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Diagrammatic scale for assessment the severity of Cercospora leaf blight on soybean (*Glycine max*) leaflets [1]

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Artículos

Diagrammatic scale for assessment the severity of Cercospora leaf blight on soybean (*Glycine max*) leaflets [1]

Escala diagramática para evaluar la severidad del tizón foliar por Cercospora en los foliolos de soja (*Glycine max*)

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Abstract:

Introduction. Cercospora leaf blight (CLB) caused by Cercospora kikuchii is an endemic soybean crop disease in Argentina. **Objective.** To develop and validate a scale to quantify the severity of CLB in leaflets of soybean (*Glycine max*) for the evaluation of foliar fungicides and cultivar trials. **Materials and methods.** A diagrammatic scale to assess the severity of CLB on soybean leaflets (*Glycine max*) was developed. The scale was based on the evaluation of 50 leaflets. The different severity levels of the leaflets were determined according to Weber-Fechner's stimulus-response law. The proposed scale included severity levels of 1, 4.5, 17.5, 50, 82.2, 95, and 99 %. The validation of the scale was performed by 23 raters (3 experienced and 20 inexperienced) who assessed the severity of CLB on 50 leaflets with and without the use of the scale. **Results.** The results from the three experienced raters were more precise than those obtained by the inexperienced raters. The scale improved the visual interpretation to assess the severity of CLB for the inexperienced raters, who obtained estimated severity values close to the actual severity values. When the inexperienced raters used the proposed scale to estimate the severity of CLB on leaflets, they were accurate having determination coefficients (R2) between 0.74 and 0.91. According to the absolute errors, the greatest deviation was observed between -2.49 and 2.46. However, these deviations were not significantly different from the population average. To speed the process of cultivar evaluations we also propose the use of scales based on grades. **Conclusions.** The proposed scale to assess the severity of CLB on leaflets can be used for the assessment of foliar fungicide trials and soybean cultivars. The scale showed specificity for assessing the severity of Cercospora leaf blight (CFB) in soybean leaflets.

Keywords: *Cercospora kikuchii* , epiphytology, pathometric, plant pathology, disease evaluation.

Resumen:

Conclusiones.

Notas de autor

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Palabras clave: *Cercospora kikuchii* , epifitiología, patometría, fitopatología, evaluación de enfermedades.

Introduction

Cercospora leaf blight (CLB) caused by *Cercospora kikuchii* (Matsumoto & Tomoyasu, 1925) is an endemic soybean disease in Argentina (Ivancovich & Botta, 2000). Although CLB is not yet considered a yield limiting disease, under specific environmental conditions and/or the characteristics of the pathogen could become a serious threat in the main production region of Argentina (Ivancovich & Botta, 2000; Lavilla et al., 2021; March et al., 2010). CLB and purple seed stain were found to be widespread in all soybean regions of Argentina, with a prevalence of 100 % (Lavilla et al., 2021). In 2015 and 2016, the severity of Cercospora leaf blight was statistically lower in the southern pampa region compared to the other regions (Lavilla et al., 2021). *Cercospora kikuchii* produces different kinds of symptoms in soybean (*Glycine max* L. Merr.). Seed infection known as purple seed stain produces a purple discoloration of the seed coat ranging from small spots to complete coverage (Pathan et al., 1989). Seed are infected when the pathogen grows from the pods into the seed coat (Wrather et al., 2001). On seeds *C. kikuchii* produces a purple discoloration (Velicheti, 1994). Severe seed infection can lead to seedling death reducing stands and yields (Sinclair & Backman, 1989).

Foliar symptoms known as CLB appear during reproductive growth as red purple spots on both sides of the leaflets (Ivancovich, 2011). Under severe infection purple necrosis on stems, petioles and leaflets occurs. The purple discoloration is associated with a photoactive mycotoxin (cercosporin) (Hartman et al., 1988; Kuyama & Tamura, 1957; Upchurch et al., 1991). Severe infection can result in early defoliation reducing yields (Bluhm et al., 2010; Ross, 1975; Walters, 1980). On this way, Albu et al. (2014) consider CLB and purple seed stain as two different diseases caused by *C. kikuchii*.

