Article

Diagrammatic scale for assessing foliar symptoms of alternaria brown spot in citrus

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ABSTRACT

Alternaria brown spot (ABS) is the main fungal disease of mandarins. Cause damage to fruits, branches and leaves. For determinations of disease severity in fruit, there is a specific diagrammatic scale, which does not occur for foliar lesions. In order to standardize leaf reviews of ABS in citrus, a diagrammatic scale was developed from the collection of symptomatic Dancy tangerine and Murcott tangor leaves, with ten levels, from zero to 97% of the area damaged by the pathogen. They were tested by five evaluators who analyzed 100 pictures of symptomatic leaves with and without the use of the proposed scale. To validate the scale, precision and accuracy of assessors in rating the images were compared. Results showed that the use of the scale improved the accuracy of the evaluators, with increased accuracy. This shows that the leaf scale can be used to determine the severity of alternaria brown spot in citrus.

Key words: Pathometry, *Alternaria alternata*, fungal disease, mandarin.

INTRODUCTION

The alternaria brown spot (ABS), caused by a pathotype of the *Alternaria alternata* fungus, produces a host-specific toxin (HST) that affects tangerines, mainly of the Dancy (*Citrus reticulata* Blanco) variety, and its hybrids. It also affects some tangors such as the Murcott (*C. reticulata* Blanco x *C. sinensis Osbeck*), and sometimes pomelos (*C. paradisi* Macf.), in the most humid and semi-arid citrus cultivation regions (Timmer et al., 2003). It damages leaves, branches and new fruit, and the symptoms are characterized by necrotic stains with or without the presence of chlorotic halos around the lesion (leaves and branches), and dark and cortisol on the fruits' surface (Akimitsu et al., 2003).

For diseases epistemological studies, it is important to use tools or techniques that guarantee standard results to make possible the comparison of the trials realized regardless of the team thus preventing subjectivity that can have a negative effect (Campbell and Madden 1990). Plant disease quantification, also called phytopatometrics, is an important phytopathology area. The terms "incidence" and "severity" are highly used in phytopathology to refer to the percentage of sick plants or sick parts of the plant and the volume or tissue area with symptoms, respectively. To quantify disease incidence is easier, precise and simple. However, for severity, the adoption of descriptive keys, diagrammatic scales or scanned images analysis by computer programs are necessary. Among these, the use of diagrammatic scales is more common, consisting of illustrated representations of a series of plants or parts of plants with symptoms at different levels of severity (Belasque Júnior et al., 2005).

The objective of this work was to prepare and validate a diagrammatic scale to determine severity levels of alternaria brown spots in tangerine leaves.

MATERIAL AND METHODS

To develop the diagrammatic scale, 197 leaves (new, fully expanded at the top) were collected from Dancy tangerine and Murcott tangor plants, at several levels of alternaria brown spot (Figure 1), in the county of Cordeirópolis, Sao Paulo State, Brazil, at the Citrus Germplasm Active Bank (BAG-Citros). The county is located at 22º 32' of latitude S and 47º 27' of longitude W; with an altitude of 639 m; climate type Cwa, by the Koppen classification (Ortolani et al., 1991). These plants are 20 years old and were implanted, with a spacing of 7.5 m between the lines x 3.0 m between the plants, and grafted in the Cleopatra tangerine (*Citrus reshni* hort. Ex Tanaka). These leaves were scanned individually with the help of a Sony Cyber-shot digital camera at a resolution of 7.0 megapixels and zoom factor three, in a laboratory, fixing the camera height with the same lighting. Next, the percentage of each lesioned area was determined in each image with the help of the free-of-charge ImageJ software (2010). From these lesioned area percentages, the following extremes were determined: the lowest and highest percentage for the lesioned foliar area of the diagrammatic scale. For the intermediate levels, it was necessary to convert the percentages into log values, and, based on the converted values frequency, intermediate levels were established with the help of the Horsfall and Baratt table (1945).

