Diagrammatic scale for the assessment of blast on wheat spikes

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ABSTRACT

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The correct quantification of blast caused by the fungus *Magnaporthe oryzae* on wheat (*Triticum aestivum*) spikes is an important component to understand the development of this disease aimed at its control. Visual quantification based on a diagrammatic scale can be a practical and efficient strategy that has already proven to be useful against several plant pathosystems, including diseases affecting wheat spikes like glume blotch and fusarium head blight. Spikes showing different disease severity values were collected from a wheat field with the aim of elaborating a diagrammatic scale to quantify blast severity on wheat spikes. The spikes were photographed and blast severity was determined by using resources of the software ImageJ. A diagrammatic scale was developed with the following disease severity values: 3.7, 7.5, 21.4, 30.5, 43.8, 57.3, 68.1, 86.0, and 100.0%. An asymptomatic spike was added to the scale. Scale validation was performed by eight people who estimated blast severity by using digitalized images of 40 wheat spikes. The precision and the accuracy of the evaluations varied according to the rater ($0.82<R^2<0.90$, $-6.12<a<2.94$, $0.85<b<1.16$), and systematic errors in overestimating or underestimating the disease were not found among the raters, demonstrating that the developed scale is suitable to evaluate blast on wheat spikes.

Additional keywords: *Magnaporthe oryzae*, *Pyricularia grisea*, ImageJ, severity, epidemic.

RESUMO

Maciel, J.L.N.; Danelli, A.L.D.; Boretto, C.; Forcelini, C.A. Escala diagramática para avaliação de brusone na espiga de trigo. ***Summa Phytopathologica,*** v.39, n.3, p.162-166, 2013.

A quantificação correta da brusone causada pelo fungo *Magnaporthe oryzae* em espigas de trigo (*Triticum aestivum*) é um componente importante para o entendimento do desenvolvimento dessa doença com vistas ao seu controle. A quantificação visual baseada em uma escala diagramática pode ser uma tarefa prática e eficiente que já tem demonstrado utilidade em diversos patossistemas, inclusive doenças da espiga do trigo como a mancha das glumas e fusariose. Espigas apresentando diferentes valores de severidade da doença foram coletadas em uma lavoura com o objetivo de elaborar uma escala diagramática para quantificar a severidade da brusone em espigas de trigo. As espigas foram fotografadas e a severidade da brusone foi determinada usando os recursos do programa computacional ImageJ. Uma escala diagramática foi elaborada com os seguintes valores de severidade de doença: 3,7; 7,5; 21,4; 30,5; 43,8; 57,3; 68,1; 86,0; e 100,0%. Uma espiga assintomática foi adicionada à escala. A validação da escala foi realizada por oito pessoas que estimaram a severidade da brusone em imagens digitalizadas de 40 espigas de trigo. A precisão e a acurácia das avaliações variaram de acordo com o avaliador ($0,82<R^2<0,90$; $-6,12<a<2,94$; $0,85<b<1,16$), não ocorrendo erro sistemático na superestimativa ou subestimativa da doença entre os avaliadores, demonstrando que a escala desenvolvida é adequada para a avaliação da brusone em espigas de trigo.

Palavras-chave adicionais: *Magnaporthe oryzae*, *Pyricularia grisea*, ImageJ, severidade, epidemia.

Blast caused by the fungus *Magnaporthe oryzae* B. Couch (*Pyricularia oryzae* anamorph. dig) is one of the major limits to food production in the world, affecting several crops of agricultural importance. For South America, since the mid-1980s, when it was first reported to affect wheat plants, this disease has also been characterized as one of the most serious problems for wheat crops, especially in Brazil, Bolivia and Paraguay (5, 10, 12). Difficulties for its control are enormous. Management practices such as crop rotation, balanced fertilization and treatment of aerial parts with fungicides are not sufficient to prevent the economic damage caused by this disease, especially in seasons of severe epidemics (6, 7, 13).