A correct evaluation and quantification of CLB are important to develop strategies for disease management. Disease severity scales are an effective tool in accurately determining the severity of foliar pathogens, these scales must have the following characteristics (Ivancovich et al., 1998):

a. Be appropriated for and related to the disease to be evaluated under different environmental conditions and crop growth stages.

b. Be reproducible, easy, and fast to use and accurate and precise.

The accuracy of the assessment is how closely the assessment is to the true level of disease (Nutter, 1993).

Several scales are available to evaluate soybean leaflets diseases, but none of them are specific for CLB (Godoy et al., 2006; Martins et al., 2004; Soares et al., 2009). The goal of this study was to develop and validate a scale to quantify CLB on soybean leaflets to be used for the evaluation of fungicides trials and soybean cultivars.

Materials and methods

The processing of the leaflets and their subsequent evaluation were carried out at Universidad Nacional del Noreste de la Provincia de Buenos Aires (UNNOBA), Argentina, in 2017.

The accuracy and precision of the assessments of each rater were determined by linear regression, where actual severity was the independent variable and estimated severity the dependent variable. The accuracy of the estimates of each rater was determined by t-test (Di Rienzo et al., 2011; StatSoft Inc., 2005) applied to the slope coefficient (b), to check whether they were significantly different from 1.0, and to the intercept (a) to check whether they were significantly different from 0. The precision of the assessments of the raters was estimated by the coefficient of determination (R2) of the same regression line and by the variance of the absolute errors (estimated severity minus actual severity) for each assessment (Nutter, 1993; Nutter & Schultz, 1995).

Cercospora leaf blight (CLB): severity scale development

The scale to evaluate of Cercospora leaf blight (CLB) (*Cercospora kikuchii*) on leaflets, was developed based on a sample of 50 soybean leaflets (cultivar Don Mario 5009) with different levels of severity collected during the years 2015 and 2016. Todefine the severity value of each leaflet three plant pathologists evaluated them under naked eye. Severities values were determined with and without a scale (Figure 1).