Figure 1. Typical symptoms of alternaria brown spot in Murcott tangor leaves.

To validate the scale, five assessors with no previous experience in assessing the disease determined the percentage of the foliar area lesioned by the *A. alternatae*, from a total of 100 leaves, with or without the help of the diagrammatic scale. Precision and accuracy were measured by the application of a t-test to the slope (b) and the line intersection value (a) obtained in a linear regression among the severities estimated by each assessor for each stage, with real severity values. Precision was determined by analyzing the line coefficient of determination (R^2) and error distribution of each assessor for each stage (Campbell and Madden 1990).

From the material collected in the field, photographed and processed by ImageJ (2010), a diagrammatic scale was developed representing ten grades of symptoms levels duly illustrated. In this scale, '0' represents leaves with no symptoms and grades 1 to 9 represent 0.3; 3.5; 8; 15; 34; 61; 80; 90 and 97% of the foliar area taken by the spot caused by *Alternaria alternata* (Figure 2).
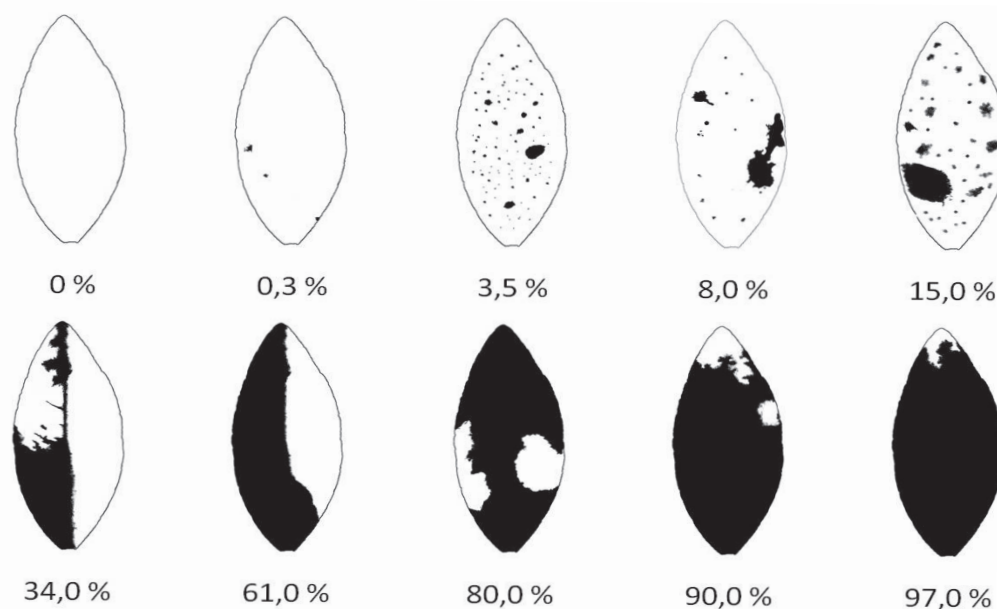
Figure 2. Ten-level diagrammatic scale representation to determine the percentage of leaf area with symptoms of alternaria brown spot caused by *Alternaria alternata*.

The only brown spot damage assessment scale published was for the tangerine fruit, with seven levels, developed by Renaud et al. (2008), varying from 0 to 25% of lesioned fruit area, which was inexistent for leaf assessments. Scales for citrus canker on leaves can be found in the literature (Belasque Júnior et al., 2005). There are several more recent

scales for other cultures such as for pine cone anthracnose (Correia et al., 2011), rice brown spot (Lenz et al., 2010) and coffee Phoma leaf spot (Salgado et al., 2009).

