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Diagrammatic scale to assess downy mildew severity in melon

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ABSTRACT

The downy mildew, caused by *Pseudoperonospora cubensis*, is an important melon disease in Northeast Brazil. Considering the lack of standard methods for its assessment, a diagrammatic scale was developed with 2, 4, 8, 16, 32, 64, 82, and 96% of affected leaf area. The scale was then checked for its accuracy, precision, and reproducibility in estimating downy mildew severity. The diagrammatic scale was validated by eight disease raters; using 50 leaves with different severity levels, previously measured using the software Assess®. Two evaluations were performed on the same set of leaves, but in a different sequence order, by the same raters, within a 15-day interval. The accuracy and precision of each rater was determined by simple linear regression between the actual and the estimated severity. The scale provided good levels of accuracy (means of 87.5%) and excellent levels of precision (means of 94%), with absolute errors concentrated around 10%. Raters showed great repeatability (means of 94%) and reproducibility (≥90% in 90.3% of cases) of estimates. Therefore, we could conclude that the diagrammatic scale presented here was suitable for evaluating downy mildew severity in melon.

Keywords: *Cucumis melo*, *Pseudoperonospora cubensis*, foliar disease, epidemiology, phytopatometry.

RESUMO

Escala diagramática para avaliação da severidade do míldio do meloeiro

O míldio, causado por *Pseudoperonospora cubensis*, é uma importante doença do meloeiro no nordeste brasileiro. Devido à inexistência de métodos padronizados para quantificação desta doença, foi elaborada uma escala diagramática com os níveis 2; 4; 8; 16; 32; 64; 82 e 96% de área foliar lesionada, avaliando-se a acurácia, a precisão e a reproducibilidade das estimativas de severidade da doença. A escala diagramática foi validada por oito avaliadores que utilizaram 50 folhas com sintomas da doença em diferentes níveis de severidade, mensuradas previamente pelo programa Assess®. Foram realizadas duas avaliações em um intervalo de 15 dias, em que seqüências diferentes das mesmas folhas foram estimadas visualmente pelos mesmos avaliadores. A acurácia e a precisão de cada avaliador foram determinadas por regressão linear simples entre a severidade real e a estimada. A escala diagramática proporcionou bons níveis de acurácia (média de 87,5%) e excelentes níveis de precisão (média de 94%), com os erros absolutos concentrando-se na faixa de 10%. Os avaliadores apresentaram elevada repetibilidade (média de 94%) e reproducibilidade (≥90% em 90,3% dos casos) das estimativas. Portanto, a escala proposta mostrou-se adequada para avaliação da severidade do míldio do meloeiro.

Palavras-chave: *Cucumis melo*, *Pseudoperonospora cubensis*, doença foliar, epidemiologia, fitopatometria.

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The Northeast region of Brazil responds for nearly 95% of the national melon (*Cucumis melo* L.) production. Located at this region, States of Rio Grande do Norte and Ceará supply respectively 55 and 28% of the total melon harvested in Brazil (FNP, 2007). The expansion of the area used for melon production in this region, in addition to an intensive year-round growing system, contribute to rise the intensity of several diseases, amongst them downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rost. (Santos *et al*., 2000). Downy mildew is one of the most important melon diseases in Northeast Brazil. It causes up to 60% reduction in fruit production (Cardoso *et al*., 2002a) and 49% in the content of soluble solids (Cardoso *et al*., 2002b).

Downey mildew symptoms start showing up in older leaves, as angular light-yellow spots, limited by the veins. Following, lesions may coalesce and rot the tissue, which assumes a bronze to brown hue. Olive-green to purple fungi frutifications, the pathogen sporangiophores and spores, can be seen in the adaxial leaf surface. Severe infection results in early leaf dropping, raquitism, and malformed and underdeveloped fruits (Rego & Carrijo, 2000; Tavares, 2002).

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. & Schultz, 1995). Considering that the melon downy mildew is a leaf 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).

Downy mildew severity in melon has been assessed mainly using descriptive score scales (Pan & More, 1996; Cardoso *et al*., 2002a; Santos *et al*., 2004). 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 & 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.

Due to the lack of downy mildew quantification methods in melon that went through rigorous validation procedures, this work aimed at both developing a diagrammatic scale for assessing disease severity, and also analyzing the quality of the estimates it brought about.

MATERIAL AND METHODS

Development of the diagrammatic scale

To develop the diagrammatic scale, 100 melon leaves (cultivar AF-682), with distinct levels of mildew severity, were collected in an experimental field in Rio Largo County, State of Alagoas. Leaf images (200 dpi) were produced using the software Assess[â] (The American Phytopathological Society, St. Paul, MN, USA). 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 eight disease severity levels, based on the Weber-Fechner visual accuracy law (Horsfall & 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

To validate the diagrammatic scale, we used colored photocopies of 50 melon leaves, with different levels of mildew severity. Eight agronomists (A to H), without experience on assessing diseases, were asked to estimate severity using the scale. To evaluate the reproducibility of the estimates, 15 days after the first evaluation, a new sequence of the same leaves was assessed by the same group, again using the scale.

