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Short communication

A new diagrammatic scale for the assessment of northern corn leaf blight

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ABSTRACT

A diagrammatic scale was developed to assess the severity of northern corn leaf blight (NCLB), which is caused by the fungus *Setosphaeria turcica*. The validation of this new scale in relation to accuracy and precision was carried out by eight evaluators who estimated the severity of the infection on maize leaves showing NCLB symptoms, with and without the use of the scale. The new scale was also evaluated in relation to a published NCLB assessment scale. The precision and accuracy of the assessments were determined by linear regression, relating the estimated *versus* actual severity of NCLB as determined by image analysis. Using the new diagrammatic scale, evaluators were able to improve the precision and accuracy of NCLB assessments.

1. Introduction

Northern corn leaf blight (NLB) is caused by the fungus *Setosphaeria turcica* (Luttrell) K.J. Leonard & E.G. Suggs (anamorph: *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs) and is a major disease of maize (*Zea mays*) in humid climates wherever corn is grown. NCLB causes long, elliptical, gray-green lesions measuring 3–15 cm in length. As the lesions mature, they become tan with distinct, dark zones of fungal sporulation. As the disease develops further, the lesions may coalesce, forming large blighted areas, and entire leaves may become blighted (White, 1999).

Diagrammatic scales are useful resources to plant disease epidemiology and control because they help to reduce the subjectivity in visual estimates of disease severity. Visual scales are expected to be easy to use for a wide range of conditions, with reproducible, accurate and precise results (Berger, 1980). In plant breeding, diagrammatic scales are a valuable tool for identifying genetic variations in disease resistance among plant genotypes. The accuracy and precision of quantitative assessments provides less experimental errors and consequently a higher reliability of heritability estimates for disease resistance, increasing the potential gains from selective breeding. For NCLB, the first diagrammatic scale for assessing NCLB was developed by Elliot and Jenkins (1946); however, their diagrams depict the whole-plant, which can sometimes limit its use due to simultaneous occurrence of different leaf diseases. Another published diagrammatic scale, developed by Pataky (1992), has diagrams representing the severity levels on leaves with arithmetic increments, which may confuse evaluators.

Severity is undoubtedly the most sensitive criteria for screening maize genotypes based on differences in levels of resistance to NCLB. Based on what we have experienced in a popcorn breeding program through the assessment of germplasm possessing polygenic resistance against NCLB, it would be pertinent to develop and validate a new diagrammatic scale for this disease, aiming to improve resolution.

It has been widely accepted that the development of diagrammatic scales for plant disease assessment must follow some basic procedures, such as: (i) defining upper and lower limits according to the minimum and maximum severity levels observed in the field; (ii) the symptoms represented must be as close to those observed in the field; and (iii) its intermediate severity levels must be defined in accordance to the limits of human acuity, defined by Weber-Fechner stimulus-response law (Campbell and Madden, 1990). Thus, this study aimed to develop a new diagrammatic scale for NCLB assessment and to validate it for accuracy, precision and repeatability.

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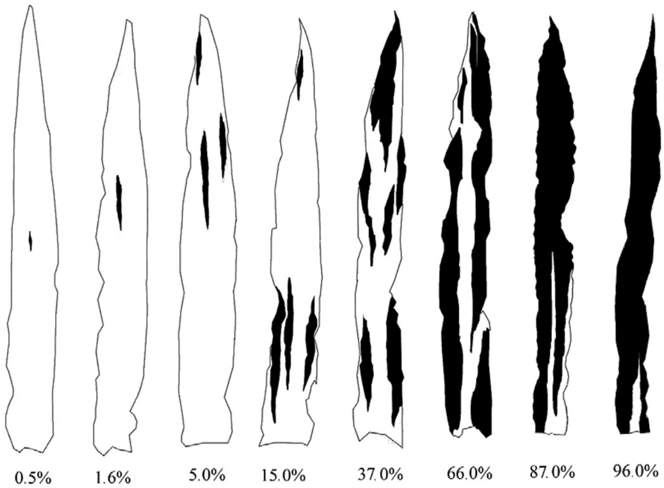

Fig. 1. Diagrammatic scale for the assessment of northern corn leaf blight severity on maize leaves.

2. Materials and methods

To develop the scale, 80 maize leaves with different levels of NLB severity were collected from the field. The leaves were photographed with a digital camera and the images were used for assessing the percentage of leaf area affected by the disease with the software package Quant v.1.0.1 (Vale et al., 2003) in order to get the actual severity data. Nine images were chosen to represent the minimum, maximum and intermediate levels of disease severity occurring in the field.

The proposed scale was validated based on an analysis of precision, accuracy and reproducibility of assessments. For this, four people without previous experience and four people with previous experience carried out a severity assessment of 40 maize leaves with different levels of NCLB symptoms. The order of the assessments was: without using a scale, new scale and Pataky's scale (Pataky, 1992).

