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Validation of a diagrammatic severity scale to quantify *Botrytis fabae* damage in faba bean

Ernesto Alonso López-Reyes, Álvaro Castaneda-Vildózola, Jesús Ricardo Sánchez-Pale*, Juyma Mayvé Fragoso-Benhumea, Universidad Autónoma del Estado de México. Facultad de Ciencias Agrícolas. Ctra. Toluca-Ixtlahuaca Km. 15.5, El Cerrillo Piedras Blancas, Toluca, Estado de México, C.P. 50200. **Rómulo García-Velasco**, Centro Universitario UAEM Tenancingo. Carretera Tenancingo - Villa Guerrero Km. 1.5 Tenancingo, C.P. 52400. **Andrés Quezada-Salinas**, DGSV, CNRF. Carretera Federal México-Pachuca km 37.5, Tecámac. C. P. 55740. Estado de México, México.

Corresponding Author:
Jesús Ricardo Sánchez-Pale
jrsanchezp@uaemex.mx

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ABSTRACT

Background/Objective. Faba bean production (*Vicia faba*) in the Toluca Valley has been considerably affected by *Botrytis fabae*, therefore, it is necessary to have a diagrammatic severity scale designed to validated and to quantify its damage.

Materials and Methods. 150 leaflets were collected, extracted from three commercial plantations, a visual preselection was carried out based on the presence of symptoms. 138 leaves were digitized to quantify the value of the real damage with the APS PRESS ©Assess 2.0 software. A first visual evaluation (without diagrammatic scale) of the leaves with different degrees of damage was carried out by 20 inexperienced evaluators. A second evaluation was carried out with 10 evaluators, but with support from the scale. The obtained values were analyzed by means of a simple regression.

Results. A diagrammatic scale was generated consisting of 6 classes 0(0) 1(0.1-10.0), 2(10.1-20.0), 3(20.01-35.0), 4(35.01-58.0), 5(58.01-100). The results obtained of the simple linear regression, by evaluator, showed for the non-scale evaluation, values of r^2 (accuracy) between 0.07-0.78, β1 (precision)=0.0001-0.85 and among evaluators β1 (reproducibility)=0.16-1.54. In contrast, the scale evaluation showed values of r^2 =0.88-0.99, β0=0.0001-0.93 8 (by evaluator), and β1=<0.0001 (significant to slope 1.0). These results supported scale validation, respect to its accuracy, precision and reproducibility.

Conclusion. A reliable six-classes diagrammatic logarithmic scale was successfully developed and validated to assess disease severity of chocolate spot caused by *Botrytis fabae*.

Keywords: Chocolate spot, *Vicia faba*, Reproducibility

INTRODUCTION

In the State of Mexico, the faba bean (*Vicia faba*) crop is destined for consumption, both dry and fresh. It is an important source of protein, with a content between 14 and 35%. In addition, it has great economic and social importance for the areas in which it is produced (Calixto *et al*., 2020). In 2023, the surface of green fava bean planted nationwide was 10,080.5 ha, out of which 68,984.76 t were obtained. In the same year, the State of Mexico recorded a production of 694.65 t in a surface of 4,835.14 ha, making it the leading producing state (SIAP, 2023). The chocolate spot (*Botrytis fabae*) and the brown spot (*Ascochyta fabae*) affect the foliar area between 25 and 80% (Omar, 2021). In the High Valleys of Mexico, chocolate spot is one of the main diseases (Hernández y Sandoval, 2015). Colak *et al*. (2017) mention that this disease can appear irregularly in stems and leaves, which limits the photosynthetic activity and causes losses in production of 50 to 100%.

The behavior of the pathogen in space and time is carried out with epidemiological studies, the most important of which include the estimation of damage caused by the causal agent over time in a given area (Olivares *et al*., 2021). Bock *et al*. (2022) points out that there are diverse methods to estimate the severity of a phytopathogen, defined as the proportion of the tissue affected in regard to the total area of the host. However, many of these methods contain limitations concerning precision, accuracy and reproducibility errors. Alves (2018) indicates the estimating severity requires accurate studies, such as the use of previously evaluated and validated diagrammatic scales. In this sense, the use of diagrammatic scales has become established in recent years as a useful tool for estimating various diseases, allowing for a quick assessment (Acco *et al*., 2020). These scales are structured in intervals that represent the progress of the disease, which helps make decisions and implement management strategies. Additionally, its practicality, ease of use and applicability are important advantages (Lavilla and Petta, 2022). Due to this, the aim of this study was to design and validate a diagrammatic scale to determine the severity of chocolate spot (*Botrytis fabae*) in faba bean crops in the Toluca Valley.

