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Development and validation of a diagrammatic scale to assess the severity of cucumber downy mildew

Juan Carlos Gonzalez-Acevedo[1], **Santiago Domínguez-Monge**[2]*, **Dagoberto Guillen-Sánchez**[1†], **Víctor López-Martínez**[1], **Porfirio Juárez-López**[1], **Irán Alia-Tejacal**[1], **Julio David Mendoza-García**[3]. [1]Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Av. Universidad No. 1001, Col. Chamilpa, Cuernavaca, Morelos, C. P. 62209, México. [2]Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, INIFAP – Campo Experimental Ixtacuaco. Carretera Martínez de la Torre – Tlapacoyan Km. 4.5, Cong. Javier Rojo Gómez, Tlapacoyan, Veracruz, C. P. 93655, México. [3]Maestría en Ciencias en Protección Vegetal. Departamento de Parasitología Agrícola, Universidad Autónoma Chapingo, Km 36.5 Carretera México-Texcoco, Texcoco, Estado México, C. P. 56230, México.

Corresponding author:
Santiago Domínguez-Monge
dominguez.santiago@inifap.gob.mx

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ABSTRACT

Background/Objective. Cucumber downy mildew (*Cucumis sativus*), caused by *Pseudoperonospora cubensis*, is one of the major foliar diseases affecting cucumber production in Morelos, Mexico. Due to the lack of standardized and validated methods for quantifying disease severity, the objective of this study was to develop and validate a diagrammatic scale for its assessment.

Experimental development. A diagrammatic scale composed of eight severity levels (2, 4, 8, 16, 24, 32, 40, and 47% affected leaf area) was developed using naturally infected leaves whose actual severity was determined through digital image analysis with Assess® software. Validation of the scale was conducted with the participation of 20 raters, who visually estimated the severity of 50 leaves with different damage levels in three successive evaluations: one without the aid of the scale (WO) and two with the aid of the scale conducted two (A1) and four weeks (A2) after the initial evaluation.

Results. The use of the diagrammatic scale significantly increased the accuracy of severity estimates, with Pearson´s correlation coefficients of $r = 0.91$ and $r = 0.93$ in A1 and A2, respectively, compared to the evaluation without the scale ($r = 0.34$). Likewise, generalized bias was lower when the scale was used ($C_b > 0.9$), which was reflected in greater overall agreement between visual estimates and real severity values ($\rho_c > 0.9$).

Conclusion. The developed diagrammatic scale is a reliable, accurate, and reproducible tool for estimating cucumber downy mildew severity under field conditions.

Keywords: *Cucumis sativus,* Downy mildew, Foliar disease, Phytopathometry, Severity

INTRODUCTION

The cucumber (*Cucumis sativus*) is one of the most important vegetable crops worldwide, for both its wide distribution and its value in intensive production systems. In Mexico, its production has become significantly intensified under open field and protected agriculture conditions, which has intensified the incidence of diseases related to environments with high humidity levels and high temperature (Naegele and Wehner, 2017).

In the main phytosanitary problems, the genera *Pythium*, *Phytophthora*, *Rhizoctonia*, *Colletotrichum*, *Alternaria*, *Fusarium* and *Pseudoperonospora* (Almándoz *et al*., 2016; Ramezani *et al*., 2017) stand out. These diseases are an important limitation to productivity, particularly in protected production systems, where the environmental conditions favor their development (Alhasnawi *et al*., 2020). Downy mildew, caused by *Pseudoperonospora cubensis* (Peronosporales: Peronosporaceae), is one of the most destructive leaf diseases in the cucumber crop. The pathogen infects cotyledons and real leaves, leading to chlorosis, necrosis and premature defoliation, which reduces the area of photosynthesis and directly affects yield (Cohen *et al.*, 2015; Salcedo *et al.*, 2020; Sun *et al.*, 2022). Its development is favored by conditions of relatively high humidity (> 85%) and moderate temperatures (15-22 °C), common during the rainy season in several production areas in Mexico such as Sinaloa, Sonora and Nayarit (Cristóbal *et al*., 2006; Ruiz *et al*., 2008; Pouzeshimiyab and Fani, 2020). In addition, the ability of the pathogen to spread via seeds and fruits has been proven and increases the risk of spreading and establishing in new production areas (Cohen *et al*., 2014; Cohen *et al*., 2019; Félix-Gastélum *et al.*, 2023; Hammer *et al.*, 2025).