RESULTS AND DISCUSSION

Without the use of a scale, values corresponding to the lesioned foliar area percentage estimate (severity) related to the slope (*a*), angular coefficient (*b*), linear regression (R^2) and residues distribution varied between 2.32 and 19.56; 0.84 and 0.97; 0.66 and 0.97; and, –10.66 and 77.99. In the next evaluation, with the help of the diagrammatic scale, values varied from between –2.17 and 0.49; 0.94 and 1.04; 0.94 and .96; and –22.34 and 18.60 (Tables 1 and 2).

Table 1. Line intersection coefficient values (a), slope (b) and coefficient of determination (R^2) for the linear regression *y= a+bx*.

ASSESSOR	Without the scale			With scale		
	a[(i)]	b[(ii)]	R^2	A	b	R^2
1	2.74***	0.90***	0.97	-0.60[NS]	0.95***	0.96
2	4.49***	0.88***	0.95	-2.17**	1.04***	0.96
3	2.22***	0.90***	0.97	0.49[NS]	0.96***	0.95
4	2.32***	0.84***	0.96	-0.77[NS]	0.94***	0.94
5	19.56***	0.97***	0.66	-2.04*	1.03***	0.94

[(i)] asterisks show that the line intersection value (a) was different from zero by the t test (*p*=0.05); [NS] shows that there was no significant statistical difference between b and one by the t test (*p*=0.05); ***(0.1%), **(1%), and *(5%).

[(ii)] asterisks show that the slope (b) was different from one by the t test (*p*=0.05); [NS] shows that there was no significant statistical difference between b and one by the t test (*p*=0.05); ***(0.1%), **(1%), and *(5%).

Table 2. Minimum and maximum values for the residues distribution of linear regression y= a + bx corresponding to assessor 1 to 5, before and after using the diagrammatic scale.

ASSESSOR	Without the scale		With the scale	
	Minimum	Maximum	Minimum	Maximum
1	-10.66	12.23	-17.35	11.16
2	-21.52	14.27	-13.44	16.53
3	-11.98	12.19	-15.65	19.04
4	-18.64	9.41	-22.34	16.63
5	-24.89	77.99	-19.42	18.60

When accuracy indexes from the two stages were compared, they showed significant differences. Although the angular coefficient values (*b*) were statistically different from one in both stages, there was an improvement in regards to this item for all participants when the scale was used, since the difference between the values for the intersection coefficient (*a*) was reduced. Assessors '1', '3' e '4' stand out for presenting intersecting straight lines values with no statistical difference at 5% of probability by the t test, showing that its accuracy was better with the use of the scale (Table 1).

In regards to assessor precision, whenever the correlation coefficients (R^2) corresponding to the data were assessed, the use of the diagrammatic scale had an impact on results consistency (Table 1). Probably because it was easy to distinguish the leaf's lesioned area from the healthy area. Weber and Jorg (1991) report that the R^2 for the regression equations, that relate visual estimates with real levels, varied from 70 to 95% among assessors. The R^2 from the estimated linear regressions between the best assessor severity and the other assessors were satisfactory in this work ($R^2 > 0.94$). Several studies confirm the precision of the severity visual assessments with the use of the diagrammatic scales (Barbosa et al., 2006; Godoy et al., 2006).

