

## Standard area diagram set for quantification of septoriosis in fruit of sour passion fruit

Anne Pinheiro Costa<sup>1</sup>, José Ricardo Peixoto<sup>1</sup>, Luiz Eduardo Bassay Blum<sup>1</sup>, Michelle Souza Vilela<sup>1</sup>, Isadora Nogueira<sup>1</sup>, Ana Paula Gomes de Castro<sup>2</sup>

<sup>1</sup> Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária, Brasília, DF, Brasíl. E-mail: e-mail: annecosta@gmail.com (ORCID: 0000-0002-8818-6123); peixoto@unb.br (ORCID: 0000-0002-8885-2886); luizblum@unb.br (ORCID: 0000-0002-3968-6615); michellevilelaunb@gmail.com (ORCID: 0000-0002-0417-568X); i.nogueiraa@gmail.com (ORCID: 0000-0002-7018-4736)

<sup>2</sup> Exército Brasileiro, Diretoria de Patrimônio, Departamento de Engenharia e Construção, Brasília, DF, Brasil. E-mail: apgcastro7@gmail.com (ORCID: 0000-0002-3989-369X)

**ABSTRACT:** A standard area diagram set (SADs) for severity evaluation of septoriosis (*Septoria passiflorae*) in fruit of sour passion fruit (*Passiflora edulis* Sims) was developed and validated in this study. The SADs presented eight severity levels (0.3; 0.5; 1; 2; 4; 10; 18; and 30%). For its validation, 20 raters were divided into groups (G1 and G3, inexperienced; G2 and G4, experienced) who initially estimated the disease severity without the aid of the SADs. Subsequently, G1 and G2 performed the second evaluation without the SADs, and G3 and G4 completed the second evaluation with the proposed SADs for each disease. The accuracy and precision of the evaluations were determined by simple linear regression and by Lin's concordance correlation coefficient. The proposed SADs allowed accurate and precise quantification of septoriosis severity, increasing the agreement between estimated and actual values. The increase in accuracy and precision in the non-aided groups, when present, was less pronounced than those increments observed in the SADs-aided groups. Lin's concordance correlation coefficient confirmed the increases in accuracy and precision analysis.

Key words: fruits; Passiflora edulis Sims; phytopathometry; Septoria passiflorae

# Escala diagramática para a quantificação da severidade da septoriose em frutos do maracujazeiro azedo

**RESUMO:** Uma escala diagramática para a avaliação da severidade da septoriose (*Septoria passiflorae*) em frutos do maracujazeiro azedo (*Passiflora edulis* Sims) foi desenvolvida e validada neste estudo. A escala diagramática apresentou oito níveis de severidade (0,3; 0,5; 1; 2; 4; 10; 18; e 30%). Para a sua validação, os 20 avaliadores foram divididos em grupos (G1 e G3, sem experiência; G2 e G4, com experiência), que inicialmente estimaram a severidade da doença sem auxílio da escala. Posteriormente, G1 e G2 fizeram outra avaliação sem escala, e G3 e G4 realizaram a avaliação com a escala proposta. A acurácia e a precisão das estimativas foram determinadas por regressão linear simples e pelo coeficiente de correlação de concordância de Lin. A escala diagramática proposta permitiu quantificar a severidade da septoriose de forma acurada e precisa, aumentando a concordância entre os valores estimados e os reais. O aumento da acurácia e precisão nos grupos que realizaram dupla avaliação sem escala, quando ocorreu, foi mais discreto que os incrementos observados nos grupos que utilizaram a escala. A análise de concordância de Lin confirmou os incrementos da acurácia e precisão detectados pela análise de regressão linear.

