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Diagrammatic scale to quantify the severity of Ascochyta blight in broad bean crops

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ABSTRACT

Objective/background: The objective of this study was to design and validate a diagrammatic severity scale of brown spot on broad bean.

Materials and methods: We collected 120 leaflets with different level of brown spot damage from commercial crops in the Toluca Valley, which were visually selected based on the expressed symptomology. Sixty leaflets were scanned for evaluation with the software APS PRESS ©Assess 2.0 to determine the real severity value for each leaflet.

Results: The damage values allowed us to generate a diagrammatic scale consisting of six different classes: 0(0.0), 1(0.1-6.0), 2(6.1-10.0), 3(10.1-15.0), 4(15.1-40.0), 5(> 40.1-100). The leaflets were visually examined by evaluators who had no prior experience. The results from each evaluator were analyzed with a simple linear regression, obtaining r^2 values from 0.0042 to 0.8748, β0 de 0.51 a 9.11, y β1 de 0.132 a 0.925. Using a scale, r^2 values were obtained 0.9143 to 0.985, β0 de 0.001 a 0.911 y β1<0.001.

Conclusion: The generated diagrammatic severity scale was validated and reproducible, showing high reliability.

Key words: Symptoms, damage, evaluation, reproducibility.

Introduction

In 2022, Mexico's Agrifood and Fishing Information Service (*Servicio de Información Agroalimentaria y Pesquera* - SIAP) reported a cultivated area of 9,445 ha of broad beans (*Vicia faba*), out of which 9,436 ha were harvested for fresh grain, whereas 17,246 ha were planted and 17,231 ha were harvested for dry grain. The State of Mexico reported 4,397 ha planted with broad bean for green harvest, ranking first in production nationwide (SIAP, 2023). The broad bean is considered a species with nutritional, biological, and even medicinal properties (Prabhu and Rajeswari, 2018). Despite its importance, the crop is affected by diseases caused by *Ascochyta fabae, Botrytis fabae*, *Puccinia vicia-fabae* and *Rhizoctonia* spp., which have caused countless economic losses for farmers.

In Egypt, diseases such as the chocolate spot (*Botrytis fabae*) and the brown spot (*Ascochyta fabae*) have been reported to affect between 25 and 80% of the foliar area (Omar, 2021). López (2013) pointed out the brown spot as one of the main diseases that affect the broad bean crop in the Toluca Valley, containing lesions in concentric circles in leaves, stems and pods, as well as being transmitted via seeds and affecting quality and yield by 10 to 34% (Ahmed *et al*., 2016). An alternative to know the pathogens found in the important crops is to conduct epidemiological studies, such as estimating the area under the disease progress curve. These studies assess the damage caused by the causal agent over time and space, determining incidence, severity and their relationship with climate factors, management and the subsystems involved, to establish solutions for phytosanitary problems (Olivares *et al*., 2021).

For example, Meirelles and Cardoso (2012) point out that the estimation of severity is influenced by the subjectivity of the evaluator when quantifying the damage. Nowadays, the use of diagrammatic scales has become a useful tool to evaluate several diseases, for being user-friendly and because they have important intervals to represent the development of a disease, as well as helping to provide an immediate evaluation (Acco *et al*., 2020) and making quick decisions for the implementation of management strategies. Due to this, the aim of this study was to design and validate a diagrammatic scale to determine the severity of the brown spot (*Ascochyta fabae*) in the broad bean crop.

In three commercial plots of native broad beans, prior to flowering, located in the municipal areas of Calimaya (2117276.10 UTM N; 428894.07 UTM E), Toluca (2125772.03 UTM N; 428452.94 UTM E) and Zinacantepec (2124996.43 UTM N; 419277.08 UTM E) in the Toluca Valley, during the spring-summer 2021 agricultural cycle (temperate rainy climate (Cwbg) and an average temperature of 12.5 °C and predominant rains in the summer), 60 leaflets were collected from the lower stratum, along with 60 from the middle stratum of plants in the vegetative phase. Plants displaying characteristic symptoms associated with brown spot and different levels of damage were selected, as well as asymptomatic leaflets. The leaflets were preserved in a botanical press to keep them free from mechanical damage and preserve their characteristics until they were taken to the Plant Health laboratory of the Faculty of Agricultural Sciences of the Autonomous University of the State of Mexico. The leaflets collected were visually pre-selected, discarding those with mechanical damage, and 69 representative leaflets were chosen.