The efficient management of the cucurbit downy mildew requires an adequate understanding of its epidemic dynamics. For this purpose, the systematic monitoring of parameters such as disease incidence and severity is crucial. Particularly, the quantification of the severity helps estimate the degree of damage caused by the pathogen and evaluate the effectiveness of the implemented management strategies (Nutter *et al*., 1991; Silva *et al*., 2016).

Pathometry, as a discipline of phytopathology, is in charge of estimating the quantitative and qualitative estimation of diseases under field conditions (Vivas *et al*., 2012). In this context, the diagrammatic scales are a crucial tool for the visual evaluation of severity (Bock *et al*., 2010), since they help standardize the estimations between evaluators and reduce the subjectivity inherent to this type of measurements (Meirelles y Cardoso, 2012; Alves *et al*., 2015; Bock *et al*., 2016). The development of reliable diagrammatic scales has been favored by the advancement of digital technologies and computer systems (Bock *et al*., 2010; Bock *et al.*, 2021), which has helped improve the precision and accuracy of the severity evaluations in diverse pathosystems (Meirelles and Cardoso, 2012; Pereira *et al.*, 2020). Nevertheless, the use of these tools implies a high degree of methodological responsibility, since deficient scales may cause significant errors when it comes to making phytosanitary decisions (Cristiane-Delmadi *et al.*, 2018; Bock *et al.*, 2020). Due to this, diagrammatic scales must undergo validation processes that guarantee their utility and reproducibility (Capucho *et al*., 2011; Del Ponte *et al.*, 2022).

Despite the presence of mildew in cucurbits, the evaluation of the severity caused by *P. cubensis* in the cucumber crop continues to display limitations, due to the absence of specific diagrammatic scales validated under local edaphoclimatic conditions. In particular, for Morelos, Mexico, there are no standardized tools that help accurately quantify the severity of this leaf disease. Due to this, the aim was to develop and validate a diagrammatic scale to estimate the severity of cucumber mildew caused by *Pseudoperonospora cubensis*.

EXPERIMENTAL DEVELOPMENT

Study site and plant material. A total of 150 cucumber leaves with different downy mildew severities were gathered in the months of September and October, 2019. The sampling was performed in commercial cucumber (var. Naolin) plantations established in open-air fields in two locations of the state of Morelos which were Los Girasoles, municipality of Cuautla (18°50'58.7" N, 98°55'31.1" O; 1,397 m.a.s.l.) and Nacatongo, in the municipality of Tlayacapan (18°56'21.4" N, 98°58'39.3" O; 1,543 m.a.s.l.). The leaves were selected in such a way that they represented a wide range of severity of the disease and they were used for the development and construction of the diagrammatic scale.

Acquiring and analysis of images. The 150 leaves selected were individually photographed from the upper surface, using the camera of a Huawei Mate® 20 Pro mobile phone (40 megapixels). The images were captured under controlled conditions, with a blue cloth backdrop, at a fixed distance of 30 cm between the camera and the leaf, mounted on a tripod at a height of 50 cm, following the method proposed by Del Ponte *et al*. (2017). The percentage of affected leaf area was determined through digital image analysis using the Asses® v2.0 software (The American Phytopathological Society, St. Paul, MN, USA). The automatic color model based on hue, saturation and intensity (HSI) was used to delimit the total leaf area and the symptomatic area, from which the program calculated the severity percentage for each image (Del Ponte *et al*., 2017).

Determining types and development of the diagrammatic scale. The number of classes of the diagrammatic scale was established according to the criteria suggested by Del Ponte *et al*. (2017), considering a minimum of six and a maximum of 10 classes, with severity intervals of no more than 15% and distributed in an approximately linear way, taking the highest level of severity observed in the field as a reference. The lowest and highest severity levels recorded during the sampling were used as the lowest and highest limits of the scale. From these values, six intermediate classes were defined, with severity intervals that varied between 2 and 8%. The diagrams for each class were designed in a realistic way, using a base scheme of a cucumber leaf. The lesions were incorporated by hand, respecting the shape, size and distribution pattern observed in real leaves, until the severity value defined for each class was reached (Del Ponte *et al*., 2017).