Accuracy and precision of each rater were determine through simple linear regression, with the actual disease severity electronically obtained out of the images as the independent variable and the estimates of severity of each rater as the dependent variable. The accuracy of each rater and of the group of raters was determined applying the *t* test to the intercept of the linear regression (*a*), to prove the hypothesis Ho: $a = 0$; and to the line angular coefficient (*b*), to prove the hypothesis Ho: $b = 1$, both at 5% probability. The precision of the estimates was expressed by the regression determination coefficient (R^2), by the variance of absolute errors (difference between the estimate and actual severity), and by the repeatability of the estimates, which, in its turn, was determined by the regression between the second and first disease evaluation over the same sample. Reproducibility was determined based on the R^2 values of linear regressions involving severity estimates from different raters, sorted in pairs, over the same sample (Nutter Jr. & Schultz, 1995). Regression analyses were carried out using the software Microsoft[â] Office Excel 2003 (Microsoft Corporation, 2003).

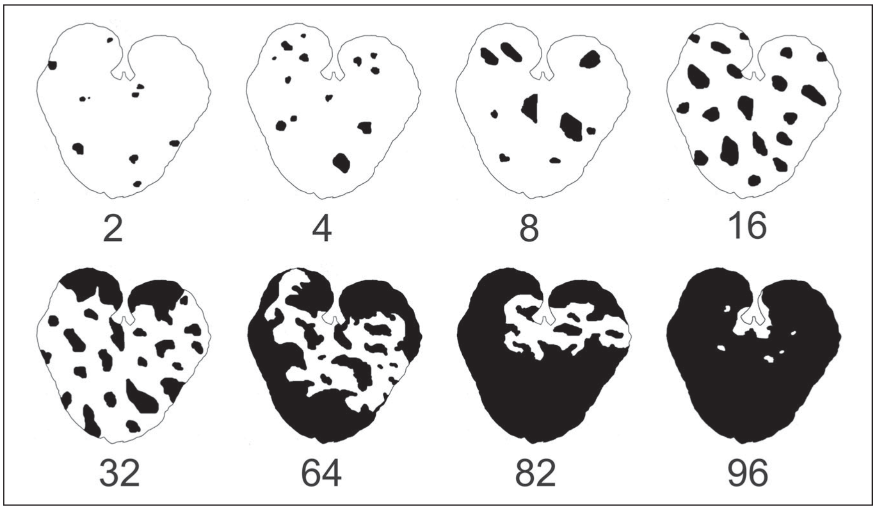

Figure 1. Diagrammatic scale of melon downy mildew showing increasing percentages of affected leaf area-severity (escala diagramática do míldio do meloeiro mostrando porcentagens crescentes de área foliar lesionada-severidade). Maceió, UFAL, 2006.

RESULTS AND DISCUSSION

The highest severity value observed in the 100 harvested melon leaves was 96%. Therefore, the diagrammatic scale developed to assess disease severity consisted of the levels 2; 4; 8; 16; 32; 64; 82, and 96% of leaf affected area (Figure 1). For the severity levels from 32% above, symptom representation included rotted tissue and coalesced spots. Spot coalescence is characteristic of the melon downy mildew, and may progress to extensive areas of leaf rotted tissue (Rego & Carrijo, 2000; Tavares, 2002).

In the diagrammatic scale validation, the intercept of 37.5 and 50% of the raters in respectively the first and second evaluations significantly ($p \leq 0.01$) differed from zero for the regression lines between the actual and the estimated severity. Most of these raters underestimated the actual severity (Table 1). The values of the line angular coefficient for 87.5% of the raters, in both evaluations, did not differed significantly from 1 ($p \leq 0.01$) (Table 1), with no systematic deviations. Each 1% increment in the melon downy mildew severity assessed electronically corresponded to an increment of 0.85 and 0.87% in the severity estimated by raters, respectively in the first and second evaluation, attesting the trend for undervaluing. The tendency demonstrated by raters to underestimate severity levels of the melon downy mildew resembles what was observed on the validation of diagrammatic scales of the cassava brown leaf spot (Michereff *et al*., 1998), yam leaf blight (Michereff *et al*., 2000), *Cercospora* leaf spot in lettuce (Gomes *et al*., 2004), and grapevine bacterial canker (Nascimento *et al*., 2005).

Table 1. Accuracy and precision of estimates of downy mildew severity in melon, represented by the intercept (a), line angular coefficient (b) and determination coefficients (R^2) of simple linear regression, for the disease evaluation carried out by eight raters, using the diagrammatic scale (acurácia e precisão das estimativas da severidade do míldio do meloeiro, representadas pelo intercepto (*a*), coeficiente angular da reta (*b*) e coeficiente de determinação (R^2) de equações de regressão linear simples nas avaliações efetuadas por oito avaliadores com o auxílio da escala diagramática). Maceió, UFAL, 2006.

