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Development and validation of diagrammatic scale to assess target spot severity in cotton

L. H. Fantin[1] · K. Braga[1] · M. G. Canteri[1] · A. R. Dias[2] · E. P. Borges[2]

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Abstract

Due to the emergence of target spot disease on cotton, caused by the fungus *Corynespora cassiicola,* the aim of this study was the development and validation of a diagrammatic scale to assess the severity of target spot on cotton leaves using Lin's statistic and linear regression models. For the design of the scale, 200 cotton leaves were collected from naturally infected plants. Severity was measured by APS assess image analysis software. The diagrammatic scale was developed with seven levels of severity: 1%, 2%, 5%, 9%, 19%, 37 and 53%. Validation was determined by severity estimates of 50 leaves by five experienced and five inexperienced evaluators. The first assessment was performance without a scale and the second with a scale aid. The data were analyzed by two methods: Lin's statistics and linear regression. The diagrammatic scale aid reduced the absolute and relative error and improved precision ($R^2 = 0.79$ and 0.91 without and with the scale, respectively). Evaluators overestimated the severity without the scale. Agreement (Lin's concordance correlation coefficient, $p_c = 0.83$ and 0.94, without and with the scale, respectively) and accuracy (Bias correction factor, $C_b = 0.93$ and 0.98, without and with the scale, respectively) were improved with the scale. The agreement between experience evaluators was higher with the scale. The use of the proposed diagrammatic scale contributed to assessment of target spot in cotton leaves by improving inter- and intra-rater reliability and accuracy of experienced and inexperienced evaluators.

Keywords *Corynespora cassiicola* · Phytopathometry · Lin's concordance, reliability, *Gossypium hirsutum*

Introduction

Cotton (*Gossypium hirsutum* L.) is cultivated in several countries, providing fiber for the textile industry, animal feed and feedstock for oil production. Many factors could be affected the productivity, especially diseases. The target spot of cotton or Corynespora leaf spot, is caused by the fungus *Corynespora cassiicola* (Berk. & Curt.) Wei. and was first identified in 1959 in the state of Alabama, USA (Jones 1961). In South America, it was first detected in 1994 in Bolivia (Metha and Barea 1994) and 1995 in the state of Mato Gross, Brazil, but epidemic levels were not confirmed (Mehta et al. 2005).

The number of target spot in cotton cases has been on the increase over recent years. Cases in many states of the USA (Fulmer et al. 2011; Conner et al. 2013; Price, Singh and Fromme, 2015; Butler et al. 2016), China (Wei et al. 2014), and Brazil (Galbieri et al. 2014; Goulart and Lamas 2016) have attracted the attention of the scientific community. The fungus is considered cosmopolitan, found spread across grown areas in Brazil, and infecting several species, such as cucumber (*Cucumis sativus* L.)(Teramoto et al. 2011b), pepper (*Capsicum annuum* L.) (Cutrim and Silva 2003), cotton (*Gossypium hirsutum*) (Sinclair 1999) and soybean (*Glycine max* L. Merril) (Yorinori and Homechin 1977). It also infects weeds, such as trapoeraba (*Commelina bengalensis* L), Lantana (*Lantana camara* L.) and *Vernonia cinerea* (L.) Less (Barreto et al. 1995; Pereira and Barreto 2001; Oliveira et al. 2007). In addition, the pathogen can survive on previous crop residues and seeds.

L. H. Fantin
fantinagro@gmail.com

[1] Agronomy Department, Universidade Estadual de Londrina, Londrina, Paraná, Brazil

[2] Fundação Chapadão, Chapadão do Sul, Mato Grosso do Sul, Brazil

Because information about yield damage in cotton is unavailable and considering the soybean-cotton crop system rotation in Brazil, Galbieri et al. (2014) isolated lesions in soybean and cotton plants and identified that the pathogen was the same for both crops. In addition, testing of resistance genes concluded that, in Brazil, all cotton cultivars are susceptible to the pathogen, which aggravates the problem because most of cotton growing areas are sowed in succession with the soybean crop (Galbieri et al. 2014).

