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Logarithmic scaling and effects of severity levels of ringspot disease on sensory quality of coffee brew

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A logarithmic scale was developed to evaluate the severity of the ringspot caused by *Coffee ringspot virus* (CoRSV) in coffee berries to investigate the effect of disease severity on the quality of brew. The scale comprised seven severity levels (0, 1 to 4%, 4.1 to 8%, 8.1 to 15%, 15.1 to 25%, 25.1 to 50%, 50.1 to 75%, 75.1 to 90%) and was evaluated for accuracy, precision, and reproducibility of severity estimation. The accuracy and precision were determined by simple linear regression between the actual and the estimated severity, considering twelve raters. Brew quality was assessed in berries with three severity levels (4.1 to 8%, 50.1 to 75%, 75.1 to 90%) and healthy berries. The selected fruits were processed and analyzed for electrical conductivity, total sugars, reducing sugars, non-reducing sugars, activity of polyphenol oxidase, and total phenol. The logarithmic scale obtained was easy to use and able to provide quick estimates of disease, good accuracy and good precision. Biochemical analysis of berries showed that polyphenol oxidase activity decreased with increasing severity, whereas total sugars and total phenol levels increased with increasing severity. This result indicated that the higher the CoRSV severity level, the greater the change in compounds directly related to the final quality of brew, which consequently contribute to coffee depreciation.

Key words: CoRSV, *Coffee ringspot virus*, phenolic compounds, polyphenol oxidase.

INTRODUCTION

Coffee industry in Brazil accounts more than 30% of the world's supply of coffee, which represents 2.70 million tons of coffee in 2011, and confirms its great potential for generating foreign exchange (FAO, 2012).However, Brazil has reported fall in exports due to inability to meet international market requirements. Effect of genotype and

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lack of appropriate methodology for processing berries and climatic conditions favoring the occurrence of pests and diseases caused by fungi and bacteria are considered the main causes of depreciation in quality of coffee brew (Silva et al., 2000, 2005, 2013; Batista et al., 2003; Bertrand et al., 2003, 2012; Poltronieri et al., 2011).

In the past ten years, beside fungi and bacteria, the *Coffee ring spot virus* (CoRSV) has been related to depreciation in quality of coffee brew (Reis and Chagas, 2001; Boari et al., 2006). Although it has been described and reported in Brazil since the 1940s, only in the mid-1990s did high incidences begin to show in commercial crops in the State of Minas Gerais and later in several other states where the coffee is grown. The virus causes considerable damage, such as defoliation, which can reduce plant yield and cause discoloration of ripe fruit up to 100% of its surface, increasing berry susceptibility to fungus attack and consequently premature fruit falling.

Studies conducted to determine the influence of ring spot on coffee biochemical compounds reported that the disease can change sugar, total phenol, and PPO contents (Reis and Chagas, 2001; Boari et al., 2006). Reis and Chagas (2001) reported increase in total sugar content in berries affected by coffee ring spot and hypothesized that disease could have increased the susceptibility to fungal infection. It is known that the secondary metabolites resulting from fungus infection, such as ochratoxin, could also have serious implication on human health (Bryden, 2007); therefore, the virus damage is beyond the simple depreciation of coffee brew.

In their paper, however, Reis and Chagas (2001) did not investigate whether changes in brew quality could only be related to ring spot or whether it could also be influenced by fungal infection. Thus, there is a need to develop logarithmic scales which allow berry separation by disease levels severity with greater accuracy, precision, and reproducibility in severity estimates (Berger, 1980; Nutter Junior and Schultz, 1995).

In this study, a logarithmic scale was initially constructed to classify berries according to ring spot severity, whereby the berries were analyzed with three severity levels along with healthy fruit to determine disease influence on the final coffee brew quality.

MATERIAL AND METHODS

Development of the logarithmic scale

Coffee cherries cv. Acaiá Cerrado were harvested from crops at the campus of Federal University of Lavras (UFLA), Lavras, Minas Gerais, with different severity levels of coffee ring spot and free of fungal infection. The berries were photographed with an 8-megapixel digital camera at DFP/UFLA laboratory, and images were processed with *IT-2.0-version UTHCSA Image tool* program. After obtaining the actual severity level of ring spot on each berry, the seven-point severity scale with intervals based on logarithmic increments was designed. Definition of track severity followed the criteria recommended by Horsfall and Cowling (1978), where the upper scale limit should correspond to the maximum intensity observed in field, while determination of actual intensity of disease in field and its scale representation should have the high precision. In addition, scale subdivisions should comply with the limitations of human visual acuity defined by the stimulus-response law of Weber-Fechner, where visual acuity is proportional to the logarithm of stimulus intensity. With the designed scale, the levels of precision and accuracy of estimates were calculated (Kranz, 1988; Campbell and Madden, 1990).