Validation of the diagrammatic scale. To validate the scale, 50 images of diseased leaves were used, with a real severity (2 to 47%) had previously been determined by the analysis of images, but unknown for the raters. The images were projected on individual PowerPoint slides, in a random sequence, with an exposure time of 30 s per image. Twenty unexperienced raters were chosen for the estimation of the severity of the disease, and before beginning the assessments, instructions were provided to the participants for the recognition of the symptoms of the disease and the severity evaluation procedure. Every rater assigned a severity value expressed as a percentage (0-100%).

The validation was carried out in three consecutive assessments. In the first one, the participants estimated the severity of the 50 images without the aid (WO) of the diagrammatic scale, recording their values in a sheet of paper numbered between 1 and 50. Two weeks later, a second assessment was carried out, in which participants estimated the severity of the same images, except this time they did have the aid (A1) of the developed diagrammatic scale. Subsequently, after an additional two-week interval, a third assessment was held, also with the aid (A2) of the scale. The conditions for the projection and assessment were the same in all three assessments, in order to minimize possible distraction or bias effects. The same 20 raters participated in all assessments. The data obtained were organized in a Microsoft Office Excel 2016 data base for their statistical analysis (Del Ponte *et al*., 2017).

Statistical analyses. To evaluate the effect of the use of the diagrammatic scale on the precision and accuracy of the severity estimations, Lin's concordance correlation coefficient (ρ_c) (Lin, 1989) was calculated for every rater. This coefficient provides a general concordance or accuracy mean, considering both the precision (Pearson's correlation coefficient, *r*) and the generalized bias parameter (C_b), a coefficient calculated from the systematic bias (υ) and the constant bias (μ) (Madden *et al*., 2007). A generalized linear model was used to test the hypothesis of the means of the concordance coefficients not having a significant difference among the three sequential assessments. The significance of the fixed effects was evaluated using Barttlet's test, with a level of significance of $\alpha = 0.05$ ($P > \chi^2$). In addition, the reproducibility between raters was determined between the coefficient of determination (R^2), according to Nutter and Schultz (1995). The statistical analyses were carried out using the R (R Core Team, 2016) and SAS 9.0 softwares (SAS Institute Inc., Cary, NC, USA).

Determining the severity and definition of types of the diagrammatic scale. The real values of severity for the mildew caused by *Pseudoperonospora cubensis* in cucumber leaves, determined by digital image analysis, varied between 2 and 47%, corresponding to the lowest and highest leaf damage levels observed during the field sampling. Based on this interval, eight types of severity were defined, represented by the following percentages: 2, 4, 8, 16, 24, 32, 40 and 47%. Each class was illustrated by a diagram that visually represents the pattern and distribution of the foliar damage, which is characteristic of each security level. The final diagrams of the scale are blank (Figure 1).

Relationship between the real severity and the estimated severity. The severity estimations obtained during the assessments performed, with the use of the diagrammatic scale, displayed a higher concordance with the real severity values determined by a digital image analysis, in comparison with the assessments carried out without the use of the scale. This relationship is observed in the dispersion diagrams that compare the estimated severity and the real severity for each assessment (Figure 2).

When the severity was evaluated without the aid of the diagrammatic scale, a high data dispersion was registered, reflected a low determination coefficient (R^2= 0.13), which indicates a low precision in the visual estimation of the severity. By contrast, when using the diagrammatic scale developed (interval of 2 to 47% of affected area), the severity estimations displayed a broad and consistent relation with the real values in both the assessment performed two weeks after the first assessment (A1; R^2= 0.93), and in the one carried out four weeks later (A2; R^2= 0.94). These results show that the use of the diagrammatic scale significantly increases the precision and reliability of the downy mildew severity assessments, reducing the subjectivity related to the visual estimation. The improvement observed coincides with reports by earlier studies, in which the use of validated diagrammatic scales helps produce a more objective and reproductible assessment of leaf diseases (Mora-Aguilera, 2013; Flores-Sánchez *et al*., 2025).

