate in the same sample (Vale et al., 2004). The diagrammatic scale for the Blister's Spot of the coffee tree permitted to repeat the measurement of the disease in the first one in relation to the second evaluation, using the scale, similar to what was observed in the diagrammatic scale proposed by Belan et al. (2014) and Custódio et al. (2011).

Using the diagrammatic scale, R^2 values for evaluators' combinations in the first and second evaluations were higher than 0.75 in 87% of the combinations, showing a good reproducibility. Similar results were obtained in validations of diagrammatic scales in other pathosystems (Gomes et al., 2004; Martins et al., 2004).

Conclusion

It was possible to build up diagrammatic scale for evaluating Blister's Spot severity in coffee leaves. The diagrammatic scale was developed to aid and quantify Blister's Spot severity in coffee leaves, providing better levels of accuracy, precision and reproducibility of the evaluations in relation to evaluations of its use.

Conflict of Interest

The authors have not declared any conflict of interest.

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