For validating the logarithmic scale, a panel of 12 raters inexperienced in quantifying coffee ring spot measured the severity of 84 images of berries with different severity levels. First, the severity level was measured without using a scale. Next, two evaluations were done using the logarithmic scale: the first was performed seven days after the assessment without scale and the second, seven days after the first evaluation with scale. Accuracy and precision of each rater were determined through simple linear regression, by using the actual severity level obtained electronically as independent variable, and the severity estimated by the rater as dependent variable. The accuracy of estimates determined by each rater and by the panel of raters was determined by *t* test applied to the intercept of the linear regression (*a*) to verify the hypothesis H_0: $a = 0$, and the slope of the line (*b*) to test the hypothesis H_1: $b = 1$, at 5% probability. Intercept values significantly different from zero described the presence of constant deviation, while values of the slope significantly different from one indicated the presence of systematic deviations. Accuracy of estimates was obtained through the regression coefficient of determination (R^2), by absolute errors (estimated severity minus actual severity), and by the repeatability of estimates determined by regression of the second assessment in relation to the first sample of the same unit. Reproducibility of the estimates was determined by R^2 values obtained from linear regressions between the estimated severity levels among the raters combined in pairs (Campbell and Madden, 1990; Nutter Junior et al., 1993; Nutter Junior and Schultz, 1995). Regression analyses were performed with SAS ® software (2009).

Classification and analysis of berries

Coffee cherries from cultivar Acaiá Cerrado collected from UFLA crops were properly conditioned in containers and transported to the Department of Plant Pathology for evaluation and classification into three severity levels (4.1 to 8; 50.1 to 75; and 75.1 to 90%), plus healthy cherries, using the logarithmic scale developed and validated previously. Subsequently, berries were sun-dried with special care to prevent it from undesirable changes. After drying and processing (light roasting and grinding), 300 g aliquots of ground coffee were taken from each severity level, with three repetitions, for analysis of the total phenolic content (TPC) by the Goldstein and Swan method (1963) described by the AOAC (1990), reducing sugars (RS), non-reducing sugars (NRS), and total sugars (TS) by the Lane-Enyon method cited by the AOAC (1990); electrical conductivity (EC) according to a method adapted from Loeffler et al. (1988) and polyphenol oxidase activity (PPO) was determined by the method of Ponting and Joslyng (1948). The analyses were performed in Lavras, at the Laboratory of Coffee Brew Quality from EPAMIG (Minas Gerais State Agricultural Research Corporation), and the comparison test was carried out in the Laboratory of Coffee Quality, UFLA. Results were statistically