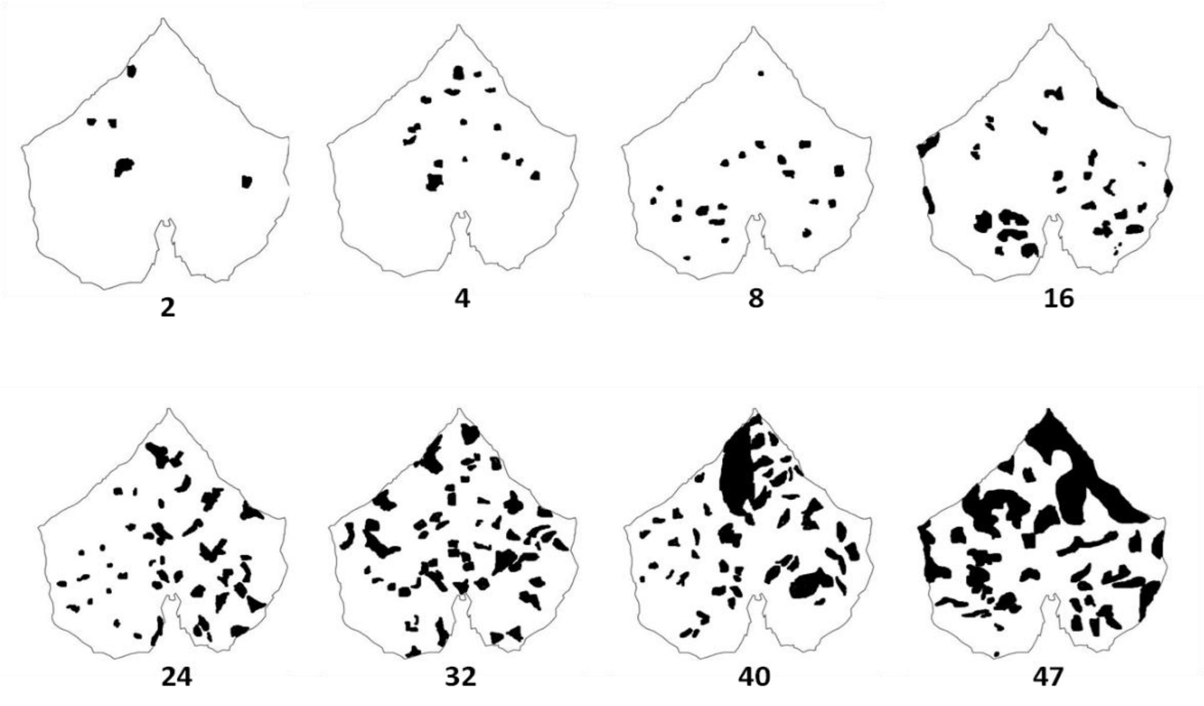

Figure 1. Scale developed for the severity of downy mildew from *Pseudoperonospora cubensis* in cucumber leaves. Numbers in the diagrams represent the percentage (%) of the area of the leaf with necrotic symptoms of the disease.