Twelve leaflets were chosen for each type, plus 9 healthy leaflets. They were grouped into similar categories or ranges and digitized fresh, aided by a multifunctional HP® LASERJET PRO, MFP M127fn printer.

In every leaflet, the damaged area was evaluated in relation to the total area and expressed as a percentage using the APS PRESS software ©Assess 2.0 2008. The area with symptoms and the asymptomatic area were determined using RGB (Red, Green, Blue) according to the methodology proposed by Massom *et al*. (2013). The actual severity of the disease (S) was estimated based on the formula proposed by Nutter *et al.* (2006): Severity = [(diseased area / total area of the digitized leaf) * 100]. The intermediate levels of the severity of the brown spot were determined according to Weber-Fechner's visual acuity law, which establishes a quantitative relationship between the magnitude of a physical stimulus and the way in which it is perceived by the subject (Horsfall and Barrat, 1945; Rivera-Zabala, 2016). This helps establish six types of severity, as well as their maximum and minimum limits in each type. For the scale, the leaf with the value closest to the average severity of each type was chosen. To validate the diagrammatic scale of severity, 69 digital images were evaluated, representative of the different degrees of damage, and inserted at random in individual slides for viewing in Microsoft 365 ® Power Point ® (2022) and projected using an EPSON PowerLite S18+ multimedia projector model V11H552021 for 20 inexperienced evaluators and without the support of a diagrammatic scale, with a viewing time of 30 seconds for each image.

In a second evaluation, conducted eight days after the first evaluation, 10 of the 20 evaluators with better eyesight used the diagrammatic scale proposed to assess 60 leaflets. In both evaluations, in order to quantify the accuracy of the severity assessments carried out by the evaluators, a simple linear regression was carried out, with the coefficient of determination parameter (r^2), as well as the margin of error ($1-r^2$). A T test was conducted on the intercept of the linear regression (β_0) to verify the following hypothesis H_0: $\beta_0 = 0$ and with the coefficient of the slope of the line (β_1) to check if it was different to 1: H_1: $\beta_1 = 1$, with a $P \leq 0.01$. The evaluation of both sets of hypotheses was carried out with a significance level of 0.01. In addition, the accuracy of the estimations obtained by calculating the coefficient of determination, accuracy and absolute errors was determined with a simple linear regression analysis using SAS® software version 9.0 (SAS Institute, 2002). Real severity was the independent variable, and the estimated severity was the independent variable. The estimates were evaluated based on precision (r^2) and the absolute errors (estimated severity minus real severity) (Nutter Jr. *et al*., 1995).

The APS PRESS© Assess 2.0 software (2008) determined percentages of damaged area, which fluctuated from 0 to 77.1 %. In the first evaluation, five of the evaluators presented a high level of accuracy when identifying the corresponding type of severity of the disease, whereas the remaining evaluators presented deficiencies in their accuracy (Table 1). In this sense, Barbosa *et al*. (2006) mention that the observed values for the accuracy of evaluators with no experience are not acceptable and emphasize that training may have a favorable influence on the quality of the evaluations.

Table 1. Values of the Intercept (β_0), slope of the line (β_1), coefficient of determination (r^2) and margin of error ($1-r^2$) of the simple linear regression equation in visual estimations of the severity in of brown spot in broad bean (*Ascochyta fabae*), with 20 unscaled evaluators and 10 scaled evaluators.