The assessment procedures were examined using linear regression analysis. The model used was: $Y_i = \beta_0 + \beta_1 X_i + \varepsilon_i$, in which Y_i is the ith severity estimated using an assessment procedure; β_0 and β_1 are the parameters, or linear and angular coefficients; X_i is the *i*th actual severity and ε_i is the experimental error to Y_i. The assumptions of this model were $\varepsilon_i+ \sim$ NID (0, σ^2), in which NID is normally and independently distributed, and σ (ε_i+ asε_i ') equal to 0 each *i* and *i'*.

Precision of the estimates was measured in each assessment procedure through the coefficient of determination (R^2), error between actual and estimated severities and mean square error (MSE). In order to examine the accuracy of the assessments, the estimates of β_0 and β_1 (linear and angular coefficients) were tested for significance using a *t*-test. The null hypothesis was $\beta_0 = 0$ and $\beta_1 = 1$, and their acceptance indicated accuracy. The reproducibility of the estimates using each assessment procedure was measured by comparing the standard deviation among evaluators for the R^2, β_0 and β_1 criteria and for the MSE.

Statistical procedures were performed using the STAT module of SAS version 9.1 for Windows (SAS Institute, Cary, NC).

3. Results and discussion

The new diagrammatic scale has severity levels of 0.5, 1.6, 5.0, 15.0, 37.0, 66.0, 87.0, and 96.0% (Fig. 1). The diagrams also depict deformations of the leaves, which are very common when the lesions coalesce. The new scale provided lower standard deviation values, slightly higher R^2 values and slightly lower MSE values between the estimate and actual severity, indicating that it was more precise than the other two procedures (Table 1). The experienced disease evaluators were also observed to be more precise than the inexperienced evaluators.

The estimative errors of severity assessments were plotted (Fig. 2), and the errors ranged from 0.1% to 30.0%. The means of the absolute errors were 4.25% with the new scale, 4.76% when no scale was used, and 4.97% when Pataky's scale was used.

Regarding accuracy, the β_0 and β_1 estimates were significant ($P < 0.05$) for some of the evaluators for all assessment procedures (Table 1). The number of evaluators who estimated significant β parameters varied considerably among the tested procedures used for NCLB assessment, indicating that some evaluators are more accurate in their assessments than others. Only one evaluator presented significant β_0 using the new scale. On the contrary, several significant β_0 estimates were verified among evaluators using other methods (four of eight not using scales; six of eight using Pataky's scale). For examining β_1, the assessments with the

Table 1
Accuracy, precision and reproducibility of NCLB assessments using the new scale, Pataky's scale and without using a scale: coefficient of determination (R^2), linear and angular coefficients and the mean square error from regression analysis for actual *versus* estimated values of severity, and standard deviation from the estimates of the parameters.

Parameters	Evaluators[a]								Mean	Standard deviation[b]
	1	2	3	4	5	6	7	8		
Without using a scale										
R^2 coefficient	0.96	0.97	0.90	0.96	0.97	0.97	0.97	0.97	0.9588	0.02
β_0	-1.92^{ns}	9.12^{*}	14.17^{*}	3.25^{*}	0.66^{ns}	-4.36^{*}	1.13^{ns}	1.20^{ns}	2.91	6.01
β_1	1.16^{ns}	0.90^{ns}	0.94^{ns}	1.08^{ns}	1.01^{ns}	1.01^{ns}	1.05^{ns}	1.00^{ns}	1.02	0.08
Mean square error	57.99	24.60	100.62	40.41	28.15	39.26	32.34	26.46	43.73	25.38
New scale										
R^2 coefficient	0.96	0.95	0.94	0.97	0.97	0.96	0.98	0.97	0.9625	0.01
β_0	-1.19^{ns}	0.34^{ns}	8.74^{*}	0.69^{ns}	-0.30^{ns}	-2.17^{ns}	1.14^{ns}	1.00^{ns}	1.03	3.32
β_1	0.98^{ns}	0.94^{ns}	0.92^{ns}	0.99^{ns}	1.00^{ns}	1.00^{ns}	1.05^{ns}	0.95^{ns}	0.98	0.04
Mean square error	43.17	46.35	57.79	28.75	25.28	45.13	26.85	25.77	37.39	12.29
Pataky's scale										
R^2 coefficient	0.94	0.95	0.77	0.95	0.98	0.95	0.98	0.97	0.9363	0.07
β_0	3.34^{*}	6.76^{*}	31.29^{*}	4.58^{*}	0.49^{ns}	-1.49^{*}	1.75^{ns}	6.98^{*}	6.71	10.36
β_1	1.00^{ns}	0.84^{*}	0.70^{*}	0.98^{ns}	1.03^{ns}	1.07^{ns}	1.07^{ns}	0.97^{ns}	0.96	0.13
Mean square error	55.86	37.58	149.59	48.08	23.08	58.29	22.30	27.85	52.83	41.57

[*]Situation in which the null hypotheses (H_0: $\beta_0 = 0$; H_0: $\beta_1 = 1$) were rejected by *t*-test ($p < 0.05$). [ns] Not significant ($P > 0.05$).
[a] From 1 to 4: inexperienced in disease assessment; from 5 to 8: experienced.
[b] Among the parameters estimated by evaluators.

