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RESEARCH ARTICLE

Development and Validation of a Diagrammatic Scale for Estimation of Cashew Blight for Epidemiological Studies

***D. Menge[1], M. Makobe[1], S.Shomari[2] and A. V. Tiedemann[3]**

1. Jomo Kenyatta University Agriculture and Technology (JKUAT); P. O. Box 62000-00100 Nairobi, Kenya.
2.Cashew Research Programme, Naliendele Agricultural Research Institute (NARI), P.O. Box 509, Mtwara, Tanzania.
3. University of Göttingen, Grisebachstrasse 6, 37077 Göttingen, Germany.

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Abstract

Cashew (*Anacardiumoccidentale* L.) blight is caused by the fungal pathogen *Cryptosporiopsis* spp. The objective of this study was to develop a scale based according to Weber-Fechner's visual acuity law, considering the lowest and highest limits of severity observed in the field. A diagrammatic scale with six levels of disease severity (0.1-3.0; 3.1-6.0; 6.1-12.0; 12.1-25.0; 25.1-50.0; 50.1-75.0%) was developed to assess cashew blight severity, caused by the fungus *Cryptosporiopsis*spp. Scale validation was performed by six raters (three inexperienced and three experienced), who estimated the severity of 48 cashew leaves showing blight symptoms, with and without the use of the scale. Precision and accuracy were determined by linear regression, relating the assessments using the scales to actual severity. Actual severity was assessed with the software Scion image. With the scale, the raters obtained better accuracy and precision levels, although the tendency to overestimate was maintained. Experienced raters were more accurate and precise than inexperienced raters, and assessment improvements with the use of the scale were more significant for inexperienced raters. Two evaluations were performed on the same set of leaves, but in a different sequence order, by the same raters. Using the diagrammatic scales, some raters were able to improve precision and accuracy. Therefore, we could conclude that the diagrammatic scale presented here was suitable for evaluating cashew blight severity.

Introduction

Cashewis an important tropical tree crop and in terms of international trade for major edible nuts it ranks second or third. The main producer countries are Brazil, India, Mozambique and Tanzania. Cashew blight is one of the most important cashew diseases in East Africa. Leaf and nut blight caused by *Cryptosporiopsis*spp is a major limiting factor affecting cashew nut production in Tanzania, causing 48.4% crop loss annually (ACRR, 2006). Blight symptoms start showing up in young tender leaves as irregular brown spots limited by the veins. Following, lesions may coalesce and become dark brown.

****Corresponding author:*** dominicmenge@yahoo.co.uk,

Cashew leaf and nut blight pathogen is typical of *Cryptosporiopsis* genus having ellipsoidal, rounded at the apex, tapering into a slightly protruding scar at the base (Menge*et al.*, 2013). Severe infection may result in leaf defoliation and malformed, underdeveloped nuts. For any program of integrated disease management to succeed, it is crucial to assess disease intensity with accuracy and precision. Accuracy refers to how faithful an estimate is from the actual amount of the evaluated disease, while precision corresponds to the confidence and/or repeatability associated to such estimate (Nutter Jr. and Schultz, 1995). Considering that blight is a leaf and nut spot, its intensity is more appropriately

expressed by assessing severity, i.e., the percent or proportion of affected leaf area. Most of the times, disease severity is assessed visually. Therefore, it is prone to large subjectivity, which can give rise to significant errors in accuracy and precision (Nutter Jr. *et al.,* 2006).Considering that disease assessment must be easy and quick to perform in a broad range of conditions, and even then produce accurate, precise, and reproducible estimates (Campbell and Madden, 1990), the use of diagrammatic scales reduces subjectivity in severity estimates and is a good aid to the disease rater, once these scales are very functional. Considering the growing need for studies on the epidemiology and control of cashew blight, associated with the absence of standardized methods for quantification of the disease, the objective of this work was to develop and validate a diagrammatic scale for rust severity assessment in cashew.

