Acorbat Revista de Tecnología y Ciencia

New proposed of standard area diagram for assessing Black Sigatoka in bananas

Andrés Mauricio Pinzón-Núñez[1*], Diego Felipe Feria-Gómez[1], Gloria Marcela Pérez-Ochoa[1], Laura Arango-Palacio[1], Vicente Emilio Rey-Valenzuela[1], Sebastián Zapata-Henao[1]

[1]Banana Research Center CENIBANANO-AUGURA, Carepa-Antioquia 057850, Colombia.

***Autor de Correspondencia:** Andrés Mauricio Pinzón-Núñez fitpatologia@augura.com.co

Contribución: Tecnológica

Sección: Fitosanidad

Recibido: 15 Diciembre, 2023
Aceptado: 15 Enero, 2024
Publicado: 16 Abril, 2024

Cita: Pinzón-Núñez AM, Feria-Gómez DF, Pérez-Ochoa GM, Arango-Palacio L, Rey-Valenzuela VE y Zapata-Henao S. 2024. New proposed of standard area diagram for assessing Black Sigatoka in bananas. *Acorbat Revista de Tecnología y Ciencia* 1(1): 22 https://doi.org/10.62498/ARTC.2422

ABSTRACT

Black sigatoka, or black leaf streak (BLS), is caused by *Pseudocercospora fijiensis* and is the most important foliar disease in *Musa* production. Our goal was to develop a new standard area diagram (SAD) with high-resolution digital images with six disease levels: 0 (0%), 1 (0.1 – 5.0%), 2 (5.01 – 13.0%), 3 (13.01 – 23.0 %), 4 (23.01 – 40.0%), 5 (40.01 – 65.0%), and 6 (>65.0%) and compare BLS severity results in banana leaves with those based on another SAD. Five evaluations were conducted by thirteen different evaluators, and each evaluation was performed at seven-day intervals. The first assessment was done without a SADs. Evaluators performed two assessments with each SAD. The statistics were analyzed with linear regression and Lin's concordance correlation. With the proposed SAD, the evaluators improved the precision, accuracy, repeatability, and reproducibility and reduced the residual distribution, as compared using the other SAD. The proposed SAD is a tool that aids users in producing a disease estimate that is close to the real value of BLS in banana leaves.

Keywords: Phytopathometry, plant disease assessment, high-resolution images, BLS, *Pseudocercospora fijiensis*, phytosanity.

INTRODUCCIÓN

In Colombia, the main diseases in banana plants are moko (*Ralstonia solanacearum*), black sigatoka or black leaf streak (BLS) (*Pseudocercospora fijiensis*) and, starting in 2019, Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense* Tropical Race 4). BLS is one of three *Pseudocercospora* leaf diseases of Musa and is caused by *P. fijiensis* (*Mycosphaerella fijiensis*) (Crous *et al*., 2021). The first report of the disease dates from 1963 in the Sigatoka Valley in Fiji; in the Americas, it was first reported in 1972 in Honduras (EPPO, 2022), spreading to other *Musa* growing countries. It was first detected in Colombia in October, 1981 in a banana farm in the

Urabá production region, and, since then, it has spread all over the country, affecting bananas as well as plantains.

Nowadays, the pathogen is present in 74 countries around the world, distributed on 4 continents (EPPO, 2022). In Ecuador, the main exporter of bananas, bunch weight losses from BLS were estimated at 20% in the rainy season and 6% in the dry season (Terrero *et al*., 2020). In Colombia, the cost of controlling this disease is 35 dollars/ha/week, which represents approximately 80 million dollars (Augura, 2022). BLS is considered the main foliar disease of *Musa* production because of its effects on plants and fruit production and its control costs. It has several effects on plants, reducing total functional leaves, bunch and fruit weight, green life and photosynthesis (Terrero *et al*., 2020).