Palavras-chave: frutos; Passiflora edulis Sims; fitopatometria; Septoria passiflorae

## Introduction

Brazil is the world's largest producer of passion fruit, having a harvested production of 703,489 t over 49,889 ha (IBGE, 2016). The sour passion fruit (*Passiflora edulis* Sims) is the most cultivated and commercialized type in the country (Faleiro et al., 2011). However, it also has a high susceptibility to different diseases, such as the septoriosis (*Septoria passiflorae*). In intense attacks, septoriosis promotes severe defoliation, reducing the productive potential and slowing the full development of the plant (Sussel, 2010).

The disease can affect fruit at any development stage, causing them to display circular lesions with well-defined edges, which are also capable of coalescing and spreading to large extensions, affecting their maturation or development. Those lesions on fruit peel affect the market quality of the fruit and can still favor their infection by other pathogens. In addition to that, at higher severity levels, the disease may cause the fall of unripe fruit to the soil (Dias, 2000).

The septoriosis quantification in sour passion fruit has been performed by descriptive scales (Kudo et al., 2012, Vilela, 2013, Castro, 2015). These scales are subjective, not permitting the visual acuity adjustment in assessing severity levels (Campbell & Madden, 1990), impairing the accurate quantification of the injured area (Santos et al., 2017). On the other hand, diagrammatic scales or standard area diagram sets (SADs) not only improve the accuracy and the precision of disease evaluations, but also demonstrate significant improvements in the visual estimates of severity (Damasceno et al., 2014; De Paula et al., 2016; Santos et al., 2017). Thus, they are valuable tools in breeding programs, whose success is achieved depending on the exact and accurate use of quantification methods of disease severity and selection of resistant materials (Librelon et al., 2015).

Several studies show that the employment of SADs makes it possible to study the genotype reaction to the different diseases and the selection of resistance sources in the development of new crops (Poltronieri et al., 2016; Aktar & Shamsi, 2018). Attained estimates from the SADs also serve as subside for the definition of pathogen inoculation methods (Parreira et al., 2016) and to quantify the efficiency of disease control methods (Marcuzzo et al., 2016).

When taking into account the lack of standardized methods for quantifying septoriosis in this crop, this study aimed: (1) to develop and validate a diagrammatic scale for assessing the septoriosis severity in sour passion fruit; (2) to compare the accuracy, precision and concordance of this disease severity estimates, scale aided and non-aided; (3) to compare the accuracy, precision and concordance between the estimates from inexperienced and experienced raters.

## **Materials and Methods**

#### **Development of the SADs**

In order to elaborate the SADs, 50 fruits of sour passion fruit (BRS Gigante Amarelo and Yellow Master FB200

commercial cultivars) showing symptoms of septoriosis were collected at the Paraná Farm commercial orchard, located in Nucleo Rural Pipiripau, Planaltina, DF (lat. 47°29'56,92" S; long. 15°30'15,08" W, and alt. 955 m). The adaxial surface of each fruit was photographed with a digital camera (Canon Powershot SX40 HS, 12.1 megapixels; Canon Inc., Tokyo, Japan), set up at the height of 45 cm from each fruit level. The resulting images were transferred to a computer and analyzed for the diseased area, using the image analysis software IMAGE J (Schneider et al., 2012). Disease severity (% of lesion area) was determined by dividing the lesion area by the total fruit area.

The SADs' upper and lower limits were based on the minimum and maximum values of septoriosis severity found in the image analysis of the 50 fruits. Intermediate levels were established following logarithmic increments (Nutter & Schultz, 1995). After establishing the disease percentages and its corresponding levels represented in the scale, a standard fruit was used as the template, and diagrams with different severity levels were created using the IMAGE J software. The patterns of lesion distribution detected on the actual fruits were maintained.

#### Validation of the SADs

The SADs was validated using images of 50 fruits with different intensities of symptoms. Twenty raters (ten with previous experience and ten without previous experience in disease quantification) were selected and divided into four groups of five raters (G1 and G3, inexperienced; G2 and G4, experienced). Initially, each group estimated the disease severity, in percentage, for each of the 50 fruit images randomly organized, without the aid of the SADs (non-aided evaluation). Subsequently, the same images were presented to G1 and G2 groups, who performed another non-aided evaluation, and to G3 and G4 groups, who conducted the evaluation using the proposed SADs (SADs-aided evaluation).

