SHORT COMMUNICATION

DEVELOPMENT AND VALIDATION OF A STANDARD AREA DIAGRAM SET FOR ASSESSMENT OF ALTERNARIA SPOT ON THE CLADODES OF THE PRICKLY PEAR CACTUS

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SUMMARY

Alternaria spot, caused by *Alternaria tenuis*, is an important disease of cladodes of the prickly pear cactus (*Nopalea cochenillifera*) in Brazil. There are no standard methods for assessment of the disease, so a standard area diagram set (SAD) was developed based on disease severity encompassing 2, 4, 7, 12, 22 and 40% of the cladode area spotted. SAD was validated by 10 raters, who analysed 50 cladodes with a range of disease severity, without and with SAD as an assessment aid. The accuracy, precision, reproducibility and repeatability of the estimates with and without the scale were determined using simple linear regression analysis. Without SAD the accuracy and precision of raters' estimates were poor, with significantly constant and systematic bias, and determination coefficients (R^2) ranging from 0.56 to 0.78. When raters employed SAD, significant reductions were observed in both constant and systematic bias, with R^2 ranging from 0.91 to 0.96. In fact, raters achieved the highest repeatability (mean R^2 = 0.95) and reproducibility (R^2 ≥0.80 in all cases) of estimates when using SAD. The proposed SAD set is suitable for assessment of Alternaria spot of prickly pear cladodes and improves the accuracy and precision of assessments.

Key words: Nopalea cochenillifera, *Alternaria tenuis*, cladodes disease, disease assessment, pathometry.

Alternaria spot, caused by the fungus *Alternaria tenuis* Nees. ex Pers., is an important disease of prickly pear cactus [*Nopalea cochenillifera* (L.) Lyons] in the dairy farms area of the semi-arid region of north-eastern Brazil. This disease was first recorded in Brazil in 2001 in the city of São Bento do Una (State of Pernambuco) and has since occurred frequently, causing serious damages to the host (Coelho *et al*., 2007). Disease symptoms consist of circular or elliptical black spots on the cladodes, 1.0 to 3.0 cm in diameter, showing abundant sporulation on their surface. With time, the spots may coalesce, causing large necrotic areas and, in more severe cases, may result in cladode perforation (Fig. 1) followed by cladode shedding (Coelho *et al*., 2007).

Accurate, precise, repeatable and reproducible estimates of disease severity are important for predicting yield losses, monitoring and forecasting epidemics, evaluating germplasm for disease resistance, and understanding fundamental biological processes, including co-evolution (Bock *et al*., 2010). Accuracy refers to the proximity level to the actual disease severity of a severity estimate. Precision is the variability associated with estimates; repeatability (intra-rater reliability) is how close are to one another repeated measures taken by the same rater, and reproducibility (inter-rater reliability) refers to the variation in estimates when the same sample is assessed by a different rater (Nutter *et al*., 2006).

Various techniques have been used to measure disease severity in plants, i.e. visual estimates, digital photography and image analysis, and hyperspectral imaging. However, visual rating methods are, and will continue to be, the single most important approach for assessing plant disease for the foreseeable future (Bock *et al*., 2010).

Inadequate visual estimates of disease severity results in inaccurate, imprecise and poorly reproducible data which can mislead from meaningful analysis and interpretation. To minimize these errors, selected assessment methods should allow an estimate as close as possible to the actual level of disease severity. Standard area diagram sets (SAD) can be employed to minimize disease assessment errors, and consists of a series of illustrations of whole plants or plant parts presenting different levels of disease severity (Nutter and Esker, 2001; Nutter Jr. *et al*., 2006; Madden *et al*., 2007).

In designing a SAD set, it is important to define the upper limit of disease severity likely to be encountered in the field, and make sure that the range of diagrams represents disease severities that, by reference to the SAD, will allow raters to accurately estimate the severity of an unknown leaf sample by a process of interpolation. Furthermore, it is critical to determine improve-

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ment in the quality of the estimates provided by use of SAD by judging the accuracy, precision, repeatability and reproducibility of assessments with and without their use (Nutter Jr. and Schultz, 1995; Bock *et al*., 2010).

Since no standard method exists for assessing the severity of Alternaria spot of prickly pear cladodes, the objective of this study was to develop a SAD to improve and standardize the assessment of disease severity and analyze the accuracy, precision and reproducibility of the estimates with its use.