Fungicide spraying and affected leaf tissue removal are the main practices to control this disease. To monitor disease status in the banana production farms, standard area diagrams are used that quantify the severity (percentage of affected leaf area) of diseases in plant populations (Lopes *et al*., 2022). Nowadays, the evaluation of BLS severity in banana leaves is based on Stover's scale, as modified by Gauhl (1989). However, this scale was obtained using black and white drawings without statistical analysis. Based on this, a new standard area diagram for evaluating BLS in banana was proposed. High resolution color photographs of diseased leaves were used, and actual disease severity was measured with the Pliman R package (Olivoto *et al*., 2022) to elucidate intervals based on the frequency distribution of the collected leaves. Systematic errors were reduced, and accuracy, precision, repeatability and reproducibility were increased, as compared to the existing scale.

MATERIALES Y MÉTODOS

Development of the standard area diagram. 204 leaves that were naturally infected by *P. fijiensis* were randomly collected in the first four months of the year (2022). The sample was collected with different severity levels of the disease in the experimental field (7°48'12.9"N 76°39'11.8"W) with Cavendish banana (*Musa* spp. AAA) cv. 'Grand Nain', localized in the Apartadó-Antioquia Department. Collected leaves were photographed with a digital camera (Nikon D3500), and the actual severity of symptomatic leaves was determined using the Pliman R package (Olivoto *et al*., 2022). Data frequency distribution was based on the minimum and maximum levels of severity (Del ponte *et al*., 2022). Subsequently, seven intervals standard area diagram were created with color photographs of leaves representing the different levels of severity.

Validation and comparison of the standard area diagram. 50 images of banana leaves were used to represent all levels of disease severity. Each image was projected randomly with the aid of a multimedia projector during 30 seconds on individual slides in Microsoft® PowerPoint® 2010 (Microsoft Corporation, Redmond, WA, USA). Thirteen evaluators with no experience with BLS quantification assessed the images five times with seven-day intervals. Previously, the evaluators were guided on how to use the Stover's scale modified by Gauhl (1989) and the proposed SAD. For the first assessment, the evaluators estimated the BLS severity without any SAD. After seven days, in the second assessment, the same evaluators assessed the images with the aid of Stover's scale, as modified by Gauhl (1989). Previously, the evaluators were guided on how to use the Stover's scale. Seven days later, a third assessment with the same scale was done to verify the repeatability of the estimates. For the fourth and fifth assessments, the evaluators were guided on how to use the proposed SAD. The images of leaves were randomly placed for each assessment.

Accuracy, precision, repeatability and reproducibility of the estimates. Lin's concordance correlation coefficient (ρc) (Lin, 1989) and linear regression were used to determine the accuracy and precision of the disease severity estimates. Analysis of amplitude, proportion and dispersion of absolute errors, correlation coefficient (r) and coefficient of determination (R^2) were used to determine the precision of the estimates. On the other hand, the reproducibility and repeatability of the estimates were evaluated using the SADs. The reproducibility of the estimates was determined by analyzing the R^2 values obtained from linear regressions between estimated severity values for the same sample unit by different pairs of evaluators (Nutter Junior and Schultz, 1995) and the analysis of the intraclass correlation coefficient (ICC, ρ) for the group of evaluators, as described by Nita *et al*. (2003). The repeatability of estimates was determined with regression analysis of the second assessment in relation to the first (Nutter Junior and Schultz, 1995).

RESULTADOS Y DISCUSIÓN

Development of the standard area diagram. The minimum and maximum values of BLS severity in the 204 leaves were 0% and 93.4%, respectively. However, 50% of the leaves presented a severity of more than 17%. Based on the frequency distribution analysis, the intervals with the highest frequency of disease severity values were represented. A standard area diagram was developed to include six levels: 0 (0.0 %), 1 (0.1 – 5.0 %), 2 (5.01 – 13.0 %), 3 (13.01 – 23.0 %), 4 (23.01 – 40.0 %), 5 (40.01 – 65.0 %), and 6 (> 65 %) (Fig. 1). The levels of the SAD were subdivided into three sections to better represent the distribution of disease intensity. In this way, the evaluator can compare his sample with the lower limit, midpoint or upper limit of each level (Núñez *et al*., 2017; Belan *et al*., 2020; Figueiredo *et al*., 2022). To develop the new standard area diagram, high-resolution digital images were used that represented actual symptoms of the disease instead of using black and white images or colored engravings. To measure actual severity on leaves, the functions of the Pliman R package were used; this package is a suite for performing several analyses in plant images, especially foliar analyses, with simultaneous processing of large sets of image collections, here, more than 200 leaves. Pliman showed great potential to produce accurate measures and accelerate studies involving plant disease severity measurements (Olivoto *et al*., 2022).