In this context, quantifying plant disease severity is necessary for the study of epidemiological models, disease progress curves, damage, evaluation of control measures, fungicide efficacy and varietal resistance tests (Amorim and Bergamin Filho 2011). The use of diagrammatic scales helps to guide visual estimation, thereby reducing the subjectivity of severity evaluations and improving the accuracy and precision of the estimates (Martins et al. 2004).

A diagrammatic scale is the illustrated representation of a series of plants, or parts of plants, with symptoms at different levels of disease severity (Bergamin Filho and Amorim 1996). Such scales are simple to use, applicable in several reproducible situations and intervals that represent all stages of development of the disease and promote the standardization of the disease evaluation methodology by providing comparisons between different studies (Bergamin Filho and Amorim 1996; Amorim and Bergamin Filho 2011).

To be useful, the scale could improve the accuracy of estimates, thus representing the closeness of disease estimate to the real disease, and reliability (Campbell and Madden 1990). Reliability can be measured by the relationship between estimates obtained by the same evaluator at different times, called intra-rater reliability or repeatability (Madden et al., 2007), and the agreement between estimates of different evaluators, defined as the linear relationship between assessments of different evaluators, called inter-rater reliability (Nutter, Jr. 1993).

Therefore, the present study aimed to: (i) develop and validate a diagrammatic scale to evaluate the severity of target spot on cotton leaves using two methods: linear regression and Lin's statistics; and (ii) assess inter- and intra-rater reliability and accuracy of experienced and inexperienced evaluators.

Materials and methods

Collection of materials

To develop the diagrammatic scale, 200 leaves of cotton plants with symptoms of target spot caused by fungus *C. cassiicola* were collected in a field located in the Chapadão do Sul, state of Mato Grosso do Sul, Brazil. The leaves were collected to represent the behavior of the disease in the field.

Image processing

The leaves were photographed individually with an image resolution of 300 dpi. The proportion of disease leaf area was processed using image analysis software ASSESS 2.0® (American Phytopathological Society, St. Paul, MN, USA), considering the necrosis lesion and the yellow halo to quantify disease severity. The data obtained by image analysis were considered the disease "real severity" and as such were used as references.

Diagrammatic scale development

Based on the maximum and minimum established by image analysis, the intermediate levels of diagrammatic scale were developed following the visual acuity law by Weber-Fechner (Horsfall and Cowling 1978). Seven levels of disease severity were established to compose the diagrammatic scale. The scale was developed using Adobe Photoshop® software.

Validation

Validation was performed through the visual estimate of the severity of leaves, performed by ten evaluators. The group comprised five evaluators with experience in disease evaluation (EX) (A, B, C, D and E) and five inexperienced evaluators (IN) (F, G, H, I and J). Evaluators were informed how to perform the assessment. A total of 50 images of leaves with target spot symptoms were uploaded to a Microsoft PowerPoint presentation. One leaf image was disposed on each slide. The assessment test was performed in two steps. For the first, the evaluators estimated the severity level without DSTC. After 1 h, the test was performed with DSTC. To validate the proposed scale, the data were analyzed by two methods: Lin's statistics and linear regression.

Validation with Lin's statistics method

Lin's statistics method was used to assess intra-rater reliability, which was defined as the agreement of visual estimates and real severity performance without and with a scale aid by the same evaluator. The measure is also called "repeatability" (Madden et al., 2007). Three parameters were calculated, Lin's concordance correlation coefficient, bias and Pearson correlation coefficient.

Lin's concordance correlation coefficient (LCC) measures the extent to which two sets of observations align on the line of concordance (45 °) (Lin et al. 2002), (Lin, 1989) and combined the precision and accuracy of each evaluator (Barnhart et al. 2002; Nita et al. 2003). The equation is defined as:

$$\rho c = C_b \times \ r$$

Where r represents the correlation coefficient (which measures precision) assessed by the Pearson's correlation coefficient and C_b is the bias coefficient.