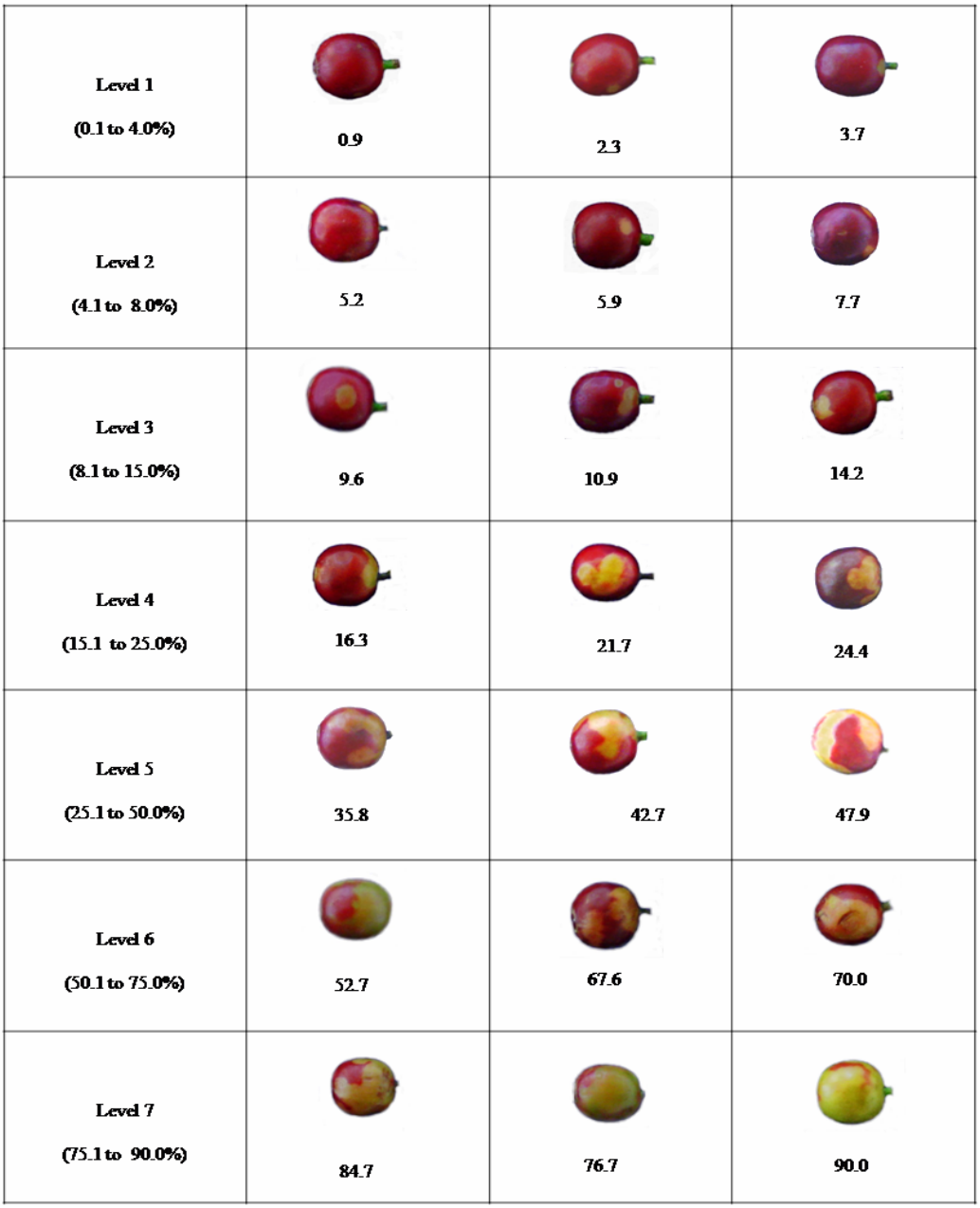

Figure 1. Logarithmic scale for coffee ringspot disease in berries with severity levels 0.1 to 4%; 4.1 to 8.0%; 8.1 to 15%; 15.1 to 25%; 25.1 to 50%; 50.1 to 75%; 75.1 to 90%. UFLA, Lavras, MG (2005).

analyzed with the Sisvar ® program.

RESULTS

The maximum and minimum severity values used for the scale development were 0.1 and 90%, respectively. Values above 90% were not found. The logarithmic scale to evaluate severity of coffee ring spot was designed with seven intervals: 0, 1 to 4%, 4.1 to 8%, 8.1 to 15%, 15.1 to 25%, 25.1 to 50%, 50.1 to 75% and 75.1 to 90% of the injured area of the berries (Figure 1).

The majority of raters (75%) showed inaccurate estimates in the first evaluation without scale (Table 1). Constant deviations were observed for 41% of the raters H, I, J, K and L, who overestimated the injured. When

Table 1. Intercept (a), slope (b), and coefficient of determination (R^2) of linear regression equations between actual severity and estimated severity of coffee ringspot disease in the evaluations performed by raters with and without a logarithmic scale. UFLA, Lavras, MG (2005).