Accuracy, precision, reproducibility and repeatability of estimates. Considering the regression analysis to the evaluation for each one of the evaluators, greater accuracy and precision were evidenced when the evaluators used the SADs than without them, which were higher with the use of the proposed SAD. The assessment of 100% of the evaluators who used the proposed SAD had a coefficient of determination (R^2) higher than that of the evaluations with Stover's scale modified by Gauhl (1989). In the 1st evaluation with the proposed SAD, 54% of the evaluators yielded R^2 values over 0.90 (0.84 – 0.92, mean 0.89). In the 2nd evaluation, 80% of the evaluators improved precision (0.88 – 0.94, mean 0.91) over the Stover scale, which did not obtain mean R^2 values over 0.70 (0.68 – 0.61 1st and 2nd assessment, respectively) (Table 1). The proposed SAD provided a higher precision in disease severity estimates.

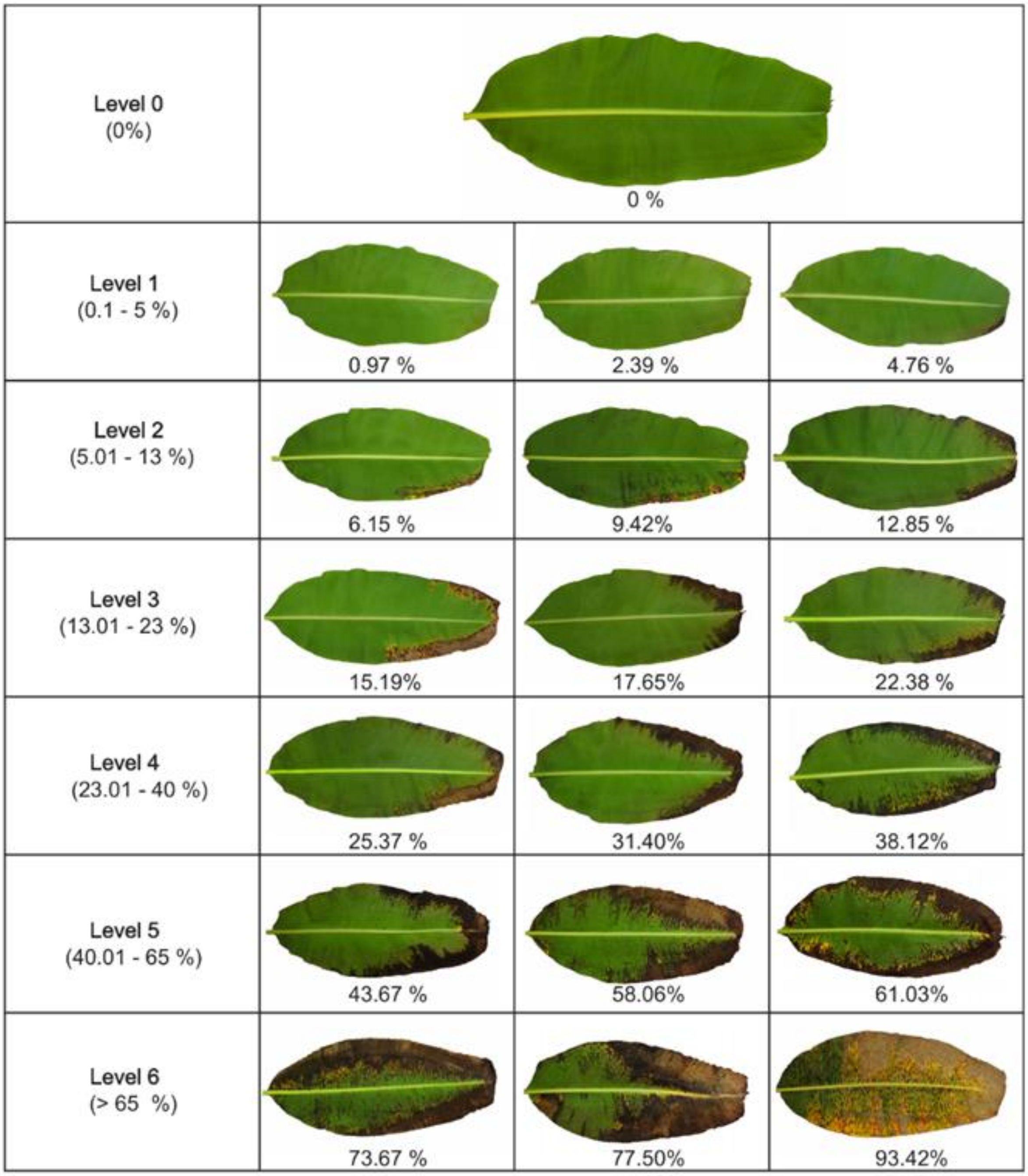

Figure 1. Standard area diagram developed to quantify black leaf streak (BLS) severity on banana leaves (Cavendish AAA). The percentage shown below each digital image represents the actual severity measured with Pliman R package (Olivoto *et al*., 2022).

Table 1 Linear regression coefficients relating actual severity vs estimated severity of black leaf streak (BLS) on banana leaves (Cavendish AAA, cv. 'Grand Nain'): Intercept (*β0*), slope of the line (*β1*) and coefficient of determination (R^2).