An important problem in Brazilian wheat fields is that the cultivars available to the producers are susceptible to blast, which demonstrates that the development of cultivars with higher resistance is an indispensable component of the strategy to control this disease. Improving the process for evaluating the reaction of genotypes to the disease is also part of this strategy. Thus, an additional difficulty is the lack of a diagrammatic scale to help evaluate the disease severity on spikes. Other fungal diseases of importance to the wheat crop have had this type of tool available for a long time, for example, fusarium head blight, caused by *Gibberella zeae*, which also infects wheat spikes (8, 11, 18, 19, 21, 22).

The quantification of a disease is a fundamental step for understanding its development and implementing strategies to manage it (1). Besides, its importance is not limited as a strategy used in the comparison of genotypes for their resistance to a disease in a breeding program. The degree of development of a fungal disease, quantified with the aid of a diagrammatic scale, may be related to the damage caused by this disease in a crop or even to the efficiency of a certain treatment, such as spraying of a fungicide for its control. However, some characteristics are essential for a diagrammatic scale to be considered efficient. Among these features are: easy use, wide applicability, reproducible results, and intervals that represent all stages of the disease development (4). Added to these aspects, Horsfal & Barrat (9) determined that the diagrammatic scales must have a sequence of severity values that satisfy the principle of logarithmic increment established in the "law of Weber-Fechner." According to this proposition, perception intensity measures of the five senses of humans, in which vision is included, are made in logarithmic magnitude.

The aim of this study was to develop a diagrammatic scale to determine the severity of blast caused by *Magnaporthe oryzae* on wheat spikes.

MATERIAL AND METHODS

One hundred spikes with blast symptoms of cultivar BRS Pioneiro were collected from a wheat field located in Londrina, Paraná State (PR), in the 2012 Brazilian wheat season. The spikes were collected, placed in paper bags and taken to the Laboratory of Plant Pathology of Embrapa Wheat, located in Passo Fundo, Rio Grande do Sul State (RS), where they were kept at -20 ºC.

Elaboration of the diagrammatic scale

Photographs were taken of each group of five spikes, which were subjected to the following procedures. They were hydrated by immersion in a beaker with water for about 30 min and allowed to dry on a paper towel for about 10 min. Subsequently, the spikes were placed on white papers, which were on a laboratory bench, with a ruler placed on the bottom or on the left side of the sheets. The spikes were positioned with the most symptomatic side in front of the camera. The images of the spikes were captured with a Sony Cybershot digital camera DSC/-H7/H9 (Sony, Brazil), which was manually held at approximately 50 cm above the spikes. Each image was compressed and saved as Joint Photographic Experts Group (JPEG) format as input for the ImageJ software (20), which was used to analyze the digital photographs. The unit used to measure the total, the healthy and the symptomatic area of the spikes was square centimeter (cm²), which was established based on the calibration dimensions obtained from the ruler scale that was below or beside the spike. A binary pattern of colors was used to determine the total area of spikes, i.e., black color on white background.

The selection and the quantification of spike areas affected by the disease were performed based on the characteristics of the ImageJ software. The symptomatic regions were marked with the mouse cursor and the diseased spike area was calculated by subtracting the non-diseased spike area (also marked with the mouse cursor) from the total spike area. The awns on the images were separated and not considered spike areas. The ImageJ software was used under the following conditions: 8 bits/pixel, 0.1% saturation of pixels, all particles were considered (0 to infinity), and photosensitivity with 65% of noise tolerance (interference accepted by the program).

The severity levels used in the scale were selected by considering minimum and maximum limits for the diseased spikes in the wheat field in Londrina 0 (zero) and 100% blast severity, respectively. Intermediate levels were defined mathematically according to the logarithmic increase and as per the "law of the stimulus" established by Weber & Fechner (9).