Rater	Without scale			With scale					
				1st Evaluation			2nd Evaluation		
	a	*b*	*R²*	*a*	*b*	*R²*	*a*	*b*	*R²*
A	-1.2	1.02	0.85	-2.8	1.04	0.89	-1.4	1.03	0.88
B	-0.9	1.03	0.91	-1.5	1.06	0.91	-1	0.99	0.92
C	3.87	1.16**	0.85	0.11	1.12*	0.87	-0.3	1.08	0.88
D	2.67	1.1*	0.91	0.74	1.02	0.89	2.08	1.05	0.92
E	3.11	1.05	0.89	4.88*	1.03	0.86	5.72**	0.97	0.9
F	1.44	1.09*	0.9	-1	0.97	0.92	0.06	1.04	0.92
G	1.66	1.13**	0.89	-4.1	1.05	0.83	-1.3	1.08	0.88
H	3.78**	1.04	0.94	-0.2	1.03	0.93	-0.4	1.02	0.95
I	2.7*	1.09*	0.93	0.18	1.06	0.94	0.87	1.04	0.93
J	4.73**	1.03	0.92	1.67	1.1	0.87	-1.2	1.02	0.92
K	10.2**	0.92	0.85	3.24*	1.04	0.92	1.98	1.02	0.88
L	3.7*	0.95	0.87	-0.8	1.04	0.92	0.17	1.05	0.91
Mean			0.89			0.9			0.91

* Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.05); ** Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.01).

using the scale, only two raters (E and K) overestimated the severity in the first assessment, while one rater (E) consistently overestimated the severity in the second evaluation, according to intercept values significantly different from zero (P ≤ 0.05). Regarding the slope values, systematic deviation was observed in 41% of the raters (C, D, F, G, and I) which showed values significantly different from 1 (P ≤ 0.05), without using the diagrammatic scale. With the scale, only one rater in the first evaluation (C) showed a slope value significantly different from 1 (P ≤ 0.05).

The absolute errors reduced with the logarithmic scale (Figure 2) when compared with the residual distribution of estimates obtained without scale. It did not show a definite undesirable pattern in spite of the wide variation range in the first, second, and third evaluations (28.61 to -45.07; 32.05 to -59.59; 25.13 to -44.78, respectively). The percentage of absolute error values found in the range of -10 to 10 rose from 75% in the evaluation without scale to 85 and 87%, respectively in the first and second evaluations with scale.

The raters showed good repeatability in the estimates, since the mean amount of variation in the first evaluation explained by the second one was 91% (Table 2). Only 17% of raters showed slope values of regression between the two evaluations significantly different from 1 (P ≤ 0.05) and intercept values different from zero (P ≤ 0.05) (Table 2). Reproducibility of estimates among raters was also used as an indicator of accuracy analysis of the scale. Without the logarithmic scale, the coefficient of determination (R^2) of the estimate regressions between pairs of raters ranged from 74 to 93% with a mean of 87% (Table 3). With the logarithmic scale, R^2 values ranged from 80 to 94% with a mean of 88%.

The biochemical analysis showed that the increasing of ring spot severity in coffee fruits also increased the levels of total phenol (TPC), reducing sugars (RS), and electrical conductivity (EC) contents, while reduced the activity of polyphenol oxidase (PPO) (Table 4). Non-reducing sugar content (NRS) and total sugar (TS) contents did not differ statistically in the Scott-Knott test at 5% probability (Table 4).

DISCUSSION

In this paper, a diagrammatic scale was devised to assess the severity of the ring spot in coffee berries. This scale was efficient when it was later applied to separate the berries with three severity degrees of virus disease, which were submitted to the biochemical tests considered indicators of the coffee brew quality. The information obtained was quite interesting, because it allowed correlating the severity of the ringspot disease with the quality of coffee brew, considering that the evaluated coffee berries were free of any apparent secondary infection by fungi.