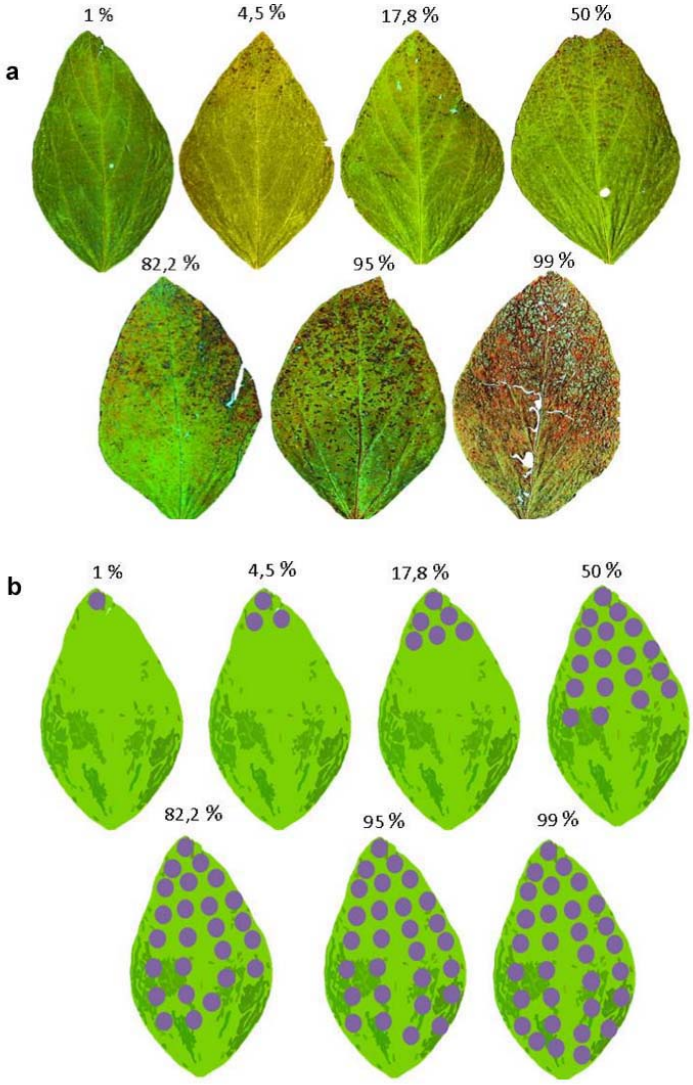

FIGURE 1

a. Scale to quantify Cercospora leaf blight (*Cercospora kikuchii*) on soybean (*Glycine max*) leaflets. b. Diagrammatic scale to quantify Cercospora leaf blight on soybean leaflets. Universidad Nacional Del Noroeste de la Provincia de Buenos Aires, Pergamino Buenos Aires, Argentina. 2017.

Figura 1. a. Escala para cuantificar el tizón foliar por Cercospora (*Cercospora kikuchii*) en los foliolos de soya (*Glycine max*). b. Escala diagramática para cuantificar el tizón de la hoja de Cercospora en los foliolos de soya. Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Pergamino, Buenos Aires, Argentina. 2017.

Validation of the scale on leaflets

The validation of the proposed scale (Figure 1a) was carried out by 20 inexperienced raters by using the scale to evaluate symptoms of CLB on the 50 soybean leaflets with different levels of severity.

Results

Scale to quantify of Cercospora leaf blight (CLB) on leaflets

The proposed scale improved the evaluation of the severity of CLB. The estimated severity values obtained by the inexperienced raters were close to the actual severity values (Table 1). The values for the lower and upper limits of the diagrammatic scale were 1 % and 99 % of the leaf area with lesions (Figure 1).

TABLE 1

Coefficients at the straight line (a), the straight slope (b), and the linear regression R^2 for the evaluation of the inexperienced raters, when using a scale to quantify the severity of Cercospora (*Cercospora kikuchii*) leaf blight on soybean (*Glycine max*) leaves. Universidad Nacional Del Noroeste de la Provincia de Buenos Aires Pergamino Buenos Aires, Argentina. 2017.

Inexperienced raters	With scale					Without scale				
	b		a		R2	b		a		R^2
1	0,98	n.s.	1,49	n.s.	0,89	0,17	*	28,72	n.s.	0,09
2	0,8	n.s.	7,48	n.s.	0,57	0,3	*	23,22	n.s.	0,19
3	0,82	n.s.	4,41	n.s.	0,63	0,16	*	23,62	n.s.	0,07
4	0,74	n.s.	7,61	n.s.	0,56	0,22	*	18	n.s.	0,14
5	0,77	n.s.	4,68	n.s.	0,51	0,23	*	14,2	n.s.	0,16
6	0,86	n.s.	10,2	n.s.	0,65	0,38	*	4,59	n.s.	0,4
7	0,63	n.s.	24,8	n.s.	0,34	0,1	*	10,98	n.s.	0,13
8	0,68	n.s.	19	n.s.	0,46	0,31	*	9,82	n.s.	0,24
9	0,74	n.s.	11,11	n.s.	0,55	0,56	n.s.	11,32	n.s.	0,31
10	0,72	n.s.	10,91	n.s.	0,5	0,63	n.s.	14,02	n.s.	0,4
11	0,71	n.s.	21	n.s.	0,4	0,24	*	32,29	n.s.	0,14
12	0,78	n.s.	7,7	n.s.	0,59	0,58	n.s.	18,34	n.s.	0,36
13	0,8	n.s.	8,12	n.s.	0,6	0,48	n.s.	19,16	n.s.	0,3
14	0,75	n.s.	7,35	n.s.	0,57	0,37	n.s.	16,89	n.s.	0,17
15	0,77	n.s.	6,51	n.s.	0,57	0,52	n.s.	11,51	n.s.	0,33
16	0,69	n.s.	8,01	n.s.	0,59	0,35	*	12,08	n.s.	0,27
17	0,7	n.s.	4,1	n.s.	0,43	0,39	n.s.	21,9	n.s.	0,22
18	0,65	n.s.	3,19	n.s.	0,46	0,4	n.s.	20,93	n.s.	0,25
19	0,58	n.s.	15,9	n.s.	0,3	0,59	n.s.	6,5	n.s.	0,4
20	0,78	n.s.	2,38	n.s.	0,6	0,43	n.s.	13,54	n.s.	0,28