Unscaled										With scaled				
ID	**β_0**	**β_1**	**r^2**	**$1-r^2$**	**ID**	**β_0**	**β_1**	**r^2**	**$1-r^2$**	**ID**	**β_0**	**β_1**	r^2	$1-r^2$
1	8.224	0.227	0.191	0.808	**11**	9.370	0.094	0.112	0.887	**1**	0.055*	<0.0001**	0.9851	0.0149
2	5.707	0.302	0.325	0.674	**12**	10.425	0.066	0.051	0.948	**2**	0.307*	<0.0001**	0.9845	0.0155
3	7.170	0.195	0.171	0.828	**13**	9.995	0.116	0.408	0.591	**3**	0.452*	<0.0001**	0.9847	0.0153
4	6.347	0.305	0.350	0.650	**14**	6.009	0.188	0.335	0.664	**4**	0.911*	<0.0001**	0.9843	0.0157
5	0.510*	0.921*	0.811	0.188	**15**	10.008	0.065	0.050	0.949	**5**	0.887*	<0.0001**	0.9843	0.0157
6	0.941*	0.925*	0.8748	0.125	**16**	11.717	0.044	0.007	0.993	**6**	0.001*	<0.0001**	0.9143	0.0857
7	0.941*	0.925*	0.8748	0.125	**17**	11.613	0.031	0.010	0.989	**7**	0.106*	<0.0001**	0.9543	0.0457
8	0.941*	0.925*	0.8748	0.125	**18**	9.831	0.069	0.055	0.944	**8**	0.524*	<0.0001**	0.9684	0.0316
9	0.941*	0.925*	0.8748	0.125	**19**	10.842	0.111	0.029	0.971	**9**	0.013*	<0.0001**	0.9464	0.0536
10	9.116^{ns}	0.132	0.119	0.880	**20**	11.872	0.030	0.0042	0.995	**10**	0.279*	<0.0001**	0.9407	0.0593
					Average			**0.3263**	**0.672**		**Average**		**0.9647**	**0.0353**

*significant: situation where the null hypothesis (β_0=0 o β_1=1) was rejected by test t ($P \leq 0.01$).

The results of the second evaluation helped established minimum and maximum limits in each one of the six types of severity expressed in percentages: **C0**: (0; 0= Without visible symptoms), **C1**: (0.1-6.0; 3.0 mid-value= Small circular lesions), **C2**: (6.1-10.0; 8.0= Small lesions increase, with brown edges), **C3**: (10.1-15.0; 12.5= lesions begin to coalesce, they become a gray and/or chocolate brown color with a light colored center), **C4**: (15.1-40.0; 27.5= The majority of lesions begin to coalesce, and begin to turn from dark to necrotic), **C5**: (>40.1-100; 70.0= The lesions coalesce and develop a dark brownness with a complete sinking of the center); these levels were used to determine the limits of the scale. The minimum severity value was 0% (healthy leaflet) and a maximum severity value of 40.1 % (damaged leaf). The latter displays characteristics of coalescence of the lesions and becomes black, with a complete sinking of the center (Figure 1). The scale created coincides with what was indicated by Campbell and Madden (1990), who defined the scale of severity as a series of types that must contain the ranges of severity for a determined disease, providing an adequate resolution to the behavior of severity.

Class 0	**Class 1**	**Class 2**	**Class 3**	**Class 4**	**Class 5**
Range : 0 %	**Range** 0.1-**2.9**-6.0 %[z]	**Range** 6.1-**8.0**-10.0 %	**Range** 10.1-**12.5** 15 %	**Range** 15.1-**27.5**-40.0 %	**Range** 40.1-**70.0**-100 %

Figure 1. Diagrammatic scale of brown spot severity (*Ascochyta fabae*) in broad bean. [z]Lower limit-average-upper limit.

The coefficient of determination values (r^2) for the 20 inexperienced evaluators ranged from 0.0042 to 0.8748 with an average of 0.3267, whereas the margin of error ($1\text{-}r^2$) ranged from 0.125 to 0.995 and an average of 0.673 (Table 1). In the validation with the aid of the scale, the coefficient of determination values (r^2) ranged from 0.9143 to 0.9851 and an average of 0.9647 (Table 1), above the results obtained in the first evaluation (Table 1), therefore prior training influenced the quality of the evaluations. These results coincide with a report by Ortega-Acosta *et al*. (2016), who obtained averages greater than 0.80% for evaluations conducted with the use of diagrammatic scales. Therefore, this scale is reproducible and accurate, in line with findings by Belan *et al*. (2014), in which absolute errors obtained in the evaluation with the scale ranged from 0.0 to 54.96% (Figure 2), higher than the ranges of 0.0 to 37.41% from the first evaluation in the absence of the scale. The margin of error ($1\text{-}r^2$) in the second stage tanged from 0.0149 to 0.857, with an average of 0.053, indicating that it is significant and acceptable