The accuracy and precision of the raters were determined by linear regression between the actual severity (independent variable) and the visually estimated severity (dependent variable). The accuracy of estimates of each rater was determined by the t-test applied to the intercept of linear regression (*a*) to verify if it was significantly different from 0, and to the slope of the line (*b*), to test if it was significantly different from 1 ( $P \le 0.05$ ). Intercept values significantly different from 0 indicate the presence of constant errors, whereas values of the slope of the line different from 1 indicate the presence of systematic errors (Nutter & Schultz, 1995). Thereby, the most accurate raters were considered those whose estimates provided linear regression equations with values of "*a*" and "*b*" not significantly different from 0 and 1 by the t-test.

The precision of estimates of each rater was obtained by the coefficient of determination of the regression analysis ( $R^2$ ) and the variance of absolute errors (the difference between estimated and actual severities) (Kranz, 1988). Absolute errors were compared by the t-test (P  $\leq$  0.05). Raters with higher values of  $R^2$  were considered as of higher precision. Evaluations of the absolute errors considered the criteria used in disease quantification training programs [Distrain (Tomerlin & Howell, 1988) and Disease.Pro (Nutter & Worawitlikit, 1989)], which classify raters as excellent (errors up to 5%) or good (errors up to 10%). Moreover, the mean maximum error (absolute value) was also recorded for each group, indicating, in absolute value, the difference between the farthest estimate and the actual severity value. The reproducibility or inter-rater reliability was measured using the  $R^2$  values for each pair of raters, based on estimates of non-aided evaluations and SADs-aided evaluations (Nutter & Schultz, 1995).

Accuracy and precision of the estimates of each rater, with and without the use of the SADs, was also determined based on the Lin's concordance correlation coefficient (LCCC;  $\rho_c$ ). The LCCC combines measures of accuracy and precision to assess the fit of pairs of observations to the line of concordance (with intercept = 0 and slope = 1), or line 1:1. LCCC is defined by  $\rho_c$  =  $C_b$ . r, where  $C_{b'}$  is a coefficient calculated based on systematic and constant errors, measures how far the best-fitting line deviates from 45° and is thus a measure of accuracy; and ris the correlation coefficient between estimated severity (Y) and actual severity (X), which measures precision (variation). When there is perfect concordance between estimated and actual severity, the points fall on the concordance line. As a result, r = 1,  $C_b = 1$ , and  $\rho_c = 1$  (Lin, 1989; Bock et al., 2010).

Linear regressions and absolute errors analyses were performed using the Genes software (v. 1990.2017.37). The LCCC was calculated using the MedCalc software (v. 17.9.7).

## **Results and Discussion**

The SADs to quantify septoriosis in sour passion fruit was elaborated with eight levels of severity (0.3; 0.5; 1; 2; 4; 10; 18 and 30%) (Figure 1). Septoriosis occurs in a large part of passion fruit crops in Brazil, in addition to nurseries, but only sporadically causes significant damage (Dias, 2000), since



**Figure 1.** Standard area diagram set for evaluation of septoriosis (*Septoria passiflorae*) severity (%) in fruit of sour passion fruit (*Passiflora edulis* Sims). Brasília, DF, Brazil, 2018.

chemical control has proven to be efficient so far. Probably, due to this reason, values higher than 30% of disease severity were not observed in the area where the fruit of this study were collected.

The septoriosis severity was not very accurate in the first evaluation, with no SADs aid. The raters presented constant and/or systematic errors and overestimated the severities (Table 1, Figures 2 to 5). There is a tendency to overestimate disease severity due to the impression caused by the size and number of lesions. As a direct consequence, higher values of severity are assigned to smaller lesions when compared to values attributed to larger lesions, which are in lower number (Bock et al., 2008).