Materials and methods

Assessment of disease severity

Leaflets were collected in late June, when a wide range of severities are observed in the field.Leaflets came from different plants and were selected so that a wide range of severity values would be obtained. Selection of leaflets was done by an individual not rating the leaflets for severity. All leaves were obtained from naturally infected plants reflecting a range of disease occurring in the field. Leaf and lesion age varied.To develop the diagrammatic scale, 210 cashew leaves, with distinct levels of leaf blight severity, were collected in an experimental field in Mtwara, Tanzania.

A total of 210 leaves were digitized on a flatbed scanner (HP Officejet 4500 Wireless) at 200dpi resolution, and the images were printed (4 per page) to provide images close to the actual size of the leaf. Infected leaves were scanned as previously described and saved as TIFF files. The images were assessed by six raters (three experienced and three inexperienced) and by image analysis using Scion image analysis Software. Image analysis provides a way of assessing disease that can remove considerable bias and error, and performs lesions counts and measures the % area infected. The percent of disease affected area was determined in each image and used as the disease severity level. A diagrammatic scale was then developed, with sixdisease severity levels, based on the Weber-Fechner visual accuracy law (Horsfall and Cowling, 1978) and on the highest severity level observed in the collected leaves. In the scale, we tried to reproduce patterns, shapes, and the lesion scattering standard, as observed in the sampled leaves.

Validation of the diagrammatic scale

The validation of the proposed scale was performed based on images of 48 cashew leaves showing blight symptoms at different severity levels, assessed by four persons, and divided into two groups: inexperienced and experienced in disease scoring. In order to estimate severity, the colorized leaf images were used, where necrotic tissue were considered as diseased area. The four raters performed an initial estimate without the scale and another immediately afterward using the scale proposed in this work. By comparing the data estimated by the raters with and without the aid of the diagrammatic scale (estimated severity), with the actual severity obtained with Scion Image, the accuracy and precision of assessments was verified, as well as the influence of the use of the diagrammatic scale. The accuracyand precision of the assessments of each rater were determined by linear regression, where actual severity was the independent variable and estimated severity the dependent variable. The accuracy of the estimates of each rater was determined by t-test applied to the slope coefficient (*b*), to check whether they were significantly different from 1.0, and to the intercept (*a*) to check whether they were significantly different from 0. The precision of the assessments was estimated by the coefficient of determination (R) of the same regression line and by the variance of the absolute errors (estimated severity minus actual severity) for each assessment (Nutter Jr. &Schults, 1995).

Results

The values for the lower and upper limits of the diagrammatic scale devised in this work, with six severity levels, were 0.2 and 67.4% of the leaf area with lesions (Fig 1). The symptoms of the disease were chlorotic spots on the youngest tender leaves on both sides. Leaf spots occurred on both sides of the leaves and varied in size, shape and color, within and between cashews. The highest severity value observed in the 210 harvested cashew leaves was 67.4%. Values above 70% are rarely found in the field, since blight severity reduces with the maturity of the leaves. All raters showed a tendency to overestimate severity without the aid of the diagrammatic scale (Fig 1). This tendency was observed in both experienced and inexperienced raters. There was a tendency to overestimate the disease severity when the number of lesions is higher and their size smaller. The analysis of accuracy was applied to verify how close the estimate severity levels were to actual severity levels (Nutter Jr. and Schultz, 1995). Figure 1 shows the linear regressions

obtained between actual and estimated severity for all raters, without the scales. Without aid of the diagrammatic scale, the values of the slope coefficients (b) were significantly different from 1.0 for raters 1, 2, 3, and 4, meaning that the assessments done by these raters were more accurate.