In the municipalities of Zinacantepec (2124996.43 UTM N; 419277.08 UTM E), Toluca (2125772.03 UTM N; 428452.94 UTM E) and Calimaya (2117276.10 UTM N; 428894.07 UTM E), of the Toluca Valley, with a temperate rainy climate (Cwbg) and an average temperature of 12.5 °C, with predominant rainfalls in the summer, three commercial faba bean plots were chosen, prior to flowering. A total of 150 leaves were collected, 50 leaflets from the lower stratus, and the same number from the middle and upper strata, with characteristic symptoms of chocolate spot and with different levels of damage. Similarly, 10-15 asymptomatic leaves were collected. The leaflets were stored in a botanical press to reduce mechanical damages and preserve its characteristics. They were transported to the Plant Health Laboratory of the School of Agricultural Sciences at the Universidad Autónoma del Estado de México. The leaflets were visually preselected, discarding those damaged by insects, torn o with similar symptoms, and 138 representative leaflets were selected. These were grouped into six similar categories or ranges and digitized using an HP® LASERJET PRO multifunction printer, model MFP M127fn.

The damaged area of each leaflet was evaluated in relation to the total and expressed as a percentage, using APS PRESS© Assess 2.0 (Lamari, 2008). Likewise, the areas with symptoms and asymptomatic areas were determined using RGB (Red, Green, Blue), following the methodology proposed by Passador *et al*. (2013), and the real severity of the disease (S) was estimated, using the formula proposed by Nutter *et al*. (2006): Severity = [((diseased area) ⁄ (total area of the digitized leaf))*100]. The intermediate levels of chocolate spot severity were determined according to the Weber-Fechner visual acuity law, which establishes a quantitative relationship between the magnitude of a physical stimulus and how it is perceived by the subject (Horsfall and Barrat, 1945), thereby establishing the six severity classes, as well as the maximum and minimum levels for each. To validate the severity scale, 138 digitized images of leaves with different degrees of damage, including asymptomatic ones, were evaluated. Each image was randomly placed on individual slides to be projected and viewed for 30 seconds with the aid of Microsoft 365® Power Point® (2022) and an EPSON® PowerLite S18+ multimedia projector, model V11H552021, by 20 evaluators with no experience in severity quantification and without the support of a damage scale.

The second evaluation was performed eight days after the first one, with 10 of the 20 evaluators, who displayed better visual acuity and used the proposed diagrammatic scale; they evaluated 138 leaflets. In both evaluations, a simple lineal regression was performed to determine the accuracy of the evaluators, with the support of the parameters of coefficient of determination (r^2), margin of error ($1-r^2$) and carrying out a T test on the intercept of the linear regression (β0) to test the hypothesis $H0 : \beta0 = 0$, as well as on the slope coefficient (β1) and show that it differed from: $H1 : \beta1 = 1$, with $P \leq 0.01$. Both hypothesis tests were conducted at a significance level of 0.01. The precision of the estimations obtained was evaluated based on the coefficient of determination (r^2), accuracy and the absolute errors (estimated severity minus real severity) (Nutter *et al*., 2006) obtained with a simple linear regression using SAS® version 9.0 (Tang, 2020), considering real severity as the independent variable and estimated severity as the dependent one.

The percentages of damaged area fluctuated between 0 and 58.1%. In the first evaluation, 10 evaluators showed a high level of precision identifying the class that corresponds to the severity of the disease, whereas the rest presented deficiencies in this aspect (Table 1). In this sense, Fragoso-Benhumea *et al*. (2022) point out that the levels of precision of evaluators that lack experience are not acceptable and require previous training that could improve the quality and reliability of the evaluations.

Table 1. Values of the Intercept (β_0), slope of the line (β_1), determination coefficient (r^2) and margin of error ($1-r^2$) of the simple linear regression equation in the visual estimations of the severity of *Botrytis fabae*, with 20 evaluators without a scale and 10 evaluators with a scale.