During the analysis of the errors distribution of assessors (residues) and residue maximum and minimum values interval, it was possible to verify that the distribution of errors for most images analyzed was within the range of ±10 with the use of the diagrammatic scale, except for the 30% to 60% range of lesioned leaf area (Figure 3 and Table 2). Such information shows that, despite the high dispersion of errors between the 30-60% in the scale, there is no precision problem, since it discriminated most of the lesions assessed adequately. In addition, most of the grades attributed with the help of the scale was concentrated in the residual range of ±10, an important factor that helps classify the scale as technically acceptable (Belasque Júnior et al., 2005).
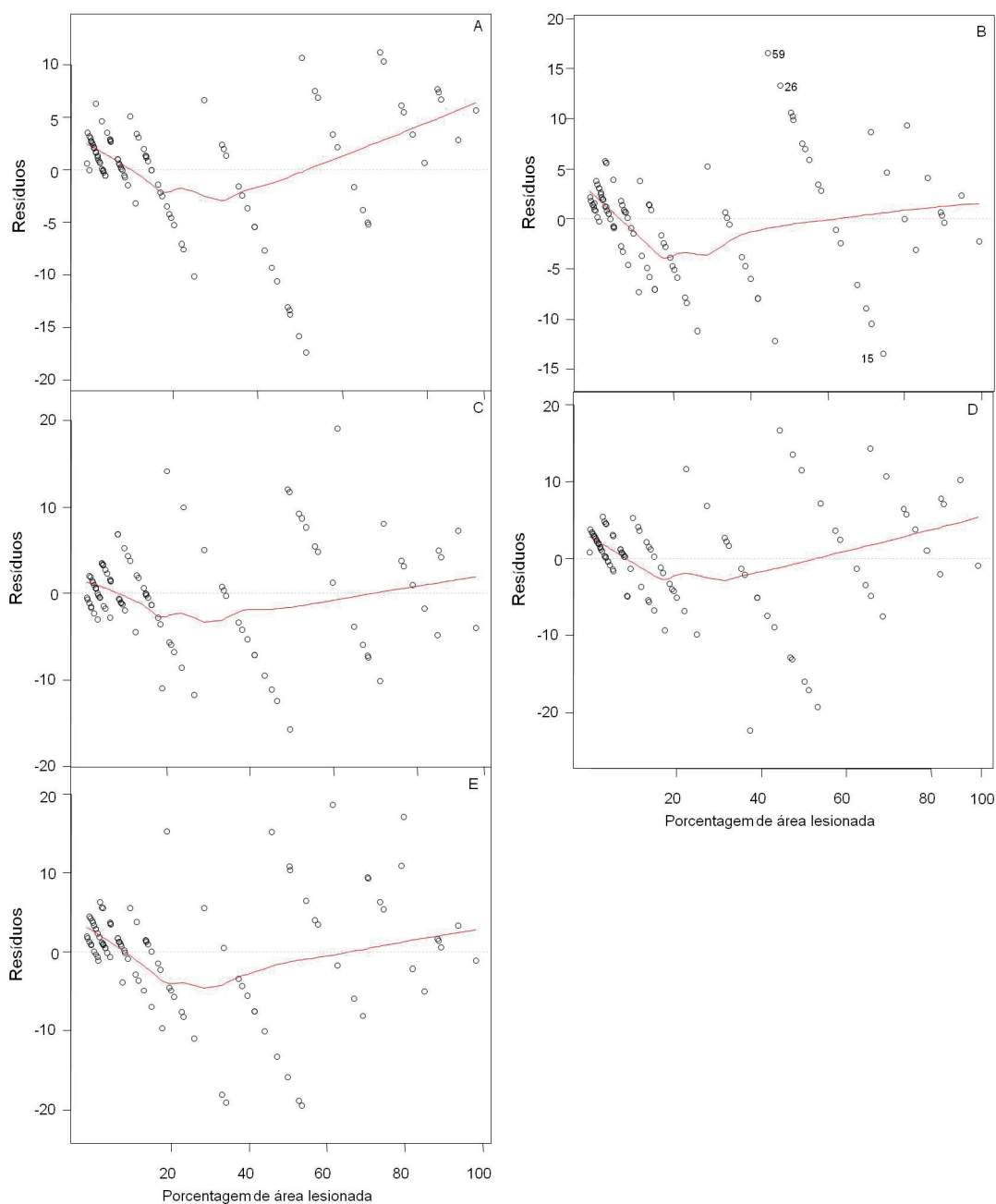
Figure 3. Distribution of values corresponding to residues from each assessor, with scales (A) 1, (B) 2, (C) 3, (D) 4 and (E).

CONCLUSION

The diagrammatic scale provides sufficiently accurate and precise assessments of alternaria brown spot leaf lesions in citrus.

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