With the aid of a diagrammatic scale, overestimation was decreased (Fig 2). The precision of the visual estimates of blight in cashew with the aid of the scale was similar to those observed in recent studies for validation of diagrammatic scales (Sami *et al.,* 2009; Barbosa *et al.,* 2006; Godoy *et al.,* 2006). A standard system to guide severity evaluation for blight disease is a considerable responsibility. Systems that are defective have heavier costs compared to benefits (Leite and Amorim, 2002). The use of a standard system for disease evaluation is the most effective way to allow the confrontation of results from different groups, institutions and places (BergaminFilho and Amorim, 1996). The analysis of precision was applied to verify the repeatability or variation associated with an estimate, regardless of the mean value. Precision is estimated by the coefficient of determination (R^2) and by the variance of the absolute errors (estimated severity minus actual severity) (Nutter and Schultz, 1995). R^2 values for experienced raters ranged from 0.77 to 0.96 with the scale and 0.64 to 0.71 without the scale. As for the inexperienced raters, the R^2 values ranged from 0.24 to 0.59 without the use of the scale and 0.71 to 0.91 with the scale. Precision estimated by the coefficient of determination ($R^{2)}$ and the variance of the absolute errors was higher with the use of the diagrammatic scale for both experienced and inexperienced raters (Table 1, Figures 3 and 4). The degree of proximity of a mean estimate and reality measured by the intercepts (a) and by the slope coefficients (b) of the regression line between actual and line estimated severity, was higher for experienced raters with and without use of the scale (Table 1, Figures 1 and 2).

With the aid of the diagrammatic scale the differences between absolute errors of inexperienced raters were concentrated in the 0-20% range while the experienced raters in the 15 % range (Fig 3). There was a decrease in the absolute error of the estimations with the aid of the diagrammatic scale proposed, where the points of residuals were well distributed throughout when compared to the values of absolute error of obtained estimates without the use of the proposed scale. The absolute errors of the estimates for the diagrammatic scale represented here varied in with and without the use of diagrammatic scale between -40 and 24, and - 86 and 24, respectively. Without the use of diagrammatic scale the absolute errors of inexperienced raters were concentrated in the range of 20-40%, and for experienced raters in the range from 5-20% (Fig 3).

TABLE 1 - Intercepts (*a*), slope coefficients (*b*), and coefficients of determination (R) of the regression line for actual (independent variable) versus estimated severity (dependent variable) of cashewblight for six raters (1 to 3 inexperienced and without previous contact with the scale, 4 to 6 experienced and with previous contact with the scale) with and without the diagrammatic scale

	without scale			**with scale**		
Rater	**a**	**b**	**R^2**	**a**	**b**	**R^2**
Inexperienced						
1	10.91	1.08	0.49	-1.05	0.94	0.91
2	3.30	0.87	0.59	-0.68	0.89	0.90
3	48.17	0.82	0.24	13.60	1.05	0.71
Experienced						
4	7.56	1.00	0.64	1.90	0.92	0.77
5	13.60	1.05	0.71	1.19	0.97	0.95
6	11.83	0.99	0.67	1.66	0.96	0.96

Figures

FIG. 1 - Estimated severity of cashew (*Anacardiumoccidentale*)blight without the aid of the diagrammatic scale and linear regressions obtained between actual and estimated severity (solid line). Dotted lines represent an ideal situation, with estimated severity equal to actual severity. Raters 1 to 3 are inexperienced, without previous contact with the scale; raters 4 to 6 are experienced and have used the scale previously.