Validation of the diagrammatic scale

Eight people estimated blast severity in the digitalized images of 40 wheat spikes with the aid of the diagrammatic scale developed in the present study. Using the same procedures described above for the quantification of diseased areas affected by blast to elaborate the diagrammatic scale, the disease severity on 40 digitalized images of symptomatic spikes was determined (one spike per image). The accuracy and the precision of visual estimates of each rater were determined by linear regression analysis, considering the actual and the estimated severity as the independent and the dependent variable, respectively. The accuracy of each rater was determined by *t* test applied to the linear (a) and angular (b) coefficients of the straight line obtained from the linear regression between the two above-mentioned variables. The precision of the estimates was assessed by determining the regression coefficient (R^2) and the variance of absolute errors (estimated severity minus actual severity) (15, 16).

RESULTS AND DISCUSSION

A cultivar with awns on the spikes (BRS Pioneiro) was chosen to elaborate the diagrammatic scale because most wheat cultivars used in Brazil have this characteristic. However, to obtain the severity values shown in the scale, the awns were not considered part of the spikes (Figure 1). This convention was used for both the scale elaboration

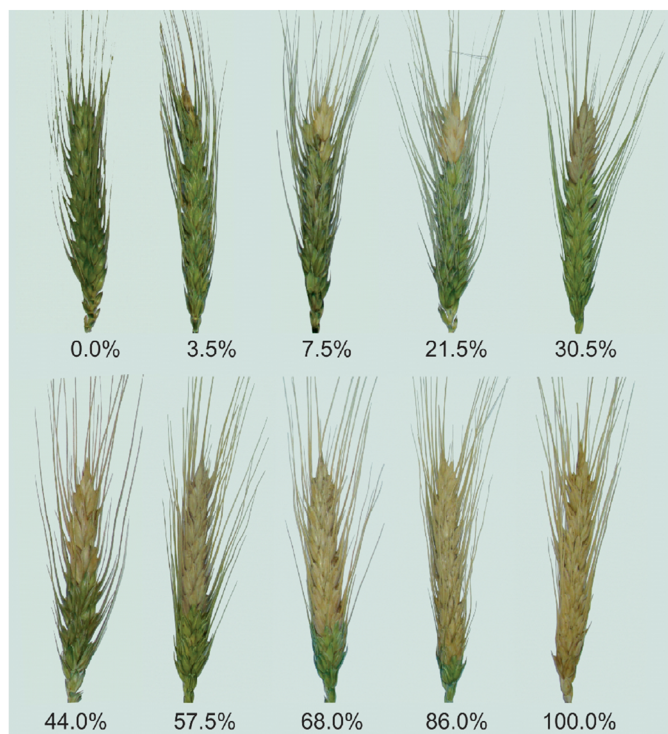

Figure 1. Diagrammatic scale for assessing blast severity caused by *Magnaporthe oryzae* on wheat spikes. The awns were not considered in the determination of severity on the spikes.

and the validation procedures and was adopted by considering that, in general, the infection process caused by the fungus *M. oryzae* does not reach this segment of the spike.

Minimum and maximum blast severity levels found for the collected spikes were 3.7% and 100%, respectively. These values were considered lower and upper limits, respectively, in relation to the symptomatic spikes of the diagrammatic scale (Figure 1). In total, 10 spikes formed the diagrammatic scale, one of which is asymptomatic (0% blast, i.e., the first one in the scale) and the other nine show different blast severity values.

The adopted sequence of severity values in the scale was proposed to meet the logarithmic increment principle established by Weber-Fechner. Based on this principle, Horsfall & Barratt (9) suggested that the diagrammatic scales should have severity levels inserted in the following 12 intervals: 1 (0), 2 (0%-3%), 3 (3%-6%), 4 (6%-12%), 5 (12%-25%), 6 (25%-50%), 7 (50%-75%), 8 (75%-88%), 9 (88%-94%), 10 (94%-97%), 11 (97%-100%), and 12 (100%). According to Campbell & Madden, cited by Martins et al. (14), some authors have hesitated to use scales designed based on this principle mainly because there are only two severity level intervals ranging from 25% to 75%. However, these intervals can be subdivided into shorter intervals, as proposed by Amorim et al. (2), Martins et al. (14), and in the present study. This means that the diagrammatic scale presented here followed the "law of Weber-Fechner" (logarithmic increments) without necessarily having used only one severity level of each interval suggested by Horsfall & Barratt (9). This applies to spikes showing 30.5% and 43.8% severity, which belong to interval 6 (25% -50%), as well as to spikes showing 57.3% and 68.1% severity, which belong to interval 7 (50% -75%).