The bias coefficient measures how far the fitting line is from the concordance line and represents the accuracy. $C_b = 0$ indicates the perfect match between estimates and real severity. The parameter was calculated as:

$$C_b = {}^{2}/_{(\omega+1|\omega+\upsilon^2)}$$

Where

$$\omega = \frac{\sigma_y}{\sigma_x} \text{ and } \upsilon = \frac{\left|\mu_{y-\mu_x}\right|}{\sqrt{\sigma_y} \times \sigma_x}$$

Where quantity ω is the *scale shift*, υ is the *location shift relative to scale*; μ_y and μ_x are the means of evaluated and real severity, respectively; and σ_y and σ_x are the standard deviations.

Perfect agreement of estimates was found when $\rho c = 1$, $C_b = 0$, scale shift = 1 and location shift = 1. Different values can be interpreted, such as to indicate bias, imprecision and loss of accuracy (Lin et al. 2002; Nita et al. 2003).

Inter-rater reliability represents the agreement between evaluators in estimates of the severity of the same leaf. The term is also called reproducibility (Madden et al., 2007). The overall concordance correlation (OCCC) was used to calculate the agreement between multiple evaluators (Barnhart et al. 2002) and was calculated by the IN and EX group, without and with the scale.

Validation with linear regression

Regression analysis was performed based on a visual estimate without and with the proposed scale and real severity. Accuracy and precision by estimates were calculated. Accuracy was calculated by linear regression using image severity, such as the independent variable (x), and the visual estimate, such as the dependent variable (y).

The parameters were tested by *t-test* applied to the intercept (β_0) and the angular coefficient (β_1). The null hypothesis was that $\beta_0 = 0$ and $\beta_1 = 1$, with a significant level of 0.05. Intercept values (α) greater than zero (>0) indicated severity estimated above the real and values below zero (<0) severity estimated below the real (Nutter and Schultz 1995). For angular coefficient values, nearly to one negative (−1) and one positive (+1) were considered underestimated and overestimated, respectively.

Precision was estimated by the coefficient of determination (R^2) and absolute error, which was calculated by the difference of the visual estimate and image analysis (Kranz 1988; Campbell and Madden 1990; Nutter and Schultz 1995). In addition, the relative error (absolute error divided by real severity*100) was calculated (Yadav et al. 2013).

Data analysis

The analyses were performed with R software (R core team 2017) using "epiR" package (Stevenson et al. 2017) functions "epi.ccc" and "epi.occ".

Results

Development of the scale

The real severity obtained from 200 field leaves ranged between 0.8 and 53%. Based on Webner-Fechner's law of visual acuity, seven levels determined were 1%, 2%, 5%, 9%, 19%, 37 and 53% (Fig. 1).

Intra-rater reliability

The results of the estimates' reproducibility indicated that the values of the *t*-test for the linear coefficients without the diagrammatic scale were significant from zero ($P < 0.01$), which indicates the presence of constant deviations. All evaluators overestimated the severity values (Table 1). The inexperienced evaluators showed higher estimated levels, varying between 3.15 and 11.81, while the estimated levels of the experienced evaluators ranged between 3.02 and 7.46. The regression coefficient (β_1) was statistically different from 1.0 for all evaluators, ranging from 0.65 to 1.17 (Table 1). The coefficient of determination (R^2), which explains the variation in assessment, indicated values between 0.66 and 0.89 for IN and 0.80 and 0.72 EX.