However, the use of SADs can reduce the tendentious pattern of severity overestimation, as verified in this and other studies (Valeriano et al., 2015, Ortega-Acosta et al., 2016; Nuñez et al., 2017). Although the overestimation tendency

**Table 1.** Intercept (*a*), slope of the line (*b*) and determination coefficient ( $R^2$ ) of linear regression equations calculated between actual severity and estimated severity of septoriosis (*Septoria passiflorae*) in fruit of sour passion fruit (*Passiflora edulis* Sims). Brasília, DF, Brazil, 2018.

Raters	E1			E2			
Unexperienced	Non-aided			Non-aided			
	а	b	<b>R</b> <sup>2</sup>	а	b	<b>R</b> <sup>2</sup>	
G1							
1	7.87*	2.28*	0.89	6.24*	1.08	0.90	
2	2.26*	1.23*	0.81	0.90*	0.60*	0.89	
3	0.07	0.80*	0.94	1.08*	1.04	0.93	
4	3.25*	1.44*	0.85	7.18*	1.01	0.56	
5	9.83*	1.30*	0.74	5.24*	1.06	0.73	
Mean	4.65	1.41	0.85	4.13	0.96	0.80	
Experienced	No	Non-aided			Non-aided		
		G2					
6	2.44*	0.91*	0.93	0.0007	0.89*	0.91	
7	11.44*	1.42*	0.78	11.24*	0.98	0.78	
8	2.24*	1.38*	0.92	1.61*	1.48*	0.93	
9	8.06*	1.36*	0.84	4.63*	1.00	0.86	
10	2.92*	1.24*	0.74	2.41*	1.13	0.86	
Mean	5.42	1.27	0.84	3.98	1.10	0.87	
Unexperienced	No	Non-aided			SADs-aided		
		G3					
11	2.53	2.11*	0.71	0.68	0.81*	0.87	
12	5.57*	2.11*	0.84	0.22	1.48*	0.96	
13	8.09*	1.93*	0.89	1.91*	1.09	0.82	
14	9.02*	1.59*	0.85	0.84	1.21*	0.92	
15	-0.04	1.52*	0.79	0.87	1.29*	0.86	
Mean	5.04	1.85	0.82	0.90	1.18	0.89	
Experienced	No	Non-aided			SADs-aided		
G4							
16	7.21*	2.36*	0.82	3.41*	0.99	0.80	
17	4.55*	0.71*	0.74	1.72*	1.08	0.90	
18	1.84*	0.59*	0.96	1.07*	0.92*	0.96	
19	8.11*	1.83*	0.84	0.31	1.11	0.85	
20	12.69*	1.35*	0.67	1.79*	1.04	0.90	
Manu	6 00	1 27	0.91	1 66	1 02	0 00	

E1 = evaluation 1; E2 = evaluation 2.

\* Indicates that the null hypothesis (a = 0 or b = 1) was rejected by the t-test (P  $\leq 0.05$ ).



**Figure 2.** Septoriosis (*Septoria passiflorae*) severity in fruit of sour passion fruit (*Passiflora edulis* Sims) estimated by inexperienced raters, without the aid of the standard area diagram set in the first (A-E) and second (F-J) evaluations. Solid line = linear regression of actual severity x estimated severity. Dashed line = perfect agreement (linear regression of actual severity = estimated severity). Brasília, DF, Brazil, 2018.

persisted in all groups in the second evaluation, the groups that used the proposed SADs managed to reduce it (Table 2, Figures 2 to 5). When aided by the SADs, 80% of the raters in G3 presented intercept values equal to 0 ( $P \le 0.05$ ), indicating absence of constant errors, and 20% presented absence of systematic errors, with slope of the line values equal to 1 ( $P \le 0.05$ ). In G4, 20% of the raters presented intercept values equal to 0 ( $P \le 0.05$ ), while 80% presented slope of the line values equal to 1 ( $P \le 0.05$ ), while 80% presented slope of the line values equal to 1 ( $P \le 0.05$ ) (Table 1).