Rater	1st evaluation			2nd evaluation		
	A	B	R^2	a	b	R^2
A	1.48	0.92	0.97	-0.67	0.93	0.98
B	-1.98	0.87	0.98	-1.96	0.92	0.98
C	-1.63	0.81	0.96	-1.63	0.86	0.98
D	-3.39 *	0.76 *	0.95	-3.93 *	0.83	0.97
E	0.03	0.83	0.90	4.84	0.75 *	0.93
F	-0.15	0.83	0.92	5.09 *	0.94	0.92
G	-4.41 *	0.87	0.95	-3.42 *	0.80	0.95
H	-6.68 *	0.88	0.88	-6.63 *	0.89	0.91
Average	-	0.85	0.94	-	0.87	0.95

*The star identifies cases in which the nullity hypothesis (a= 0 or b= 1) was rejected by the *t* test, p≤0.01 (o asterisco representa as situações em que a hipótese de nulidade (*a*= 0 ou *b*= 1) foi rejeitada pelo teste *t*, p≤0,01).

Table 2. Reproducibility of the estimates of downy mildew severity in melon as function of the frequency of determination coefficients (R^2) of simple linear regression relating estimates from distinct raters in two evaluations, 15 days apart. (reprodutibilidade das estimativas da severidade do míldio do meloeiro, representada pela freqüência dos coeficientes de determinação (R^2) de equações de regressão linear simples relacionando as estimativas entre avaliadores, em duas avaliações separadas de 15 dias). Maceió, UFAL, 2006.

Determination coefficient Interval (R^2)	Frequency (%)*	
	1st Evaluation	2nd Evaluation
0.70 - 0.79	3.3	2.7
0.80 - 0.89	7.4	6.2
0.90 - 1.00	89.3	91.1

* Calculated considering the number of occurrences in the interval in relation to 28 total possible combinations among the eight different raters, in each disease severity evaluation (calculada considerando o número de ocorrências do intervalo em relação ao total de 28 combinações possíveis entre os oito avaliadores em cada avaliação da severidade da doença).

In the precision analysis, the visual severity estimates in the first evaluation explained from 88 to 98% of the variation (R^2) observed in the electronically disease assessment, scoring an average of 94%. In the second evaluation, visual estimates explained from 91 to 98% of the electronically assessed variation, with an average of 95% (Table 1). Most of the raters improved estimate precision from the first to second evaluation, which might indicate that as raters get better acquainted with the disease or are trained for disease assessment, estimate accuracy and precision are expected to improve (Michereff *et al*., 2000; Tovar-Soto *et al*., 2002). The precision of the visual estimates of downy mildew severity in melon with the aid of the scale was similar to those observed in recent studies for validation of diagrammatic scales (Barbosa *et al*., 2006; Godoy *et al*., 2006), getting fairly close to an ideal agreement, which denotes high precision (Kranz, 1988; Nutter Jr. & Schultz, 1995). Most of the raters reached excellent estimate repeatability when using the scale: in average, 94% of the variation in the first evaluation was explained by the second evaluation.

The absolute errors (residue) in both evaluations, when the diagrammatic scale was used, were concentrated around 10%, which, in addition to be fairly acceptable in evaluations of diagrammatic scales, can be reduced by training the raters (Nutter Jr. & Schultz, 1995; Nutter Jr. *et al*., 2006). The reproducibility of estimates of disease severity was higher than 90% in 89.3% of the cases in the first evaluation, and in 91.1% of the cases in the second evaluation (Table 2). Such a high reproducibility level is good evidence that the diagrammatic scale can be placed into practice to compare different experiments, carried out by different raters.

The difference among raters in the assessment of the melon downy mildew severity confirms the observations by Nutter Jr. & Schultz (1995) about the human distinct ability to discriminate disease levels. The quality of the disease estimate is influenced not only by psychological stimulus and response, but also by factors such as the complexity of the sample, lesion size and shape, color and number of lesions in a sample (Kranz, 1988), and rater fatigue and difficulty to keep the focus on the task (Shokes *et al*., 1987).

To put forward a proposal of a standard system to guide severity evaluation for a given disease is a considerable responsibility. If the system is defective, the costs related to its use may be heavier than the benefits it granted (Nutter Jr. & Schultz, 1995; Leite & Amorim, 2002). Nevertheless, standardization should be pursued. 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 (Bergamin Filho & Amorim, 1996).

The diagrammatic scale presented here to assess downy mildew severity in melon was very straightforward. At the same time, it was effective in providing a quick disease estimate, with reasonable accuracy and excellent precision and reproducibility. Therefore, we consider the diagrammatic scale to assess downy mildew severity in melon presented here to be a valuable tool for field surveys, epidemiological studies, and comparison among disease control methods.

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