The evaluators improved precision using the scale, estimated by the coefficient of determination (R^2), ranging from 0.81 to 0.99 (Table 1) and reduced the systematic errors of the estimates. The values of the intercept (β_0) were not significant, except for evaluator "C". The evaluators "F" and "J" showed estimates below the real severity, with intercept (β_0) equal to −0.34 and −

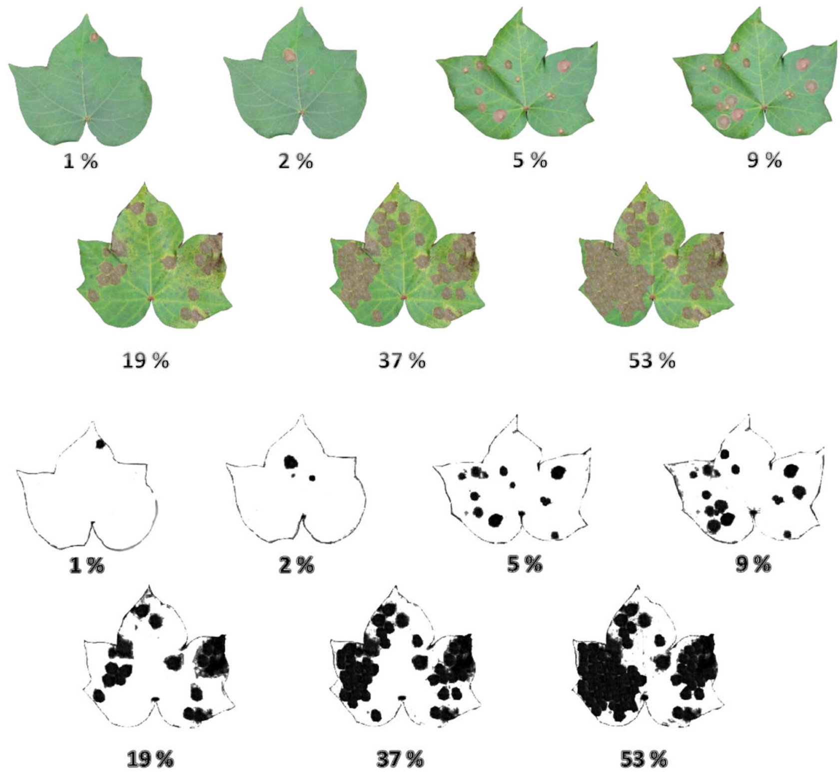

Fig. 1 Diagrammatic scale for evaluating the severity of the target spot (*Corynespora cassiicola)* on cotton leaves. Severity levels determined: 1%, 2%, 5%, 9%, 19%, 37 and 53%

0.28, respectively. With the scale aid, coefficient (β_1) was significant ($p < 0.01$) for all the evaluators.

Lin's statistics showed ρ_c between 0.71 and 0.92 without the scale, and 0.82 and 0.99 with the scale. The results showed the improvement of agreement between estimate and real severity with the scale aid. The IN evaluators showed a higher agreement mean with the scale aid, 0.92 and 0.96 for EX and IN, respectively. Accuracy (C_b) of estimates varied between 0.88 and 0.98, and 0.90 and 1.00, without and with scale, respectively (Table 2). Perfect accuracy indicated no deviation of the fitting line from the 45° line of concordance, which is obtained when $C_b = 1$. The best agreement occurred when $\rho_c = 1$.

Table 1 Linear regression coefficients of the estimated severity of target spot in cotton leaves evaluated by five experienced evaluators (A,B,C,D and E) and five inexperienced (F,G,H,I and J) with and without propose scale

	Without scale			With scale		
Evaluator	β_0	β_1	R^2	β_0	β_1	R^2
A	7.1*	0.76	0.66	0.96	0.98	0.94
B	6.57*	1.17	0.82	1.33	0.96	0.86
C	11.81*	0.86	0.77	3.37*	0.59	0.82
D	3.15*	1.01	0.89	1.39	0.99	0.93
E	4.13*	0.94	0.84	0.73	0.78	0.87
F	3.19*	0.65	0.63	−0.34	0.85	0.85
G	3.28*	1.02	0.85	1.39	0.99	0.93
H	3.02*	1.09	0.89	1.38	0.99	92
I	5.92*	0.97	0.84	0.22	0.99	0.98
J	7.46*	1.01	0.77	−0.28	0.98	0.99