For most raters, the estimated severity values were close to the actual values (Figure 2). This assertion is based on the results obtained from the *t* and R^2 statistical tests, used for evaluating the linear regression between the two variables (Table 1). Such proximity between estimation and actuality determines the precision of assessments, defined as the accuracy without systematic errors which can be measured by the linear (*a*) and angular (*b*) coefficients of the linear regression established between the actual and estimated severity (3). The exception was rater 6, for whom *a* and *b* values significantly differed from zero and one [1], respectively, according to *t* test (Table 1). In the case, this rater underestimated blast severity in the spikes considering that the angular coefficient of the linear regression between the actual and the estimated severity was significantly lower than 1.

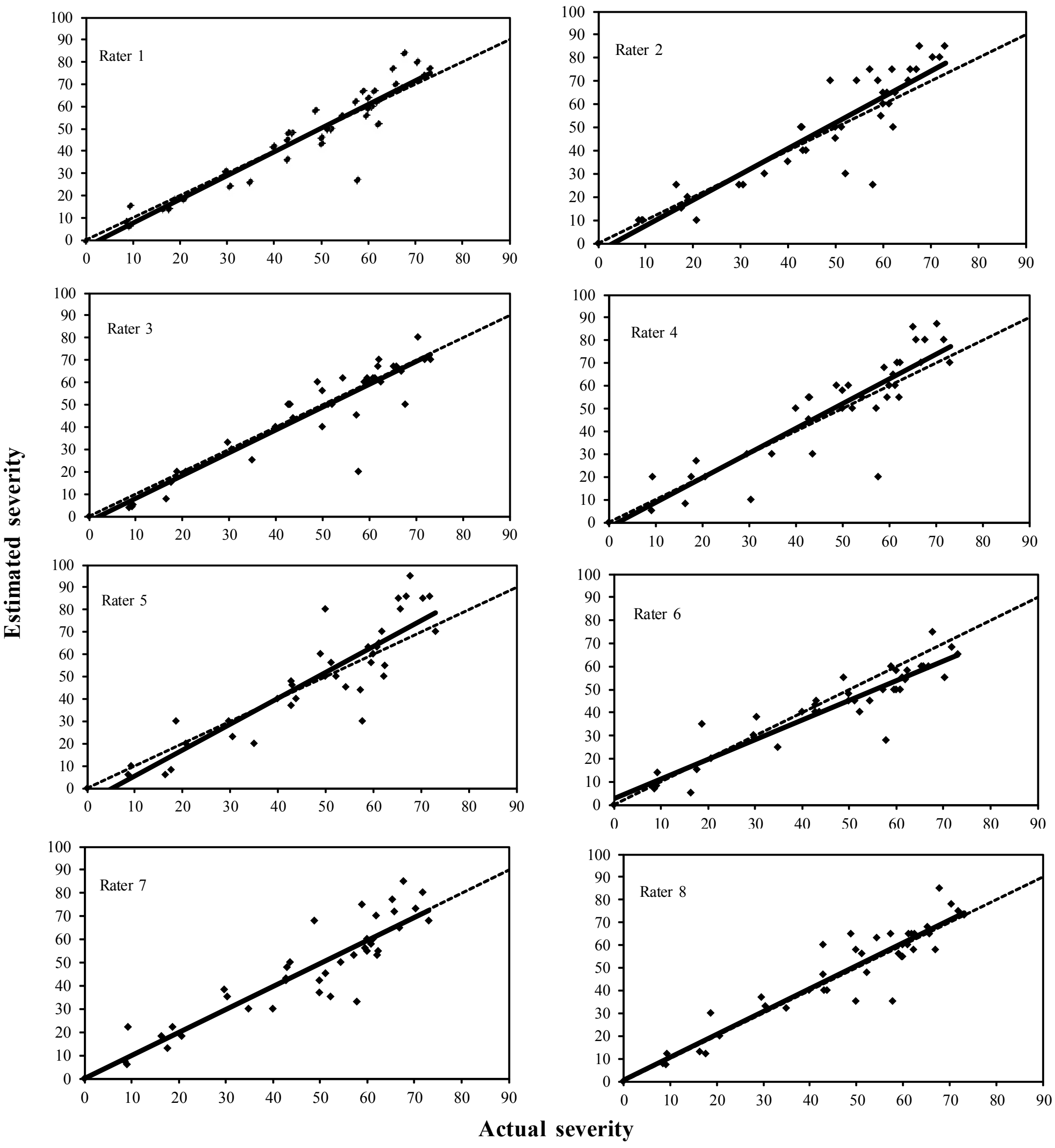

Figure 2. Estimated severity of wheat blast, caused by *Magnaporthe oryzae*, and linear regressions obtained between actual and estimated severity (solid line). Dotted lines represent an ideal situation, in which estimated severity is equal to actual severity.

Table 1. Linear (a), angular (b) and determination (R^2) coefficients of the regression line for actual (independent variable) *versus* estimated severity (dependent variable) of blast, caused by *Magnaporthe oryzae*, on 40 wheat spikes evaluated by eight raters using the diagrammatic scale

Coefficient	Raters							
	1	2	3	4	5	6	7	8
a	-3.10	-3.83	2.94	2.20	-6.12	2.78*	0.18	0.64
b	1.07	1.11	0.91	1.09	1.16	0.85*	0.99	1.00
R^2	0.90	0.83	0.87	0.82	0.82	0.86	0.83	0.87

*Hypotheses a =0 and b =1 were rejected according to *t* test, at significance of 0.05.

Precision can be evaluated based on the variation of absolute errors and on the R² coefficient, obtained from regression analysis, which becomes more accurate as it gets closer to 1. As regards the scale developed in this study, the evaluators had very good precision, with R² ranging from 0.83 to 0.90. Besides R² coefficient, the good precision was confirmed when the difference between the actual and the estimated severity values was calculated, and the result was named absolute error or residue (Figure 3). Most of the absolute error values did not exceed 10% and rarely reached 20%. Furthermore, for most raters, the highest values of absolute errors occurred for severity levels ranging from 50% to 70% and the two highest values were obtained in the evaluations made by raters 4 and 5. All these results are indicative of a good precision level of the assessments using the diagrammatic scale elaborated for wheat blast in spikes. This statement is based on criteria already used to classify the results obtained by different authors in training procedures for evaluators to estimate