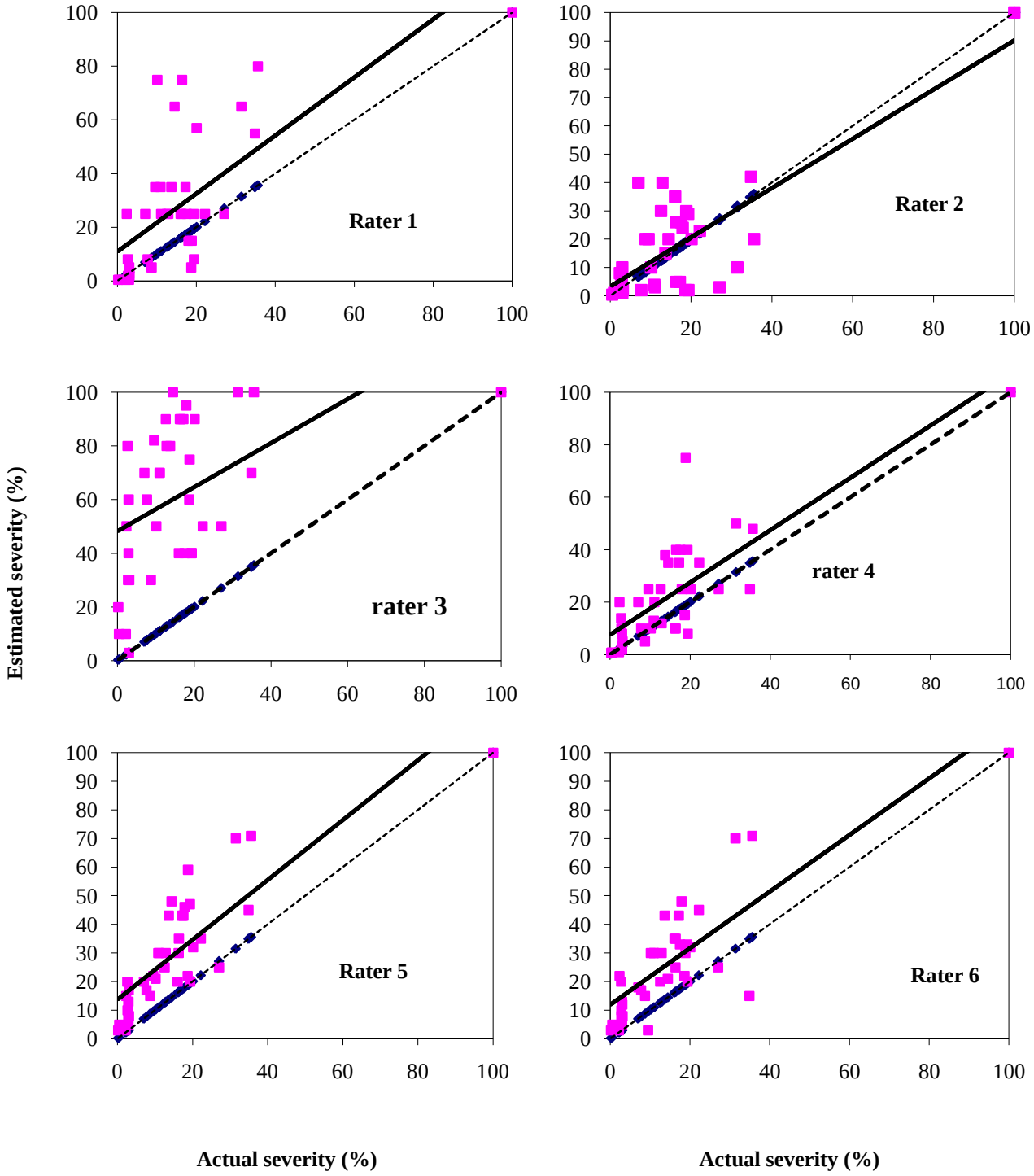
FIG. 2 - Estimated severity of cashew (*Anacardiumoccidentale*)blight with the aid of the diagrammatic scale and linear regressions obtained between actual and estimated severity (solid line). Dotted lines represent an ideal situation, with estimated severity equal to actual severity. Raters 1 to 3 are inexperienced, without previous contact with the scale; raters 4 to 6 are experienced and have used the scale previously.