*The null hypothesis ($\beta_0 = 0$ or $\beta_1 = 1$) was rejected according to the t-test (P. value <0.05)

(1) Determination coefficient (R^2) values for the regression analyses

(2) Intercept (β_0) of the linear regression

(3) slope of the line (β_1) of the linear regression

Inter-rater reliability

Inter-rater reliability was measured by correlation between pairs of raters (Table 3). The results showed a higher relationship between evaluators with a scale aid. The determination coefficient was 0.52 and 0.96, without the scale, and 0.73 and 0.99 with the scale. This suggest that the concordance between evaluators improved with the scale.

The overall concordance correlation coefficient (OCCC) showed higher concordance between experienced evaluators without and with the scale, at 0.81 and 0.95, respectively. The scale aid also improved precision and accuracy between all evaluators (Table 4).

The results suggest that the scale helps to promote standard assessment between evaluators. Agreement between experienced evaluators were more improved than that of the inexperienced evaluators with the aid of the proposed scale.

Table 2 Lin's statistics coefficient of the estimated severity of target spot in cotton leaves evaluated by 10 evaluators with and without propose scale

	Without				With			
Evaluator	Pc	Cb	E. shift	L. shift	Pc	Cb	E. shift	L. shift
A	0.78	0.96	0.93	0.29	0.97	1.00	1.01	0.05
B	0.77	0.85	1.29	0.53	0.93	1.00	1.04	0.06
C	0.71	0.80	0.98	0.7	0.82	0.90	0.65	−0.17
D	0.92	0.98	1.06	0.22	0.96	1.00	1.03	0.08
E	0.89	0.97	1.03	0.23	0.91	0.97	0.84	−0.16
F	0.77	0.97	0.81	−0.1	0.91	0.98	0.93	−0.16
G	0.89	0.97	1.11	0.23	0.95	0.99	1.02	0.08
H	0.9	0.96	1.15	0.27	0.96	1.00	1.03	0.08
I	0.85	0.93	1.06	0.37	0.99	1.00	0.99	0.00
J	0.78	0.88	1.16	0.49	0.99	1.00	0.99	−0.04

E.shift = Scale shift relative to the perfect match (1 = perfect match between x and y)

L.shift = Location shift relative to the perfect match (0 = perfect match between x and y)

Cb = Bias correction (Cb) measures how much the best-fit line deviates from the 45 line. No deviation from the 45 line occurs when Cb = 1. Cb is a measure of accuracy calculated from scale shift and location shift

Pc = Concordance correlation coefficient (*Pc*) described by Lin (1989) that combines precision and accuracy to measure agreement with the true values

Table 3 Coefficients of determination (R_2) of linear regression equations between evaluators matched in pairs and inter-evaluator reliability of visual estimates of target spot severity on 50 leaves of cotton by 10 evaluators

Evaluators	A	B	C	D	E	F	G	H	I	J
Without scale										
A	1	0.56	0.52	0.66	0.76	0.76	0.64	0.63	0.76	0.84
B		1	0.85	0.81	0.73	0.52	0.78	0.83	0.76	0.68
C			1	0.85	0.71	0.52	0.8	0.82	0.75	0.64
D				1	0.81	0.68	0.96	0.93	0.85	0.73
E					1	0.68	0.79	0.81	0.86	0.8
F						1	0.71	0.68	0.58	0.71
G							1	0.95	0.79	0.7
H								1	0.79	0.69
I									1	0.83
J										1
With scale										
A	1	0.83	0.86	0.92	0.82	0.85	0.92	0.92	0.94	0.94
B		1	0.73	0.79	0.74	0.67	0.79	0.79	0.81	0.83
C			1	0.78	0.78	0.77	0.78	0.78	0.84	0.83
D				1	0.84	0.83	0.99	0.99	0.94	0.93
E					1	0.93	0.84	0.84	0.87	0.87
F						1	0.83	0.83	0.85	0.9
G							1	0.99	0.94	0.93
H								1	0.94	0.93
I									1	0.97
J										1

Discussion

Overestimation of the results was also verified by Leite and Amorim (2002), Hirano et al. (2010), Azevedo de Paula et al. (2016) and Librelon et al. (2015), who analyzed scales of Alternaria leaf spot in sunflower, Asian soybean rust, brown eye spot in coffee and angular leaf spot in common bean, respectively.