To develop the diagrammatic scale, 300 prickly pear cladodes with a range of Alternaria spot severity were collected from commercial crops in the cities of Garanhuns and São João (Pernambuco). A digital camera was used to acquire images (resolution of 200 dpi) of the cladodes, and the percent diseased area was determined on each cladode using Assess V2.0 (American Phytopathological Society, USA).

The maximum severity of Alternaria spot on cladodes collected from a commercial plantation was 39.7%. Taking this value as the maximum disease severity, a SAD was chosen with six levels of severity, i.e. 2, 4, 7, 12, 22 and 40% of diseased cladode surface (Fig. 2). The shape and distribution patterns of the spots on the diagrams were positioned to reflect the appearance of infected cladodes observed in the field.

To validate the SAD set, 50 images of cladodes with a range of Alternaria spot symptom severity were photocopied in colour and disease severity was estimated by 10 raters with no previous experience in plant disease estimation. First, the 10 raters assessed disease severity without using SAD, and after a fortnight they assessed the same images using SAD as an assessment aid. Following a further 15-day interval, to assess the repeatability of previous estimates using SAD, the same cladodes were randomly mixed and the same raters re-evaluated disease severity aided by SAD.

The accuracy and precision of each rater's estimates were determined with linear regression analysis. Actual disease severity (measured using image analysis) was the independent variable (*X*-axis), and the severity estimated by the rater was the dependent variable (*Y*-axis). The accuracy of the estimates of each rater, and the mean accuracy of all raters combined was determined using a Student's *t*-test to determine whether the intercept (*a*) of the linear regression was = 0 (at P = 0.05), and whether the slope (*b*) of the linear regression was = 1 (at P = 0.05). Intercept values significantly different from 0 (zero) might indicate constant bias by either overestimation (> 0) or underestimation (< 0), whereas slope coefficients that deviate significantly from 1 (one) indicate

Fig. 1. Cladodes of prickly pear cactus with symptoms of Alternaria spot.

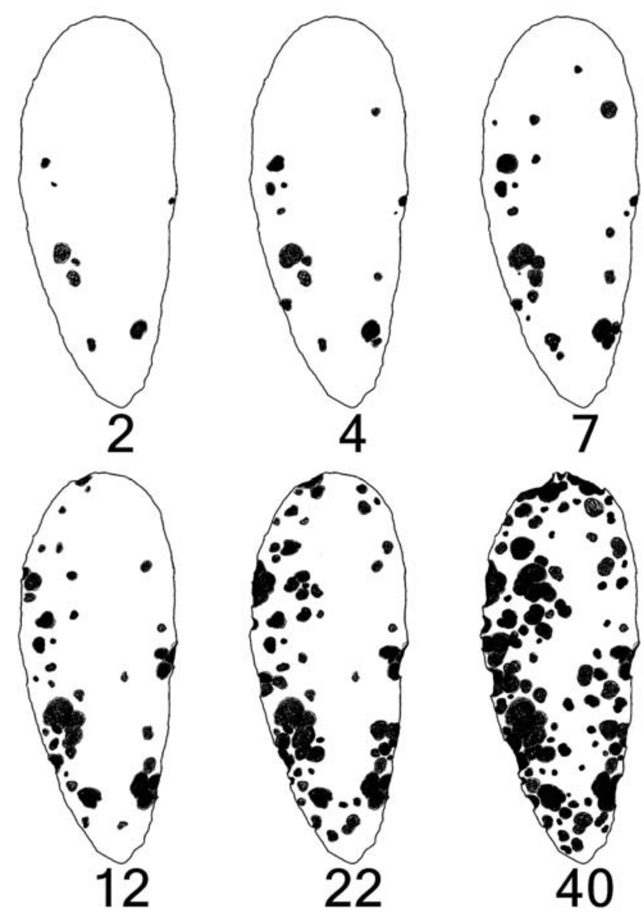

Fig. 2. The standard area diagram set for assessment of Alternaria spot on cladodes of prickly pear cactus with severity levels of 2, 4, 7, 12, 22 and 40% of the area infected.

Table 1. Accuracy and precision of estimates of the severity of Alternaria spot on cladodes of prickly pear cactus, based on the intercept (*a*), slope (*b*) and coefficient of determination (R^2) of the linear regression of actual severity (independent variable) versus estimated severity (dependent variable). Disease severity was assessed by 10 raters, once without the use of a standard area diagram set (SAD), and twice with the use of SAD.