Without Scale										With Scale				
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	0.02*	1.54	0.55	0.45	**11**	<0.0001*	1.02	0.63	0.38	**1**	0.02*	<0.0001	0.90	0.10
2	<0.0001*	0.95	0.74	0.27	**12**	0.71*	1.26	0.65	0.35	**2**	<0.0001*	<0.0001	0.93	0.07
3	0.66	1.49	0.58	0.42	**13**	0.31	0.27	0.35	0.65	**3**	0.66*	<0.0001	0.96	0.04
4	0.01	1.08	0.33	0.67	**14**	0.04	1.37	0.34	0.66	**4**	0.01*	<0.0001	0.88	0.12
5	<0.0001*	0.83	0.11	0.89	**15**	0.04	0.16	0.48	0.52	**5**	<0.0001*	<0.0001	0.96	0.04
6	0.06	0.99	0.43	0.57	**16**	0.00*	1.35	0.61	0.39	**6**	0.06*	<0.0001	0.95	0.05
7	0.93	1.15	0.33	0.67	**17**	0.01*	0.90	0.54	0.46	**7**	0.93*	<0.0001	0.90	0.10
8	0.01*	1.20	0.66	0.35	**18**	0.85	1.06	0.26	0.75	**8**	0.01*	<0.0001	0.92	0.08
9	0.00*	0.61	0.07	0.93	**19**	0.32	0.84	0.63	0.37	**9**	0.01*	<0.0001	0.97	0.03
10	0.04*	0.52	0.78	0.22	**20**	0.35	0.91	0.70	0.30	**10**	0.04*	<0.0001	0.99	0.01
							Average	**0.487**	**0.512**			**Average**	**0.936**	**0.08**

*Significant: situation where the null hypothesis ($\beta_0=0$ o $\beta_1=1$) was rejected by the test t ($P \leq 0.01$).

For the second evaluation, the minimum and maximum limits of each class of chocolate spot severity were evaluated and expressed as percentages: C0: (0 = without visible symptoms), C1: (0.1-10.0; 8.8 mean value = start of concentric circular spots smaller than

20 mm), C2: (10.01-20.0; 14.5 = spots increase, with a slight brown depression), C3: (20.01-35.0; 29.5= lesions coalesce along the length and width of the leaflet, developing a reddish brown color with brown centers), C4: (35.01-58.0; 52.5 = the spots display brick-red to brown edges, with a light-brown center), C5: (>58.01-100; 65.5 = the lesions coalesce and develop a reddish-brown color, with the plant tissue beginning to necrotize).

The minimum severity value observed was 0% (healthy leaflet) and the highest severity value observed was 58.1% (Figure 1). These values were crucial to determine the limits of each class within the scale. The severity scale created has a series of classes that contains the observable ranges of the disease and provides the behavior of the severity of the disease. This coincides with Campbell and Madden (1990), who define that a severity scale must be composed of classes that represent the observable ranges of the disease, providing an adequate resolution to the behavior of the severity.

Class	0	1	2	3	4	5
Rango	**0**	**0.01%-10.0%**	**10.01%-20.0%**	**20.01%-35.0%**	**35.01%-58.0%**	**58.01% >**

Figure 1. Diagrammatic severity scale to evaluate chocolate spot caused by *Botrytis fabae* in faba bean leaves.

The values of the determination coefficient (r^2) in the 20 unexperienced evaluators fluctuated from 0.07 to 0.78 with a mean of 0.487, while the margin of error ($1-r^2$) was between 0.22 and 0.93 and a mean of 0.512 (Table 1). In the validation supported by the scale, the values of the coefficient of determination (r^2) ranged from 0.88 to 0.99 and a mean of 0.936 (Table 1). Therefore, the results of the second evaluation were superior to those of the first evaluation (Table 1). In other words, prior training influences the quality of the evaluations. These results are consistent with those reported by Lavilla and Petta (2022), who obtained averages above 0.79% the evaluations carried out using diagrammatic scales.