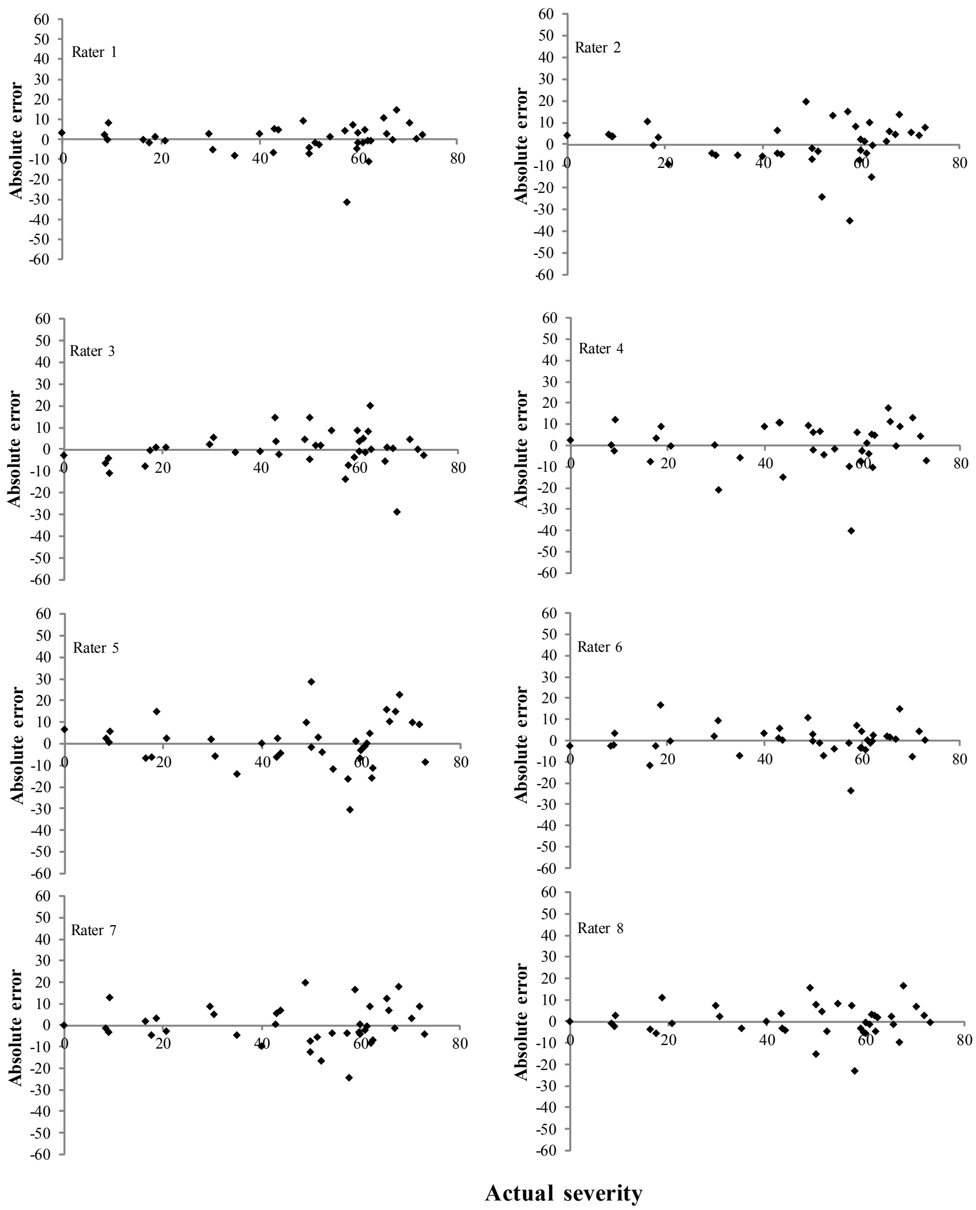

Figure 3. Absolute errors (actual severity - estimated severity) for assessments of blast, caused by *Magnaporthe oryzae*, on wheat spikes with the aid of the diagrammatic scale. Value of zero for absolute errors represents the ideal situation, in which estimated severity is equal to actual severity.

disease severity using computer programs. In this sense, the computer programs Distrain and Disease.Pro, developed by Tomerlin & Howell (24) and Nutter Jr. & Worawitlikit (17) to quantify the disease severity levels on leaves of cereals and groundnuts, respectively, classified as excellent raters those whose errors did not exceed 5% and as good raters, those whose errors were less than 10%. Stonehouse (23) also mentioned that the presence of some level of absolute error in the measurements can be compensated for by some advantages provided by the diagrammatic scale such as rapidity and standardization.

The scale presented in this study is expected to be used by a large number of students, technicians and researchers in Brazil and even abroad, in countries where wheat blast naturally occurs like Bolivia and Paraguay. There is a perception that the demand for tools that help evaluate and quantify this disease occurring on spikes is significant. In addition, it is important to emphasize that wheat blast has assumed a very large scale in terms of occurrence and damage to the wheat crops and is currently considered one of the major limiting factors to the expansion of wheat cultivation in the Brazilian Cerrado.

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