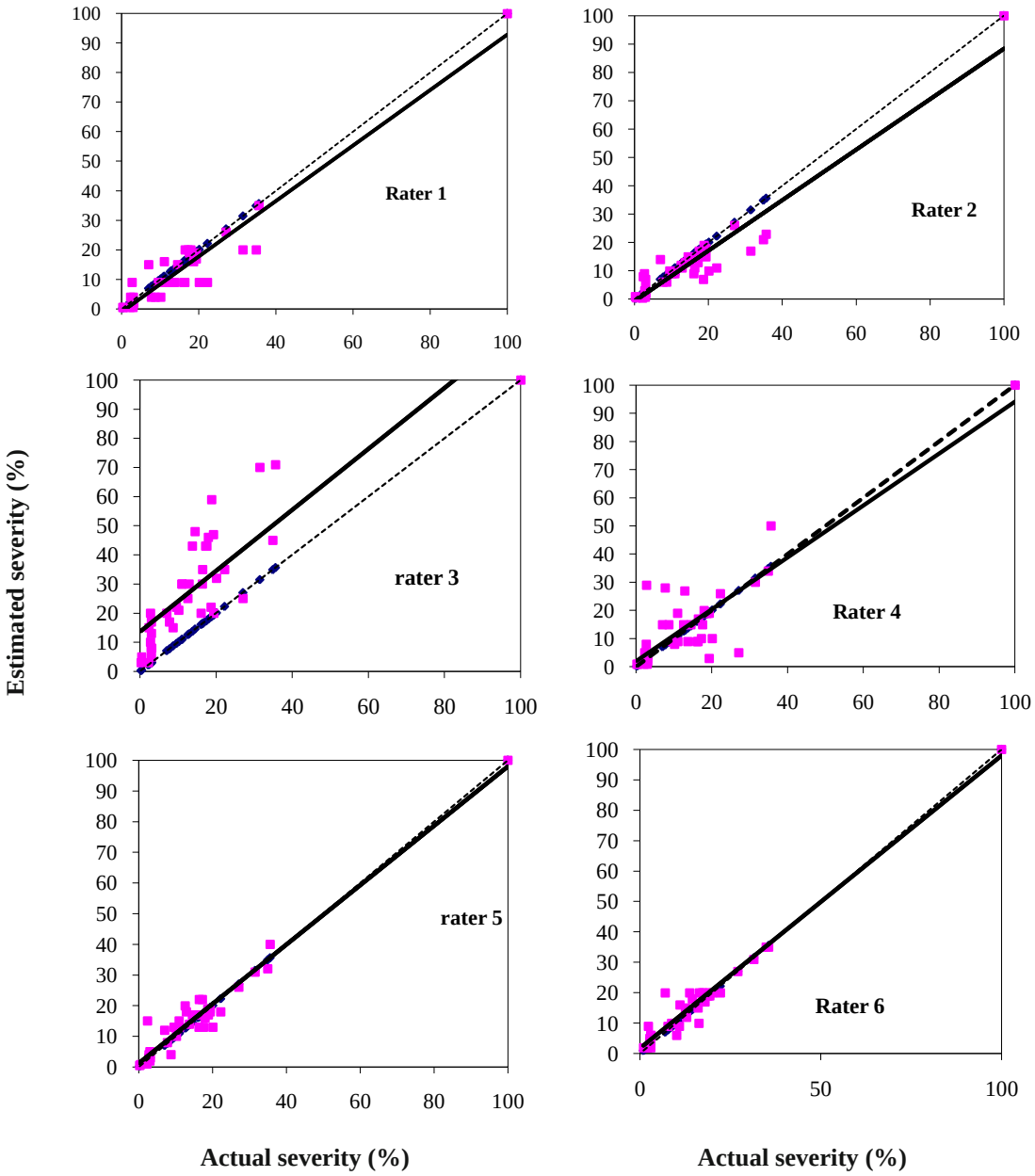
FIG. 3 - Absolute errors (actual severity – estimated severity) of cashew (*Anacardiumoccidentale*)blight for assessments with the aid of the diagrammatic scale. Raters 1 to 3 are inexperienced, without previous contact with the scale; raters 4 to 6 are experienced and have used the scale previously.