Defining criteria to standardize evaluation of coffee ring

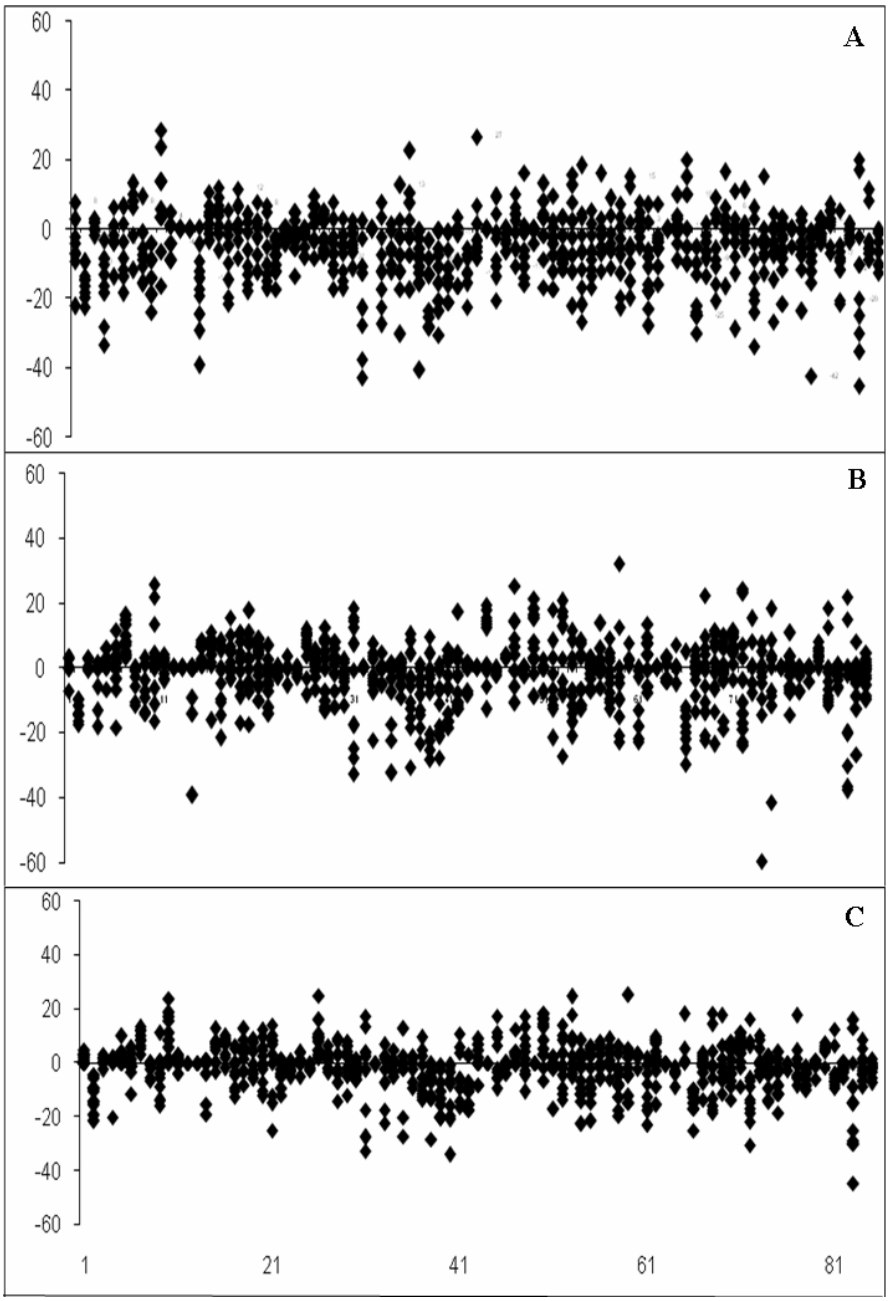

Figure 2. Absolute errors (differences between actual and estimated severity) of all raters in evaluation without logarithmic scale (a) and with logarithmic scale (b and c). Points represent the 85 estimates for each rater. UFLA, Lavras, MG (2005).

spot is necessary due to the wide variation in the severity of disease. During the evaluation, using the diagrammatic scale, there was improvement in precision in the second evaluation with scale in relation to the first, showing that training and constant use of the logarithmic scale positively affected accuracy and precision of the estimates (Corrêa et al., 2009). The percentage of absolute errors was considered a good value according to the criteria adopted in studies on logarithmic scales (Amorim et al., 1993; Corrêa et al., 2009). Some levels of absolute errors in the estimates were compensated by the speed and standardization provided by logarithmic

Table 2. Intercept (a), slope (b) and coefficient of determination (R^2) of linear regression equations correlating second and first estimates of coffee ringspot disease by the same rater, using logarithmic scale. UFLA, Lavras, MG (2005).

Rater	*a*	*b*	R^2
A	0.58	0.95	0.89
B	0.84	1.02	0.92
C	2.99	0.96	0.85
D	0.1	0.94	0.9
E	-0.4	1.04	0.91
F	0.19	0.9**	0.92
G	-2.4	0.96	0.92
H	0.16	1.01	0.98
I	0.42	0.99	0.95
J	4.33*	1.03	0.87
K	4.14*	0.93	0.88
L	1.04	0.92*	0.9
Mean			0.91

*Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.05); **Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.01).