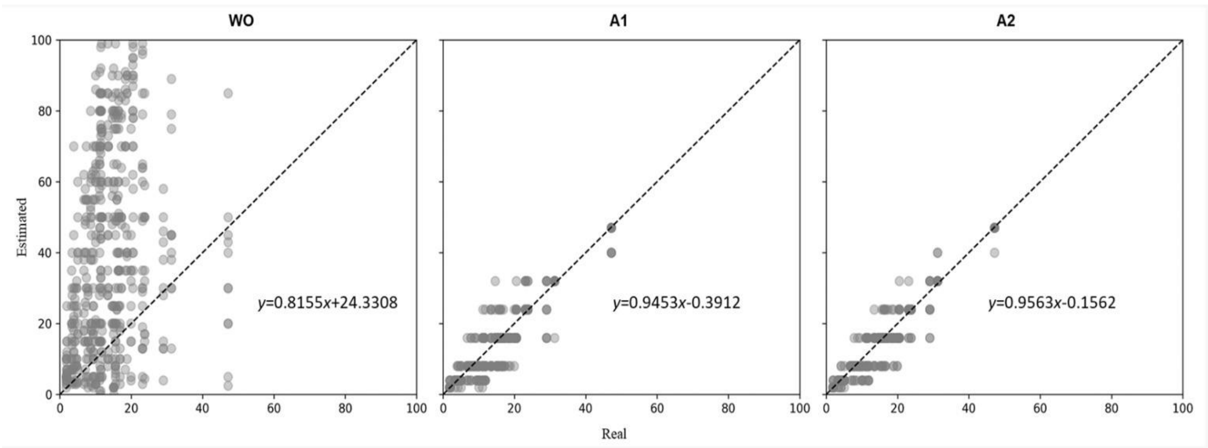

Figure 2. Relationship between estimates and real severity of the downy mildew (*Pseudoperonospora cubensis*) by 20 inexperienced raters for a set of 50 evaluated leaves (n=1000). The first assessment was carried out without the diagrammatic scale (WO), followed by two additional assessments performed two (A1) and four weeks (A2) later, using a diagrammatic scale that represents levels of severity between 2 and 47% of the leaf area affected. Dotted lines indicate the concordance line (y = x).

Accuracy, precision and concordance of the severity estimations. The comparisons of accuracy between raters, analyzed through the parameters of systematic bias (υ) and constant bias (μ), displayed a wide variation in the severity estimations during the assessment without the use of the diagrammatic scale. Nevertheless, this variability decreased progressively during the second and third assessment sessions, when the raters used the scale developed, tending to values nearer to the reference values, that is, an absence of systematic bias ($\upsilon = 1$) and of constant bias ($\mu = 0$) (Figure 3).