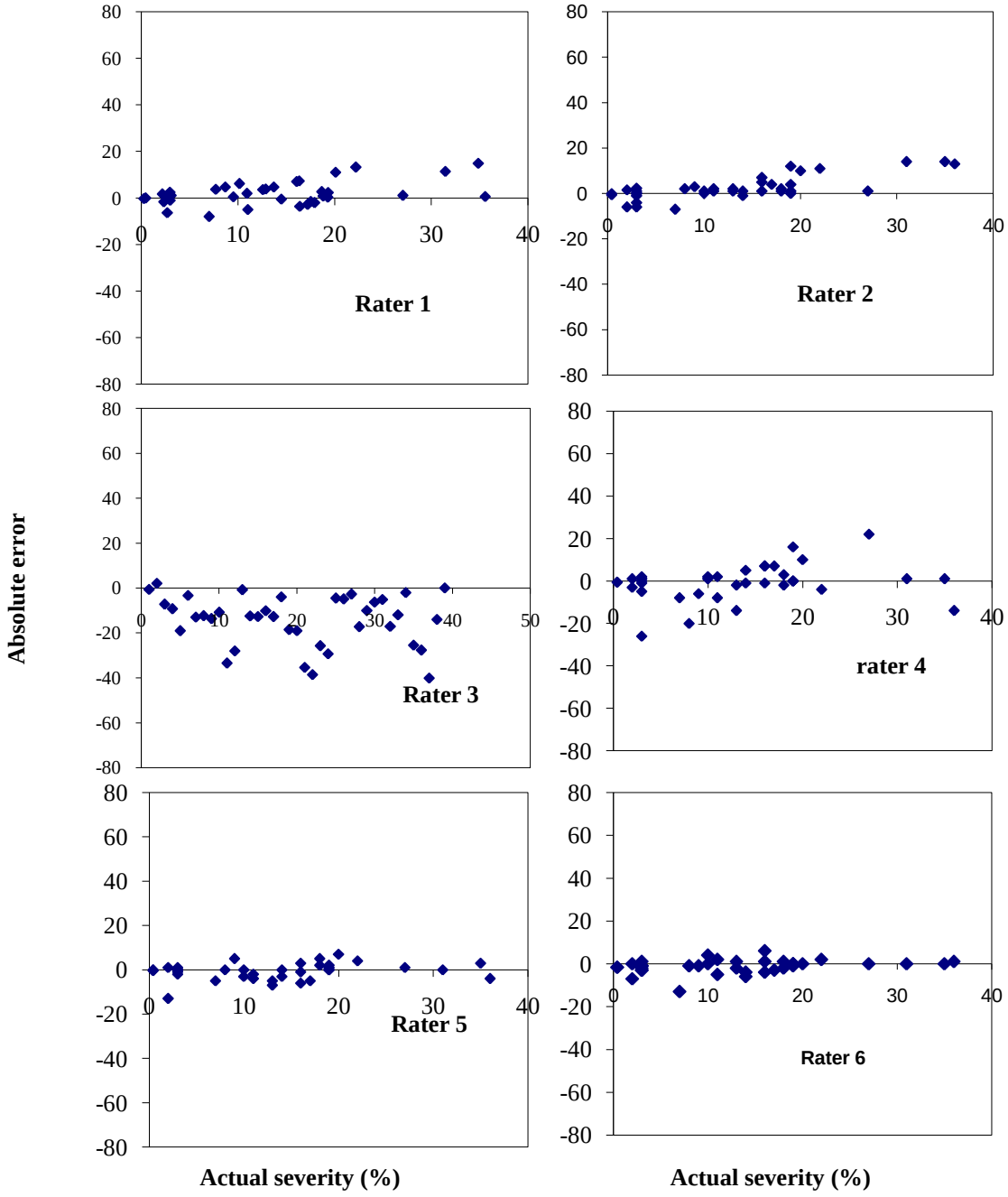
FIG. 4 - Absolute errors (actual severity – estimated severity) for assessments of cashew (*Anacardiumoccidentale*)blight without the aid of the diagrammatic scale. Raters 1 to 3 are inexperienced, without previous contact with the scale; raters 4 to 6 are experienced and have used the scale previously.