Rater	Without SAD			Using SAD					
				First assessment			Second assessment		
	a	*b*	R^2	*a*	*b*	R^2	*a*	*b*	R^2
A	0.80	0.65*	0.70	0.04	0.94	0.95	0.03	0.94	0.93
B	6.79*	0.87*	0.67	3.90*	0.93	0.92	4.22*	0.94	0.90
C	1.27	1.32*	0.70	-1.01	0.97	0.91	-1.42	1.02	0.92
D	1.95	0.79*	0.73	2.52*	1.05	0.96	2.79*	1.02	0.95
E	11.20*	1.52*	0.75	1.45	0.97	0.93	1.46	0.98	0.94
F	6.38*	1.44*	0.78	-0.31	0.88*	0.91	-0.58	0.91	0.90
G	-2.10	1.44*	0.71	-1.01	1.12*	0.92	-0.04	1.04	0.91
H	-2.54*	0.59*	0.70	0.11	0.93	0.94	-0.04	1.05	0.91
I	5.81*	1.33*	0.72	1.31	0.98	0.91	0.17	0.92	0.94
J	2.32	0.87*	0.56	0.01	0.85*	0.92	1.86*	0.94	0.92

* Indicates that the intercept (a) is significantly different from 0, or that the slope (b) is significantly different from 1 using a t-test (P=0.05).

systematic bias by either overestimation (> 1) or underestimation (< 1). The estimate precision was determined by the coefficient of determination of regression (R^2), by the absolute error of the estimates (estimated severity minus actual severity) and by the repeatability of the estimates, as determined by linear regression analysis of the first and second assessments of the same sampling units by each rater. The reproducibility of the estimates was also determined by the R^2 values obtained from linear regressions analysis between the estimated severity of the same sampling unit by different raters in all pairwise combinations (Nutter Jr. and Schultz, 1995). The statistical analyses were performed using Microsoft Excel 2003 (Microsoft Corporation, USA).

Without the use of SAD raters were less accurate (Table 1). Intercept values of the regression were significantly different from zero for five (50%) of the raters, and the slopes of the regression were significantly different from one for all ten (100%) raters. Among raters, only one (H) significantly underestimated disease severity, thus confirming the tendency of raters to overestimation, which is particularly frequent at low disease severities (Sherwood *et al.*, 1983). Overall, five raters tended towards overestimation (C, E, F, G and I) and five towards underestimation (A, B, D, H and J, Table 1). The precision of the unaided estimates was low (coefficients of determination, R^2, ranging from 0.56 to 0.78). The distribution of residuals of the estimates (the

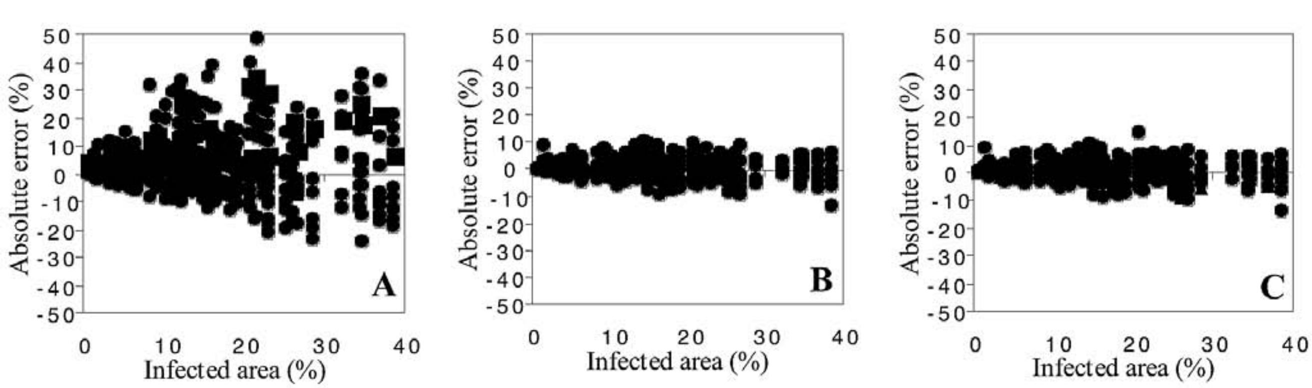

Fig. 3. Absolute errors (estimated severity - actual severity) of estimates of the severity of Alternaria spot on cladodes of prickly pear cactus without use of a standard area diagram (SAD) set (A) and with use of a SAD set for the first (B) and second (C) evaluation.