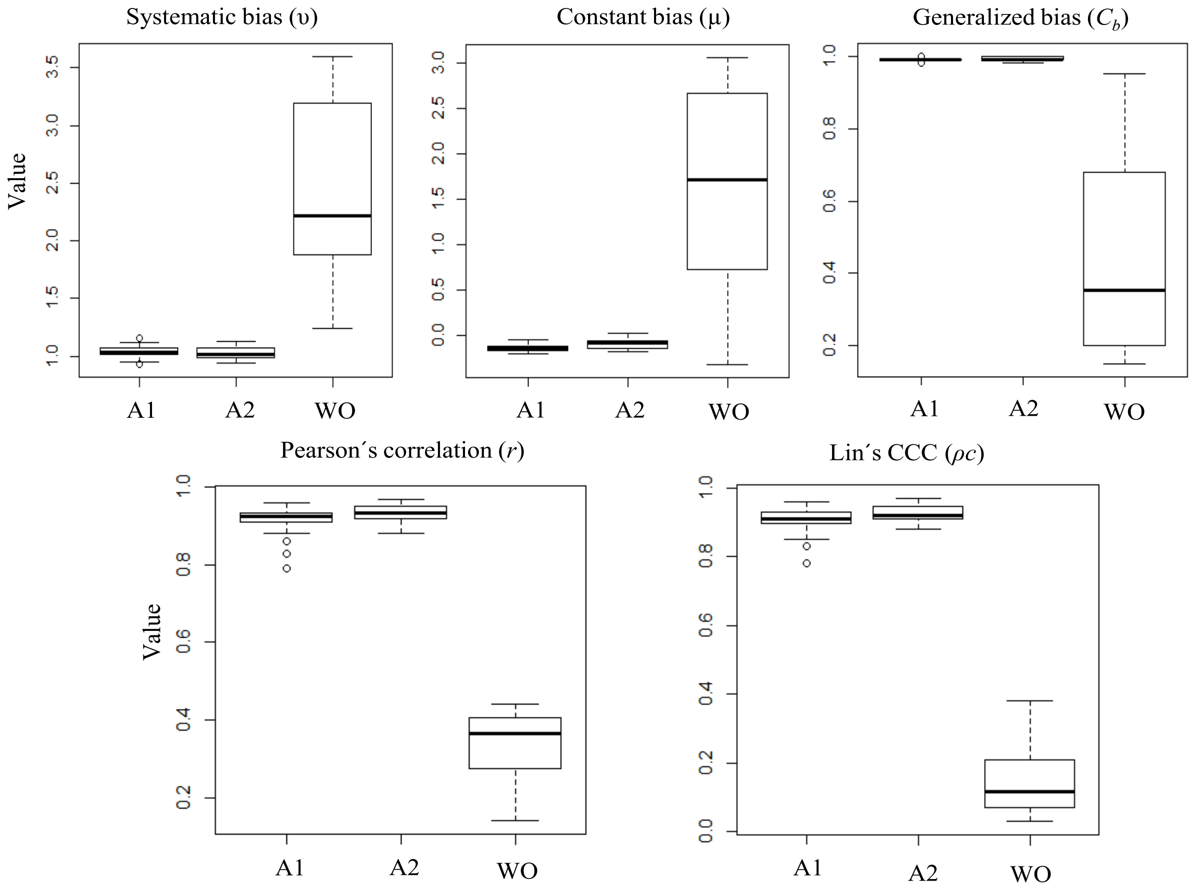

Figure 3. Distribution, mean and standard deviation of the statistics from Lin's concordance analysis used to evaluate accuracy (systematic and constant bias), precision (Pearson's correlation coefficient, r) and concordance (Lin's concordance correlation coefficient, ρ_c) of the estimations of severity of downy mildew (*Pseudoperonospora cubensis*) carried out by 20 unexperienced raters in the successive assessments, The first assessment was performed without the aid of the scale (WO), followed by two assessments with the scale two (A1) and four weeks (A2) after the initial assessment.

From the statistical point of view, both the constant bias (μ) and the systematic bias (υ) registered in the two assessments carried out with the aid of the diagrammatic scale differed significantly from those obtained in the initial assessment without any aid, which is attributed to the low precision observed in the latter one (C_b = 0.35) (Figure 3). By contrast, the precision of the estimations improved notably when the diagrammatic scale was used, reaching high correlation coefficients in both assessments with the aid of the diagrammatic scale ($r > 0.9$), in comparison with the assessment without the scale (r = 0.35).

As a direct consequence of this improvement in precision, the general concordance between the estimations and the real severity values, expressed by Lin's concordance coefficient (ρ_c), increased significantly during the assessments carried out with the aid of the diagrammatic scale. Particularly, values for ρ_c of over 0.9 were recorded in both A1 and A2, in contrast with the initial session WO (Table 1). These results show that the visual estimation of the severity of downy mildew in cucumber is considerably more precise, exact and in greater concordance when the diagrammatic scale developed in this study is used, confirming its usefulness as a similar phytopathological tool, similar to other studies

(Leiva *et al*., 2010; Savory *et al*., 2011; Zavala and Cristóbal, 2012; Mora-Aguilera, 2013; Belan *et al*., 2014; Correia *et al*., 2017; Acco *et al*., 2020; Flores-Sánchez *et al*., 2025).

Table 1. Analysis of the generalized linear model and separation of means of the statistics of Lin's concordance analysis used to evaluate accuracy (systematic and constant bias), precision (Pearson's correlation coefficient, r) and overall concordance (Lin's concordance coefficient, ρ_c) of the estimated percentages of the severity of downy mildew (*Pseudoperonospora cubensis*) in the cucumber, performed by 20 unexperienced raters in three successive assessments: without the aid of the diagrammatic scale (WO) and with the aid of the scale two weeks (A1) and four weeks (A2) after the initial assessment.

Assessment	υ[b]	μ[c]	C_b[d]	r[e]	P_c[f]
WO	1.57 a[a]	2.40 a	0.46 b	0.34 b	0.15 b
A1	-0.13 b	1.04 b	0.99 a	0.91 a	0.90 a
A2	-0.09 b	1.02 b	0.99 a	0.93 a	0.93 a
χ^2[g] (P-value)	165.40 (<0.0001)	132.90 (<0.0001)	224.80 (<0.0001)	25.61 (<0.0001)	29.82 (<0.0001)

[a]Means followed by the same letter in the same column do not differ significantly between each other (Tukey, $\alpha = 0.05$). [b]Systematic bias (υ; $\upsilon = 1$ indicates no bias in regard to the line of concordance). [c]Constant bias (μ; $\mu = 0$ indicates no bias in regard to the line of concordance). [d]Generalized bias (C_b), which measures the degree of deviation of the line of best fit from the line of concordance (Madden *et al*., 2007). [e]Pearson's correlation coefficient (r), used as a measure of precision. [f]Lin's concordance correlation coefficient (ρ_c), which integrates the measures of precision (r) and generalized bias (C_b) to evaluate accuracy. [g]Chi-squared value (χ^2) from Bartlett's test and its associated probability ($P < 0.05$).