The margin of error ($1-r^2$) in the second stage varied from 0.01 to 0.12, with a mean of 0.06, indicating it is significant and acceptable (Hernández and Sandoval, 2015). In addition, a greater precision (β0) and accuracy (β1) were determined using the proposed diagrammatic scale (Figure 2). Therefore, the proposed scale is reproducible and accurate, in agreement with what Cristiane-Delmadi *et al*. (2018) and Hernández y Sandoval (2015).

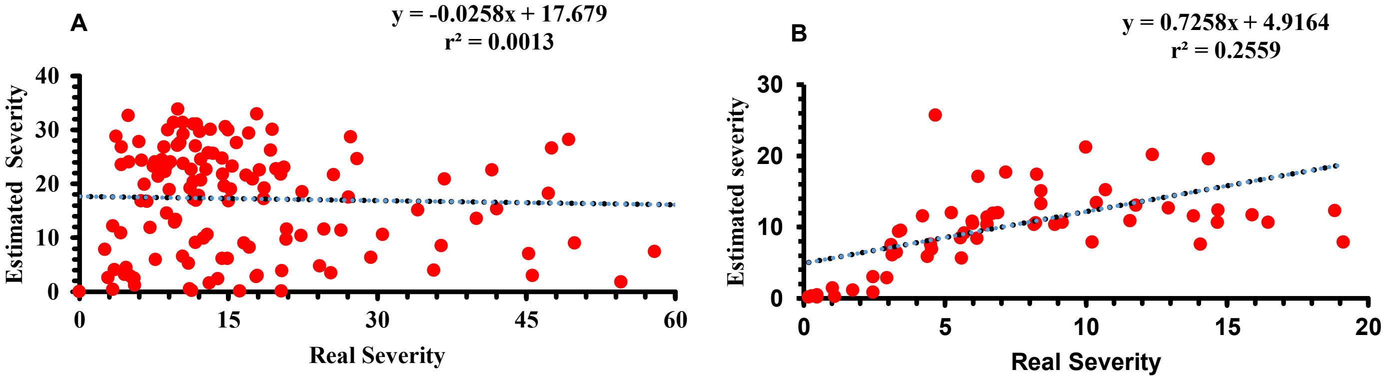

Figure 2. Distribution of residuals (estimated severity-real severity) of the chocolate spot (*Botrytis fabae*) evaluations in fava bean leaflets. **A**) Evaluation without scale. **B**) Evaluation with scale.

The precision determined by each evaluator indicates that a diagrammatic scale represents a standardized method of quantification of a disease, and this is the reason behind favorable results (Cristiane-Delmadi *et al*., 2018; Hernández and Sandoval, 2015), along with and adequate accuracy and precision between evaluators (Figure 1). Likewise, these results coincide with those reported by Muñoz *et al*. (2020), which pointed out that damage assessment supported by a scale considerably improves both precision (β0) and accuracy (β1). Fragoso-Benhumea *et al*. (2022) indicated that a diagrammatic scale can ensure reproducibility, but not the accuracy and precision of the estimation. Regarding the value of the intercept β0 and slope coefficient β1, most evaluators displayed significance, that is, statistically different and/or near to 1, with a confidence interval of ∝=0.01 (99%). This indicates that the use of the diagrammatic severity scale makes it possible to obtain precision and accuracy values close to the actual severity, even when there are slight tendencies toward underestimation and overestimation. This statement is reinforced when comparing the absolute error values of the evaluations, where a reduction in the absolute error is observed when using the proposed scale (Patricio-Hernández *et al*., 2023).

The severity scale to evaluate chocolate spot caused by *Botrytis fabae* in the faba bean crop, composed of six classes, provided a high reproducibility with acceptable levels of accuracy (0.174) and precision (<0.0001), making it a tool to standardize the evaluation of damages in different locations, as well as to evaluate the efficiency of management measurements or the response of the sensitivity of faba bean cultivars to the disease.

Limitations

The effect of variety on the intensity of damage was not considered.

Conflict of interest

The authors declare no personal or institutional conflicts of interest.

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Authors' contributions

LREA – Research, trials with evaluators, original draft writing; **CVA** - Conceptualization and review; **SPJR** – Resources, conceptualization, editing; **FBJM** - Investigation and writing; **GVR** - Conceptualization, writing and revision; **QSA** - Writing and revision.

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