*Situations in which the null hypothesis (a=0 or b=1) was rejected by the t-test, p<0.05. n.s.: non-significant statistical difference / *Situaciones en las que la hipótesis nula (a=0 o b=1) fue rechazada por la prueba t, p<0,05. n.s: diferencia estadística no significativa.

Cuadro 1. Coeficientes en la línea recta (a), la pendiente recta (b), y la regresión lineal R^2 para la evaluación de los calificadores inexpertos, al emplear una escala para cuantificar la severidad del tizón foliar por Cercospora (*Cercospora kikuchii*) en hojas de soja (*Glycine max*). Universidad Nacional del Noroeste de la Provincia de Buenos Aires Pergamino Buenos Aires, Argentina. 2017.

The coefficients (b) of the regression line between actual and estimated severity, was higher for experienced raters, with and without the use of the scale (Tables 1 and 2).

TABLE 2
Coefficients at the straight line (a), the straight slope (b), and the lineal regression R^2 for the evaluation of the experienced raters, of the severity of Cercospora (*Cercospora kikuchii*) leaf blight on soybean (*Glycine max*) leaves, based in grades. Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Pergamino Buenos Aires, Argentina. 2017.

Experienced raters	Coefficients		
	b	a	R^2
1	1.30	3.47	0.91
2	1.08	1.90	0.89
3	0.98	3.50	0.93

Cuadro 2. Coeficientes en la línea recta (a), la pendiente recta (b), y la regresión lineal R^2 para la evaluación de los calificadores expertos, de la severidad del tizón foliar por Cercospora (*Cercospora kikuchii*) en foliolos de sojas (*Glycine max*), con base en una escala. Universidad Nacional del Noroeste de la Provincia de Buenos Aires Pergamino Buenos Aires, Argentina. 2017.

Precision, estimated by the coefficient of determination (R^2) and by the variance of the absolute errors, was higher with the use of the diagrammatic scale for both inexperienced raters (Tables 1 and 3).

TABLE 3
Absolute error (estimated severity minus actual severity) obtained by 20 inexperienced raters by using the proposed scale to evaluate Cercospora leaf blight (CLB) on soybean (*Glycine max*) leaflets based in grades. Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Pergamino Buenos Aires, Argentina. 2017.

Raters	Absolute error	Actual severity	Estimated severity
1	4.2	47	51
2	0.3	47	47
3	-1.2	47	46
4	-2.1	47	45
5	-3.6	47	43
6	6.6	47	54
7	9.4	47	56
8	6.3	47	53
9	1.2	47	48
10	0.4	47	47
11	9.5	47	57
12	0.2	47	47
13	1.5	47	49
14	-1.7	47	45
15	-1.7	47	45
16	-4.2	47	43
17	-7.4	47	40
18	-1.9	47	36
19	-1.8	47	45
20	-5.3	47	42

*Values in which the hypothesis Ho of absolute error= population average: -0.02, not rejected by the t test (0.05) / *Valores en la cual la hipótesis Ho del error absoluto= promedio de la población: -0,02, no rechazada por la prueba t (0,05).

Cuadro 3. Error absoluto (severidad estimada menos severidad actual) obtenido por 20 evaluadores inexpertos usando la escala propuesta para cuantificar la severidad tizón foliar por Cercospora (*Cercospora kikuchii*) (TFC) en foliolos de soja (*Glycine max*), con base en una escala. Universidad Nacional del Noroeste de la Provincia de Buenos Aires Pergamino Buenos Aires, Argentina. 2017.

The use of the scale improved the accuracy of assessments. All showed slopes with coefficient values for the regression line statistically equal to 1 ($p<0.05$) in the assessment with the aid of the proposed scale (Table 1).