Table 3. Coefficient of determination (R^2) of linear regression equations correlating the estimates of coffee ringspot disease between raters, with and without logarithmic scale (first and second evaluations). UFLA, Lavras, MG (2005).

Rater	Without scale										
	B	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
A	**0.90**	**0.80**	**0.84**	**0.82**	**0.83**	**0.79**	**0.87**	**0.84**	**0.82**	**0.74**	**0.78**
B		0.83	0.87	0.87	0.88	0.84	0.92	0.87	0.86	0.81	0.83
C			0.91	0.89	0.85	0.88	0.86	0.89	0.86	0.82	0.82
D				0.92	0.86	0.89	0.90	0.93	0.90	0.85	0.88
E					0.90	0.91	0.90	0.91	0.93	0.84	0.86
F						0.87	0.93	0.90	0.89	0.86	0.83
G							0.87	0.92	0.87	0.84	0.86
H								0.92	0.92	0.86	0.87
I									0.91	0.87	0.86
J										0.84	0.86
K											0.80

Rater	With scale – 1st Evaluation										
	B	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
A	0.86	0.77	0.81	0.78	0.88	0.78	0.90	0.86	0.81	0.79	0.88
B		0.83	0.87	0.81	0.88	0.80	0.88	0.91	0.85	0.89	0.87
C			0.82	0.79	0.81	0.77	0.82	0.85	0.87	0.86	0.80
D				0.81	0.84	0.81	0.83	0.86	0.85	0.85	0.88
E					0.82	0.78	0.82	0.88	0.80	0.86	0.86
F						0.84	0.91	0.92	0.83	0.86	0.89
G							0.80	0.84	0.77	0.75	0.85
H								0.91	0.82	0.83	0.90
I									0.86	0.93	0.92

Table 3. Contd.

J										0.85	0.82
K											0.87

Rater	With scale – 2nd Evaluation										
	B	C	D	E	F	G	H	I	J	K	L
A	0.91	0.81	0.82	0.83	0.86	0.86	0.87	0.87	0.88	0.82	0.83
B		0.86	0.91	0.86	0.91	0.85	0.89	0.88	0.92	0.84	0.89
C			0.89	0.91	0.91	0.86	0.86	0.91	0.88	0.86	0.88
D				0.93	0.93	0.85	0.89	0.91	0.88	0.89	0.89
E					0.90	0.88	0.86	0.93	0.86	0.91	0.87
F						0.88	0.92	0.94	0.93	0.89	0.94
G							0.86	0.88	0.89	0.81	0.86
H								0.90	0.89	0.84	0.89
I									0.93	0.90	0.92
J										0.85	0.90
K											0.85

Table 4. Mean of values obtained in the biochemical analysis of coffee berries with different disease severity levels. UFLA**,** Lavras, MG (2005).

Treatments	TPC [(1)]	RS [(2)]	NRS [(3)]	TS [(4)]	EC [(5)]	PPO [(6)]
Healthy Fruit	6.0 b	0,53 d	6.26 a	7.26 a	135.9 c	64.93 a
4.1 - 8%	6.33 b	0.60 c	6.4 a	7.4 a	137.66b	64.2 b
50.1 – 75%	7.53 a	0.66 b	6.46 a	7.46 a	145.9 b	62.93 b
75.1 – 90 %	7.63 a	0.83 a	6.6 a	7.53 a	150.23 a	62.2 c
CV	1.61	18.95	2.39	2.45	2.37	0.72

Means followed by the same letter do not differ by Tukey test at 5% significance. [1]Total phenolic content; [2] Reducing sugars; [3] Non-reducing sugars; [4] Total sugars; [5]Electrical conductivity; [6] Polyphenol oxidase activity.

scales. Moreover, like most methods for quantifying disease severity, the use of this tool is subject to a certain degree of subjectivity, which can be minimized with rater training (Nutter Jr. and Schultz, 1995). The R^2 values, which presented a mean of 88%, were similar to the results found in the validation of logarithmic scale to other pathosystems. According to Nutter Junior et al. (1993), different raters using the same scale to evaluate the same material should estimate the same severity values whose significance is checked with linear regressions between pairs of values estimated by the raters.