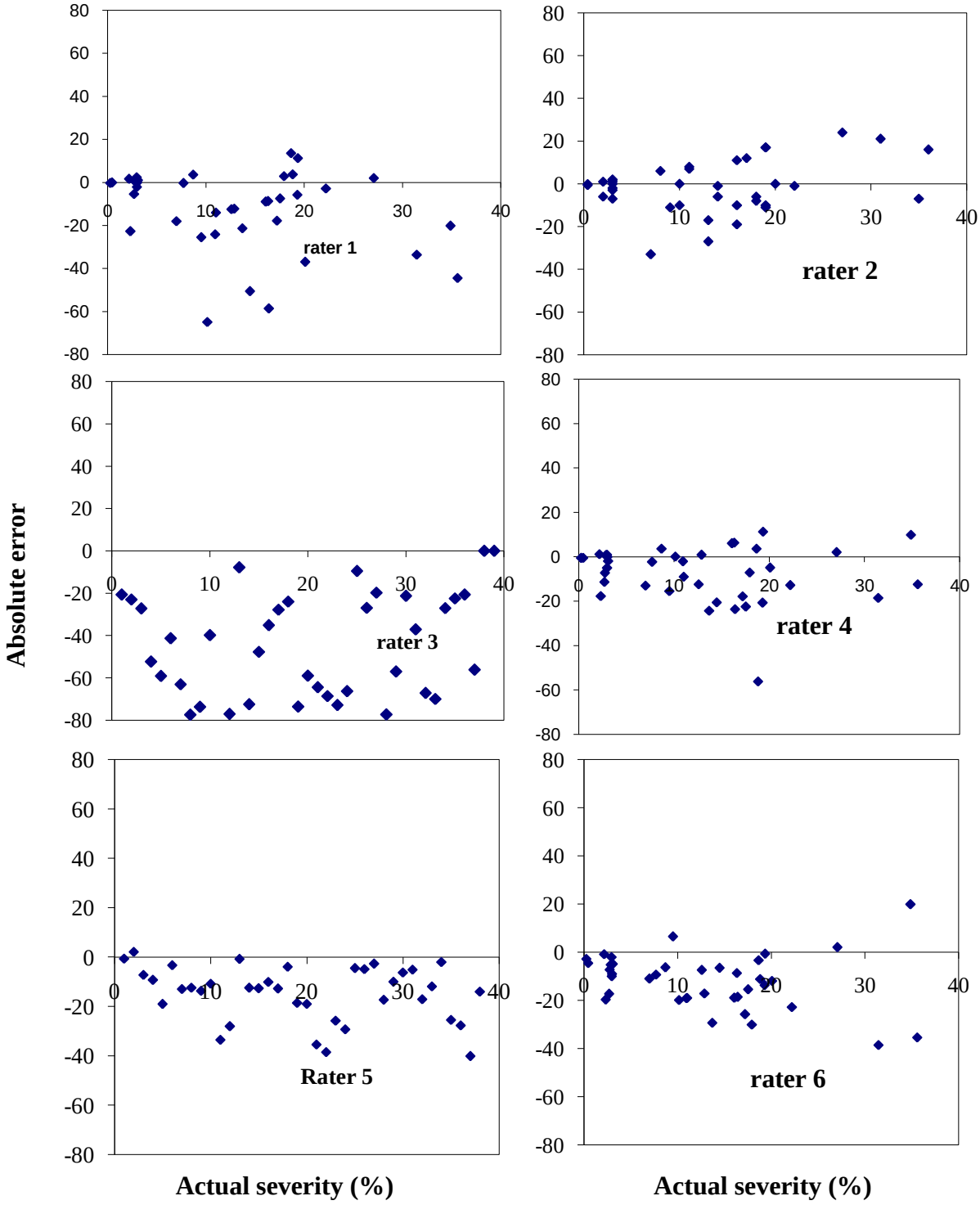
FIG. 5 - Absolute errors (actual severity – estimated severity) for assessments of cashew (*Anacardiumoccidentale*)blight without the aid of the diagrammatic scale. Raters 1 to 3 are inexperienced, without previous contact with the scale; raters 4 to 6 are experienced and have used the scale previously.

FIG. 6 – Diagrammatic scale of the severity of cashew blight


Rater 1
Rater 2
rater 3
rater 4
Rater 5
Rater 6
Estimated severity (%)
Actual severity (%)
Actual severity (%)


Rater 1
Rater 2
rater 3
Rater 4
rater 5
Rater 6
Estimated severity (%)
Actual severity (%)
Actual severity (%)


Absolute error
Rater 1
Rater 2
Rater 3
rater 4
Rater 5
Rater 6
Actual severity (%)
Actual severity (%)


Absolute error
rater 1
rater 2
rater 3
rater 4
Rater 5
rater 6
Actual severity (%)
Actual severity (%)

Level 1 (0.1 – 3.0%)	 **0.2**	 **1.0**	 **2.6**
Level 2 (3.1 – 6.0%)	 **3.1**	 **4.3**	 **5.4**
Level 3 (6.1– 12.0%)	 **7.2**	 **9.8**	 **10.6**