By analyzing the reliability between raters, a significant improvement was observed in the estimations of severity of *P. cubensis* in cucumber when the diagrammatic scale was used, in comparison with the assessment performed without the aid of the scale. This tendency was reflected in Table 2, where the mean of the determination coefficient (R^2) presented clear statistical differences between the assessments. In the first assessment, without the aid of the scale (WO), the value of R^2 was 0.12, indicating a low reliability between raters, whereas in the assessments performed on the subsequent weeks two (A1) and four (A2), with the aid of the diagrammatic scale, the R^2 values increased to 0.83 and 0.87, respectively, displaying a higher degree of consistency and reproducibility in the estimations.

The validation of a diagrammatic scale is a crucial step to guarantee its applicability in field assessments, particularly when multiple raters participate with different experience levels (Lenz *et al*., 2010). In this context, the results statistically show that the diagrammatic scale developed in this study is reliable to evaluate the severity of *P. cubensis* in cucumber, making it practical and robust tool for researchers, technicians and farmers that need accurate estimations of the disease in crops.

Table 2. Measures of reliability between raters of the estimations of the severity of downy mildew (*Pseudoperonospora cubensis*) carried out by 20 unexperienced raters in three successive assessments: without the aid of the diagrammatic scale (WO) and with the aid of the diagrammatic scale two (A1) and four weeks (A2) after the initial assessment.

Assessment	R^{2b}	95 % CI[c]	P-value
WO	0.120 b[a] (0.01)	0.09-0.14	<.0001
A1	0.834 a (0.02)	0.79-0.86	<.0001
A2	0.872 a (0.01)	0.85-0.89	<.0001

[a]Means followed by the same letter in the same column do not differ significantly between each other (Tukey, α = 0.05). [b]Means (± standard error) of all pairwise comparisons between raters, calculated from the coefficient of determination (R^2) of the relationship between two raters (Lin, 1989). The mean values were compared between assessments using a paired t-test. [c]Confidence interval corresponding to each assessment.

Similar results have been reported in other pathosystems. When evaluating the severity of leaf and calyx spot caused by *Corynespora cassiicola* in roselle (*Hibiscus sabdariffa*), Ortega-Acosta *et al*. (2016) observed that the use of a diagrammatic scale significantly improved the accuracy of the estimates. They pointed out that the greatest absolute errors and the tendency towards overestimation occurred when assessments were held without the aid of a scale. Consistently, Lima *et al*. (2017) showed that the use of a diagrammatic scale increased the reliability increased the reliability in the assessment of powdery mildew in the chestnut (*Anacardium occidentale*) caused by the fungus *Pseudoidium anacardii*, even when experienced and unexperienced raters participated, and reached determination coefficients of 0.94 and 0.91, respectively.

These findings are fully consistent with the results of this study, which obtained high determination coefficients (0.83 and 0.87) when implementing the diagrammatic scale in both assessments (A1 and A2), in contrast with the relatively low value registered in the assessment without the scale (0.12). Together, these findings confirm that the use of diagrammatic scales reduce the variability between raters and substantially improve the reliability of the visual estimations of severity in leaf diseases.

CONCLUSIONS

The diagrammatic scale, developed and validated diagrammatically to evaluate the severity of downy mildew in cucumber, helped obtain significantly more accurate exact and concordant estimations. The use of the scale is recommended to evaluate the severity in other investigations, particularly in scenarios in which multiple raters participate, since it reduces the variability between observations and substantially improves the accuracy and precision of the visual estimations.

Conflict of interest. The authors declare there is no conflict of interest.

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