	Without SAD			A. Stover's modified by Gauhl (1989)						B. Proposed SAD					
				1st evaluation			2nd evaluation			1st evaluation			2nd evaluation		
Evaluator	β0	β1	R2	β0	β1	R2	β0	β1	R2	β0	β1	R2	β0	β1	R2
A	56.89*	0.71*	0.51	5.28*	0.67*	0.81	33.97*	0.32*	0.30	5.94*	1.00ns	0.84	1.67ns	0.99ns	0.89
B	29.16*	0.93ns	0.72	10.05*	0.67*	0.71	14.64*	0.60*	0.69	6.67*	1.01ns	0.89	6.63*	0.96ns	0.89
C	12.27*	0.86*	0.78	7.89*	0.64*	0.72	19.83*	0.54*	0.52	6.34*	0.86*	0.84	4.52*	0.88*	0.90
D	16.04*	0.99ns	0.88	9.47*	0.67*	0.74	6.75*	0.68*	0.81	6.90*	1.04ns	0.84	4.32*	1.00ns	0.88
E	10.30*	0.96ns	0.90	8.14*	0.67*	0.79	7.68*	0.69*	0.79	3.87*	0.96ns	0.92	1.76ns	0.88*	0.93
F	62.39*	0.60*	0.39	27.62*	0.38*	0.38	13.89*	0.60*	0.71	1.19ns	0.98ns	0.90	2.57ns	0.91*	0.92
G	33.24*	1.02ns	0.52	18.22*	0.49*	0.41	19.69*	0.54*	0.55	4.65*	0.94ns	0.91	2.63ns	0.93ns	0.91
H	28.29*	0.92ns	0.69	16.03*	0.58*	0.67	19.30*	0.55*	0.58	4.17*	0.91*	0.92	2.26ns	0.90*	0.91
I	28.02*	0.84ns	0.61	17.46*	0.56*	0.62	17.59*	0.56*	0.62	4.47*	0.85*	0.91	4.60*	0.93ns	0.94
J	4.34*	1.15*	0.94	7.61*	0.69*	0.78	19.54*	3.08*	0.39	4.44*	0.99ns	0.92	2.33ns	0.99ns	0.93
K	17.97*	0.72*	0.88	20.93*	0.50*	0.58	15.16*	0.56*	0.74	4.48*	0.84*	0.89	4.80*	0.89*	0.90
L	31.00*	0.96ns	0.68	8.40*	0.68*	0.75	21.50*	0.53*	0.51	3.79*	0.99ns	0.92	2.83*	0.92*	0.94
M	10.74*	1.13ns	0.78	4.70*	0.69*	0.85	7.93*	0.69*	0.74	5.18*	0.89*	0.89	6.96*	0.91*	0.90

*The null hypothesis was rejected in the t-test (P = 0.05) ^{ns} the null hypothesis (β0 = 0 and β1 = 1) was accepted in the t-test (p = 0.05).