Level 4 (12.1– 25.0%)	 **13.4**	 **20.9**	 **22.2**
Level 5 (25.1 – 50.0%)	 **28.5**	 **35.6**	 **50.0**
Level 6 (50.1 – 75.0%)	 **51.2**	 **59.0**	 **67.4**

Discussion

The diagrammatic scale developed here improved precision, accuracy and reproducibility of blight disease assessment against actual values measured by image analysis. Images captured with a flatbed scanner or digital camera can be analyzed with a freely available software package, Scion Image to measure changes in leaf color caused by fungal sporulation or tissue damage. This method was adapted to quantify percent diseased leaf area ranged from 0% to 67.4% for cashew blight disease. Digital Image analysis using Scion Image can be adapted to detect early and quantify cashew blight disease.Andrade *et al.,* (2005) developed similar diagrammatic scale to assess leaf spot (*Quambalaria eucalypti*) of Eucalyptus and found improvements in the precision, accuracy and reproducibility. The scale developed here reduced error especially in inexperienced raters. This has been reported by Godoy *et al.,* (2006) in assessing soybean rust (*Phakosporapachyrhizi*) severity. With the use of the diagrammatic scale, all the raters provided more accurate values of intercept equal zero for the straight line regression between actual and estimated severity. Without the use of the scale, generalized overestimation of the cashew severity occurred. The overestimation of disease severity levels without the use of the diagrammatic scales has also been reported by some authors (Spósito*et al.,* 2004; Michereff*et al.,* 2006; Salgado *et al.,* 2009; Adriano *et al.,* 2011). The estimation of cashew blight severity with fewer larger lesions was less error prone as compared to small, random lesions. Forbes and Jeger (1987) have reported the effects of large lesions on disease severity estimation. The differences observed among raters in the estimation of cashew blight might be attributed to the human distinct ability to discriminate disease leaves. The quality of the disease estimates has been shown to be influenced by physiological stimuli and responses (Godoy *et al.,* 2006). Factors such as complexity of the sampling unit, size and shape of lesions, color and number of lesions, fatigue and difficulty to concentrate on the task have shown to affect the estimates (Kranz, 1998; Sherwood *et al.,* 1983). Rater training has a positive influence on the quality of assessments. Similar studies have been observed by Godoy *et al.* (2006) in the diagrammatic scale for assessment of soybean rust severity. The absolute errors with the aid of a diagrammatic a scale in both experienced and inexperienced raters were concentrated at around 15% which is fairly acceptable in evaluations of diagrammatic scales. Godoy *et al.,* (2006) observed most errors for assessment of soybean rust were in the range from 20 to 50% for inexperienced raters, and from 10 to 20% for experienced raters; and Andrade *et al.,* (2005) observed that most errors for assessment of eucalyptus leaf spot ranged from 15 to 30% of severity, for either inexperienced or experienced raters. The absolute errors can be further reduced by training the raters as shown by Nutter Jr. *et al.,* 2006. In order to reduce the subjectivity of disease severity visual estimates, diagrammatic scales have been developed for many crops (Amorim*et al.,* 1993; Godoy *et al.,* 1997; Giglioti and Canteri, 1998; Michereff*et al*., 2000; Leite andAmorim, 2002; Martins *et al.,* 2004; Godoy *et al.,* 2006). Some aspects must be considered in the preparation of diagrammatic scales: a) the lower and upper limits of the scale must correspond, respectively, to the minimum and maximum amounts of the disease observed in the field; b) the determination of the actual intensity of the disease in the field and its representation in the scale must have high precision; c) the intermediate levels must respect human visual acuity limitations as defined by Weber-Fechner stimulus-response law, where visual acuity is proportional to the logarithm of the stimulus intensity (Horsfall and Barratt, 1945; James, 1974; BergaminFilho andAmorim, 1996). The diagrammatic scale presented here to assess leaf blight severity in cashew was effective in providing a quick disease estimate with reasonable accuracy and excellent precision and reproducibility. The use of the scale will be important in the assessment of fungicide assays for the control of cashew blight disease and in the construction of blight disease progress curves. It will be a valuable tool for field surveys, epidemiological studies and comparison among disease control methods.

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