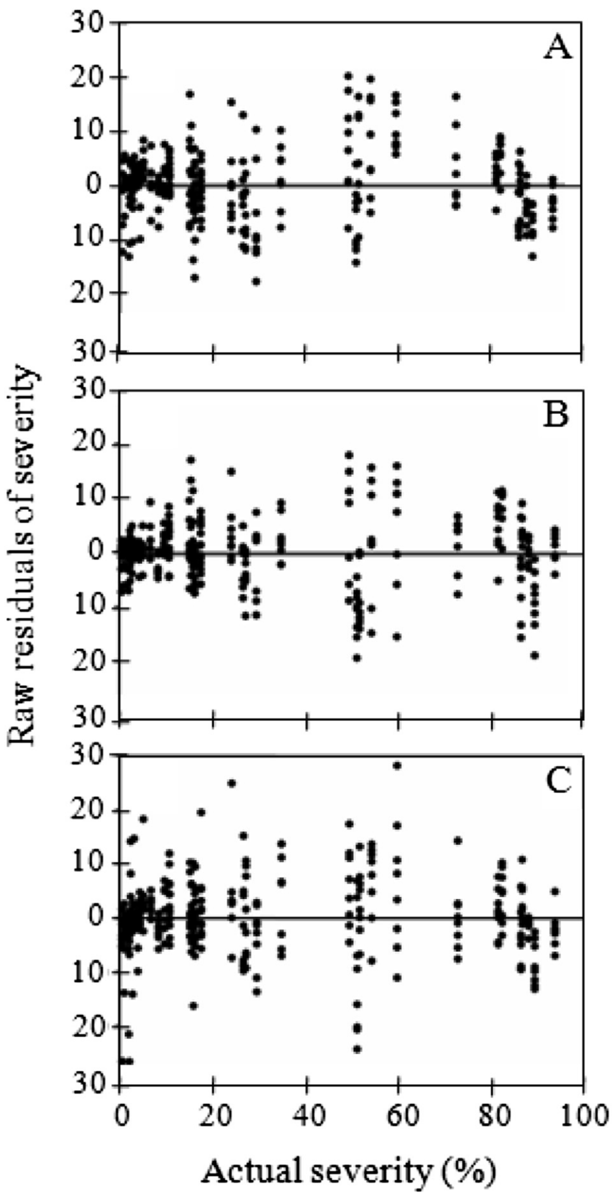

Fig. 2. Raw residuals between the actual and estimated values of northern corn leaf blight severity assessed not using scales (A); using the new scale (B); and using Pataky's scale (C). Errors of evaluators were plotted jointly.

new scale and assessments without the use of a scale did not have significant values (Table 1). On the contrary, two of eight evaluators had a significant β_1 using Pataky's scale. Thus, it was concluded that the new scale provided the highest level of accuracy with respect to the significance of the β estimates.

Moreover, it was observed that some evaluators overestimated severity when using Pataky's scale, according to the significant β_1 estimates (Table 1). Even though overestimations are not uncommon (Parker et al., 1995), the underestimation or overestimation of NCLB severity associated with the new scale was not significant (Table 1).

It is always critical to define the maximum expected severity level in diagrammatic scales because the leaves usually fall before reaching 100%. For instance, in other pathosystems such as *Phakopsora pachyrhizi*–soybean (Godoy et al., 2006) and *Phakopsora euvitis*–grapes (Angelotti et al., 2008), a maximum severity close to 90% was not found in the field. For the diagrammatic scale of maize, the maximum severity levels were 90% when using Pataky's scale (Pataky, 1992); 64% when using the scale for corn white spot (*Pantoea ananatis*) (Capucho et al., 2010) and 50% when using the scale for southern corn leaf blight (*Bipolaris maydis*) (James, 1971) and gray leaf spot (*Cercospora zea maydis*) (Smith, 1989). However, the upper limit of 96.0% was considered appropriate to NCLB because it was very common to find such a disease level in the field in popcorn, as well as in some common maize fields. The elliptical NCLB lesion has a greater expansion rate compared to other diseases, causing leaf curling, but the leaves remain attached to the plant for a longer time. This NCLB characteristic was well-rated, even without the aid of a scale. The typical shape of NCLB may facilitate severity estimates. Using the new scale, the precision and accuracy of NCLB assessments were improved.

The reproducibility of assessments among evaluators is an important criterion to judge the efficiency of diagrammatic scales (Berger, 1980). In this respect, evaluators should estimate severities as near as possible to the actual severity and these estimates should be consistent with one another. Variations among evaluators are common due to size, number and the shape of the lesion, the physiological stimulus, the sample unit, and fatigue and concentration difficulties related to the task (Sherwood et al., 1983; Kranz, 1988). In this study, reproducibility was examined as standard deviation of each parameter (Table 1). The assessments with the new scale had lower values of standard deviation than the other procedures, indicating that it was more effective in controlling variations among evaluators. Thus, the scale proposed in this paper contributes to an accurate, precise and reproducible set of tools with which to assess the severity of NLB.

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