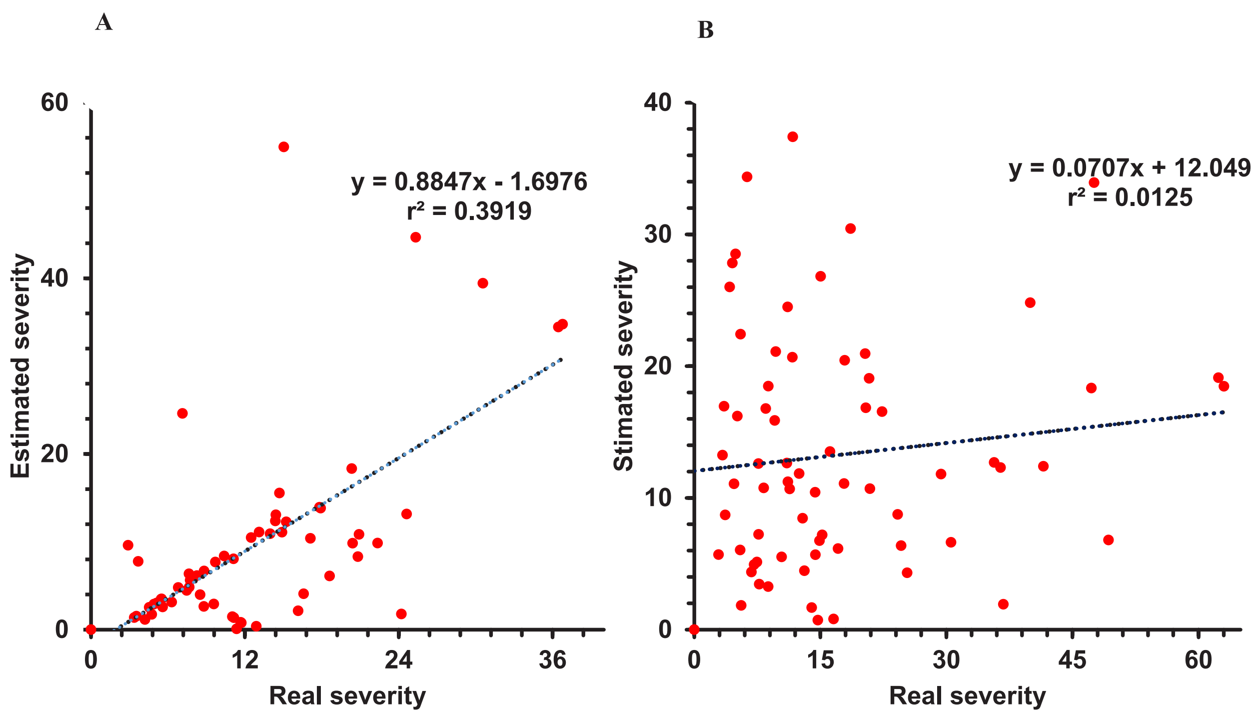

Figure 2. Distribution of residuals (estimated severity-real severity) of the brown spot evaluations (*Ascochyta fabae*) in broad bean leaflets. A) Scaled evaluation. B) Unscaled evaluation.

(Hernández and Sandoval, 2015). Furthermore, a better precision (β_0) and accuracy (β_1) were determined with the aid of the proposed diagrammatic scale.

The precision determined for each evaluator indicates that a diagrammatic scale represents a standardized method to quantify a disease, thus aiding in obtaining favorable results (Hernández and Sandoval, 2015), along with adequate levels of accuracy and precision among evaluators (Figure 1). These results align with Barbosa *et al*. (2006) indicating that damage assessment with the aid of a scale significantly improves both precision (β_0) and accuracy (β_1). In the same vein, Fragoso-Benhumea *et al*. (2022) and Ávila-Quezada *et al*. (2001) reported that a diagrammatic scale may guarantee reproducibility but not the accuracy and precision of the estimation.

In this study, the majority of the evaluators displayed significance regarding the value of the intercept value β_0 and the slope coefficient β_1; they were statistically different and/or close to 1, with a confidence interval of $\alpha = 0.01$ (99 %), coinciding with a report by Nascimento *et al*. (2005), who emphasized that an overestimation of the results in most evaluators may indicate the presence of positive deviations for all severity levels, concluding that the results presented were all statistically different from 0 and 1 based on the T tests (Pr > |t|) with a confidence interval of α = 0.01.

The diagrammatic scale to evaluate the severity of the brown spot in broad beans, created and composed of six class, provided a high reproducibility with levels of accuracy (β1<0.001) and acceptable precision (β0 from 0.001 to 0.911), making it a tool to standardize the evaluation of damages across different locations, evaluating initial symptoms to make decisions and assessing the efficiency of management measures or the sensitivity response of broad bean cultivars.

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