Belan et al. (2014) worked with bacterial blight in coffee and identified that evaluators without the scale overestimated severity values. This was because, when considering the yellow halo formed around the lesion, the evaluators observed differences in tonality between green and yellow, thereby increasing the subjectivity of evaluations. In this case, dos Santos et al. (2010) observed that evaluators overestimated disease severity for levels lower than 20%. Gomes et al. (2004) worked with Cercospora leaf spot of lettuce and observed that evaluators with the scale aid underestimated severity.

The estimated imprecision can be attributed to the size and shape of the lesion, coloration and number of lesions per area (Kranz 1988). Large-sized lesions may be attributed to precise estimation (González-Domínguez et al. 2014; Nuñez et al. 2017). Godoy et al. (2006) and Celoto and Papa (2010) observed that overestimation of severity was not accentuated in assessment of target spot in soybean and Barbados cherry, respectively. The shape of the lesion may have been affected by the evaluators in the work of Teramoto et al. (2011a), who underestimated target spot severity in cucumber, which is different from that observed in the present study.

The use of diagrammatic scales is subject to a certain degree of subjectivity, which can be minimized through the training of the evaluators (Nutter and Schultz 1995). Working with inexperienced raters Nuñez et al. (2017) observed overestimated assessment without the scale and cited that the use of the scale increased precision, accuracy,

Table 4 Inter-evaluator reliability (reproducibility) of visual estimates of target spot severity on 50 leaves of cotton by 10 evaluators measured by the overall concordance correlation coefficient

		OCCC	Precision	Accuracy
	Inexperienced	0.80	0.85	0.94
Without scale	Experienced	0.81	0.86	0.94
	Global	0.82	0.86	0.94
	Inexperienced	0.86	0.90	0.95
With scale	Experienced	0.95	0.96	0.99
	Global	0.90	0.93	0.97

OCCC - Overall concordance correlation coefficient repeatability, and reproducibility. Bardsley and Ngugi (2013) tested raters with different levels of experience and observed that the degree of experience affected accuracy more than reliability in estimates. The authors cited that assessment could be made by inexperienced and experienced raters with sufficient instruction.

Furthermore, inter-rater reliability was higher when evaluators used the proposed scale, thus demonstrated that the scale improved agreement estimates between evaluators. This means that assessment of the same leaf can result in closer estimates by different evaluators. The same results were found by Yadav et al. (2013), Azevedo de Paula et al. (2016) and Dolinski et al. (2017). Yadav et al. (2013) cited that the scale provides a method to raters for assessing disease more uniformly than with the scale.

In the present study, the use of Lin's statistic and linear regression method provided the same conclusions about intra-rater and inter-rater reliability, which are in accord with the results of a previous study (Nuñez et al. 2017). Despite this, some authors have recommended Lin's method for assessment of precision and accuracy rather than linear regression (Lin et al. 2002; Bock et al. 2010),

The diagrammatic scale that we developed to evaluate the severity of cotton target spot improved levels of accuracy, precision and reproducibility of the evaluations, proving to be an important tool for the evaluation of this disease in cotton crops. Accurate and precise disease assessments avoid wrong conclusions, which in turn can lead to incorrect decisions in management of disease (Bock et al. 2010) and enable the development of new management strategies such a fungicide time applications, selection of tolerant cultivars and comparison of results with different studies.

Thus, the use of the proposed diagrammatic scale may contribute to assessment of target spot in cotton leaves, with improved reliability inter- and intra-rater and accuracy of experienced and inexperienced evaluators.

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