The diagrammatic scale enables determination of severity levels for the pathosystem, provides a standardized method for quantifying disease severity, and makes it possible to correlate severity variation in berries with different fruit-related variables, such as brew quality. The logarithmic scale proposed to evaluate coffee ring spot was easy to use, able to provide a quick estimate of the disease, and provided good accuracy and precision of estimates.

Results of the biochemical analysis of berries showed a significant alteration in several components, such as the increasing of total phenol, reducing sugars, and electrical conductivity, and the activity decreasing of polyphenol oxidase (PPO), which are considered the key parameters in the quality of brew. Considering that those alterations were related to the increasing of ring spot severity on coffee berries, it leads to conclusion that the quality of coffee brew depends on the quantity of symptoms induced by CoRSV, being inversely proportional to the severity of ring spot in coffee berries. Similar results had already been found by Reis and Chagas (2001) and Boari et al. (2006) for most of the items evaluated herein. Boari et al. (2006), however, found a slight decrease in electrical conductivity levels in grains affected by ring spot, whereas the current results showed increase in

these values as severity levels increased. There is evidence that brew quality increases as electrical conductivity levels decrease (Prete and Abraão, 2000). However, other authors found no correlation between EC values and sensory analysis of coffee (Favarin et al., 2004). On the other hand, Malta et al. (2005) evaluated the influence of grain size and type of grain defect on electrical conductivity and potassium leaching of exudates of coffee beans and concluded that defective beans may affect test results.

It is known that coffee taste is highly dependent on organic compounds such as acids, aldehydes, ketones, sugars, proteins, amino acids, fatty acids, and phenol contents, as enzyme activity may lead to depreciation in final quality of coffee brew in cupping test (Amorim and Silva, 1968; Oliveira et al., 1977; Amorim and Melo, 1991; Carvalho et al., 1994; Mazzafera and Robinson, 2000; Silva et al., 2013). Several authors have reported a correlation between chemical composition and activity of PPO in berries, as well as peroxidase and phenolic contents in taste, aroma, and therefore in quality of coffee brew (Amorim and Silva, 1968; Oliveira et al., 1977; Amorim and Mello, 1991; Farah and Donangelo, 2006).

Phenolic compounds are considered responsible for the most significant changes in taste and aroma of coffee brew. Pinto et al. (2001) found a direct correlation between polyphenol content and brew quality. According to these authors, the higher the polyphenol content, the worse the brew quality; they found that *rio* brew and *soft* brew had the highest and lowest polyphenol contents, respectively. The authors also reported that *strictly soft* and *riada* brew had higher levels of total and non-reducing sugars. PPO activity can also significantly affect brew quality (Amorim and Silva, 1968; Oliveira et al., 1977; Amorim and Amorim, 1977).

Carvalho et al. (1994) developed a table correlating polyphenol content with coffee classification. PPO levels above 67.66 U g^{-1} of processed grains (U is the unit of enzyme activity equivalent to 0.001 optical density per minute) would be found in *extra fine* coffee, *strictly soft* brew; 62.99 to 67.66 U g^{-1} of processed grains in *fine* coffee, *soft* brew, and *only soft* brew; 55.99 to 62.99 U g^{-1} in *acceptable* coffee, *hard* brew; and under 55.99 U g^{-1} in *not acceptable* coffee, *riada* and *rio* brew. These data corroborate previous reports by several authors (Amorim and Amorim, 1977; Oliveira et al., 1977; Amorim and Mello, 1991). Based on this table, the results obtained in this work showed that grains with 0 and 8% of ring spot severity were classified as *soft* brew, while those with 75 and 90% severity were classified as a *riada* brew and *rio* brew. It demonstrated that severity of ring spot was directly related to coffee brew quality, which shows that ring spot importance goes beyond damage and losses caused by defoliation and fruit drop. Even when there is no loss in quantity, the quality loss caused by depreciation of coffee brew is certainly an additional factor in the damage caused by CoRSV infection in coffee plants.

Conclusion

This article provided a new tool for ringspot evaluation in coffee berry and also showed that the quality of coffee brew decrease when the disease severity increase. Besides that, it was possible to observe that this depreciation was not influenced by any secondary fungal infection that can be seen under visual inspection.

Conflict of Interest

The authors have not declared any conflict of interest.

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