When the inexperienced raters used the proposed scale to estimate the severity of CLB on leaflets they were accurate having determination coefficients between 0.74 and 0.91 (Table 1). However, some of the raters (5, 7, 11 and 19) were not so accurate [(rejecting the hypothesis b: 1, but not the hypothesis a: 0 (t test: 0.05)].

No differences were found between the results with and without the scale, when the experienced raters estimated the severity of CLB (Table 2).

In order to speed the process of cultivar evaluations, it was also proposed the use of scales based in grades (Figure 2).

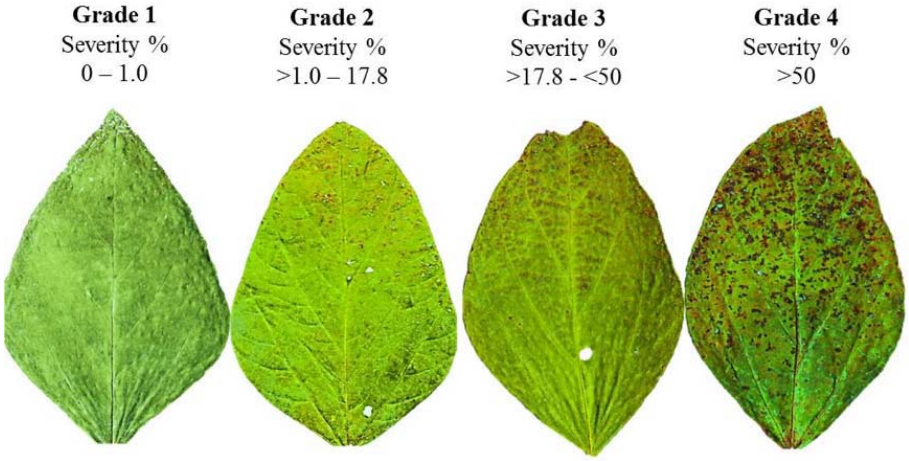

FIGURE 2
Scale for Cercospora leaf blight (CLB) on soybean (*Glycine max*) leaflets, based on severity grades. Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Pergamino Buenos Aires, Argentina. 2017.

Figura 2. Escala basada en grados de la severidad del tizón foliar por Cercospora en foliolos de soja (*Glycine max*). Universidad Nacional del Noroeste de la Provincia de Buenos Aires, Pergamino Buenos Aires, Argentina. 2017.

Discussion

The accuracy of this methodology is determined by the coefficient at the straight line (a) and the straight slope (b) between the estimated and actual severity values (Filho & Amorim, 1996; Martins et al., 2004). The accuracy can be evaluated by the determination of the regression that must be close to 100 %, and by the differences between the estimated and actual severities of the variance of the absolute errors (Table 3) (Nutter & Schultz, 1995).

An accurate disease evaluation depends not only on the used scale but also on the training of the raters. The results obtained agree with those found by Ivancovich et al. (1998) who consider that the visual scales to evaluate diseases involve evaluation errors due to the subjectivity and the inexperience of the raters.

According to the absolute errors (Table 3) the greatest deviation was observed between -2.49 and 2.46; however, these deviations were not significantly different ($p=0.9900$) from the population average. In that sense the proposed severity scale for CLB improved the evaluation of the disease.

The scale developed in this work improved the accuracy and precision of the inexperienced raters. The use of the proposed scale and the scales developed by Godoy et al. (2006), Martins et al. (2004), and Soares et al. (2009) are suggested for the assessment of fungicide trials for the control of soybean diseases and for the construction of disease progress curves.

Conclusion

The proposed scale to assess the severity of Cercospora leaf blight (CLB) on leaflets, can be used for the assessment of foliar fungicide trials and soybean cultivars. One of the advantages of this scale is the specificity to assess the severity of CLB on leaflets.

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Notes

1 This work was part of a doctoral thesis of the first author in Universidad Nacional del noroeste de la Provincia de Buenos Aires (UNNOBA).

Enlace alternativo

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