Estimates of BLS severity with the aid of the proposed SAD were more accurate, precise and close to the actual values and, consequently, with greater agreement (ρc) than with Stover's scale, as shown by the analysis of coefficients of linear regression. Precision and accuracy are determinants for the identification of symptoms and represent the most appropriate way to quantify. In this study, the values of the repeatability coefficient were 91%, close to that found in other studies on different crops and diseases (Núñez *et al.*, 2017; Figueiredo *et al.*, 2022).

Reproducibility is an indicator that evaluates the efficiency of the SADs. When the proposed SAD was used, the values of the regressions of the estimates between the pairs of evaluators in the 2nd evaluation varied from 0.82 to 0.96. This means that 100% of evaluators presented R^2 values >0.80, evidencing reproducibility of the results. With the aid of Stover's scale, 77% of evaluators presented R^2 values <0.80. Without any SADs, only 8% of the evaluators presented R^2 values >0.80 (Fig. 2).

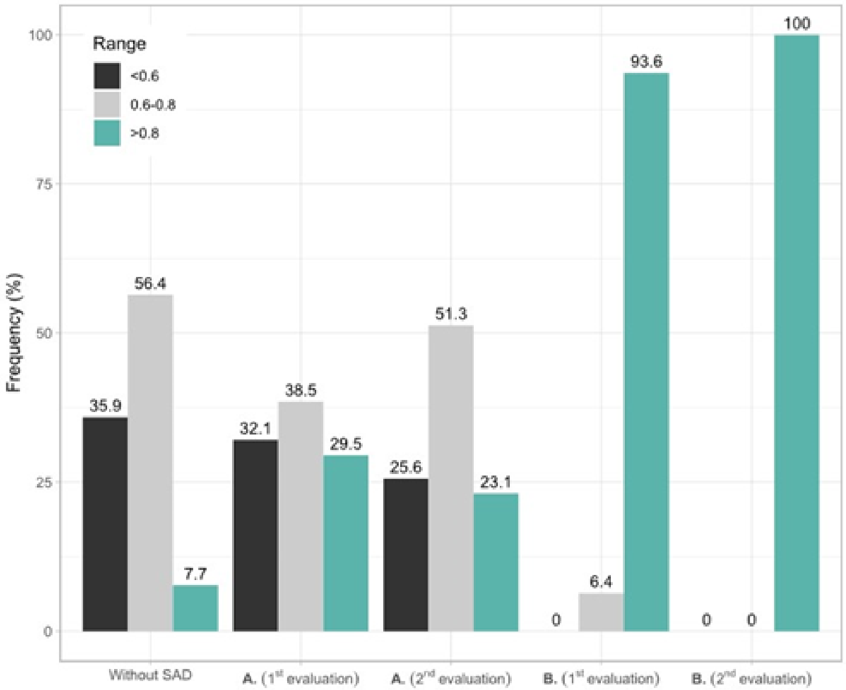

Figure 2. Graphical representation of coefficient of determination (R^2) of the linear regression equation for pairs of evaluators without and with the aid of the SADs **A**. Stover's scale modified by Gauhl (1989) **B**. Proposed SAD.

CONCLUSIONS

The validation of the proposed SAD using linear regression analysis and Lin provided similar conclusions with other studies found in the literature. There were no differences between the two validation methods, and both provided better accuracy, precision, repeatability, and reproducibility of BLS estimates in banana leaves. Therefore, the SAD proposed in this study, constructed from high-resolution digital images, is a suitable aid for visual estimates of BLS severity in banana leaves and improves the precision, accuracy repeatability and reproducibility of severity estimates.

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