Table 2. Repeatability of rater estimates of the severity of Alternaria spot on cladodes of prickly pear cactus, based on the intercept (*a*), slope (*b*) and coefficient of determination (R^2) of linear regression analysis between estimates of disease severity made by the same rater on two occasions 15 days apart and using a standard area diagram set (SAD) to aid assessment.

Rater	a	b	R^2
A	0.04	0.99	0.97
B	0.29	1.01	0.98
C	-2.49*	0.95	0.90
D	0.43	0.96	0.96
E	1.18	0.91	0.92
F	-0.20	1.04	0.98
G	0.85	0.88	0.94
H	0.55	0.89	0.95
I	0.77	0.95	0.98
J	0.39	1.00	0.96

*Indicates that the intercept (a) is significantly different from 0, or that the slope (b) is significantly different from 1 using a t-test (P=0.05).

absolute error) without the aid of SAD revealed that estimates with absolute errors ranged from -24.69 to 48.38 (Fig. 3A). The absolute error was heterogeneous, the highest occurring with increasing actual disease severity, and the lowest at disease severity <10%.

The use of SAD, improved the accuracy and precision of the estimates by most raters (Table 1). As to accuracy, only two raters (B and D) had intercept values significantly different from zero on the first assessment, and three (B, D and J) had intercept values significantly different from zero on the second assessment. The slope of the regression of the estimate was not different to 1 for 7 (70%) of the raters in the first assessment, and not different to 1 for 10 (100%) of the raters in the second assessment, respectively. SAD use improved also the precision of the estimates by all rater ($R^2 = 0.91$ to 0.96 for the first assessment, and 0.90 to 0.95 for the second assessment).

There was a reduction in absolute errors when SAD was used as an aid to assessment (Fig. 3B and C). In fact, absolute errors were more homogeneous, similar values being found across the spectrum of Alternaria spot severity, and ranged from -13.48 to 10.68 on the first assessment and from -8.56 to 12.13 on the second assessment (Fig. 3C). Absolute errors were commonly < 10% using SAD, which can be retained as a good result based on the criteria adopted in previous studies (Tovar-Soto *et al.*, 2002; Corrêa *et al.*, 2009; Michereff *et al.*, 2009; Santos *et al.*, 2010). Like the majority of methods for quantifying the severity of diseases, the use of SAD is subject to some degree of subjectivity, and computer-based training in assessment of disease severity might reduce this error further (Nutter Jr. and Worawitlikit, 1989; Nutter Jr. and Schultz, 1995; Nutter Jr. and Esker, 2001).

Raters generally demonstrated good repeatability in estimating the severity of Alternaria spot when using SAD (Table 2). In the two SAD-aided assessments, severity estimates of only one rater (C) had an intercept value significantly different from zero, whereas the estimates of all other raters had slope coefficients that did not differ significantly from 1.

Without SAD, reproducibility of assessments was poor [coefficient of determination (R^2) only 2.2% of the time] (Table 3). The contrary was true with SAD for coefficients of determinations on both assessments was ≥0.80.

In conclusion, SAD set for assessment of Alternaria spot severity on prickly pear cladodes proved easy to use and capable of providing accurate, precise, repeatable and reproducible estimates. This approach should be of value for application in field surveys, epidemiological studies and for evaluating disease control measures.

Table 3. Reproducibility estimates of the severity of Alternaria spot on cladodes of prickly pear cactus, represented by the frequency of coefficient of determination values (R^2) in particular intervals based on linear regression analysis between all parwise combinations of 10 raters, without and with the use of a standard area diagram set (SAD) to aid assessment.

Coefficient of determination intervals (R^2)	Frequency (%)*		
	Without SAD	Using SAD	
		First assessment	Second assessment
0.40 – 0.49	33.3	0.0	0.0
0.50 – 0.59	28.9	0.0	0.0
0.60 – 0.69	20.0	0.0	0.0
0.70 – 0.79	15.6	0.0	0.0
0.80 – 0.89	2.2	64.0	51.0
0.90 – 1.00	0.0	36.0	49.0

* Calculated based on the number of occurrences in the range compared to the total of 45 possible combinations among the 10 raters in each assessment of disease severity.

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