Therefore, when the evaluation was held with the aid of the SADs, there was an accuracy improvement of the raters, since the means of estimated severity were closer to the actual values obtained by the computerized analysis. As a result, an approximation between the fitted regression line (generated line between actual and estimated severity) and line 1:1 (actual severity equal to the estimated one) was detected



**Figure 3.** Septoriosis (*Septoria passiflorae*) severity in fruit of sour passion fruit (*Passiflora edulis* Sims) estimated by experienced raters, without the aid of the standard area diagram set in the first (A-E) and second (F-J) evaluations. Solid line = linear regression of actual severity x estimated severity. Dashed line = perfect agreement (linear regression of actual severity = estimated severity). Brasília, DF, Brazil, 2018.

(Figures 4 and 5). Although G1 and G2 showed an expressive reduction in systematic errors during the second evaluation, G3 and G4 generally presented intercept and slope of the line values closer to 0 and 1, respectively (Table 1, Figures 4 to 5).

When non-aided by the SADs, the raters in G3 obtained  $R^2$  values of 0.71 to 0.89 (mean of 0.82) while values between 0.67 and 0.96 (0.81) were observed in G4. When SADs-aided,  $R^2$  values ranged from 0.82 to 0.96 (0.89) for the inexperienced raters, and from 0.80 to 0.96 (0.88) for the experienced raters. This result demonstrates a precision increase in G3 (8.5%) and G4 (8.6%) higher than that observed in G2 (3.6%) (Table 1).

The reduction of the residual distribution, or absolute errors, was verified in all groups, with differences being identified among the evaluations ( $P \le 0.05$ ) (Table 2). However, in SADs-aided evaluations the reductions in the absolute errors were more expressive than the verified for groups whose





**Figure 4.** Septoriosis (*Septoria passiflorae*) severity in fruit of sour passion fruit (*Passiflora edulis* Sims) estimated by inexperienced raters, without the aid of the standard area diagram set (SADs) in the first evaluation (A-E) and with the aid of the SADs in the second evaluation (F-J). Solid line = linear regression of actual severity x estimated severity. Dashed line = perfect agreement (linear regression of actual severity = estimated severity). Brasília, DF, Brazil, 2018.

second evaluation was non-aided by the SADs. While G1 and G2 displayed reductions of 34.9 and 29.5%, respectively, G3 and G4 displayed reductions greater than 74% in the mean absolute error (Table 2, Figure 6).

The mean maximum error of the actual severity, in absolute value, decreased in 36.9 (G1) and 27.8% (G2) in the second evaluation. In the SADs-aided groups, the mean maximum errors were 59.7% lower in G3 and 62.2% lower in G4 (Table 3). Thus, the error distribution of the non-aided evaluations ranged from -5.8 to +52.6 in G3 (Figure 6C) and from -13.0 to +52.6 in G4 (Figure 6D). In SADs-aided evaluations, absolute errors ranged from -11.6 to +20.0 for inexperienced raters (Figure 6G), and from -5.0 to +19.1 for experienced raters (Figure 6H).

Moreover, with the use of the SADs, 96 and 98.4% of the estimates presented absolute errors of less than 10% (-10 to

**Figure 5.** Septoriosis (*Septoria passiflorae*) severity in fruit of sour passion fruit (*Passiflora edulis* Sims) estimated by experienced raters, without the aid of the standard area diagram set (SADs) in the first evaluation (A-E) and with the aid of the SADs in the second evaluation (F-J). Solid line = linear regression of actual severity x estimated severity. Dashed line = perfect agreement (linear regression of actual severity = estimated severity). Brasília, DF, Brazil, 2018.

+10) in G3 and G4, respectively. These values show an increase of 52.9 (G3) and 47.3% (G4) in the number of estimates with errors within the 10% range when compared to the first evaluation. In addition, the SADs aided in reducing over 89% the number of estimates outside this variation range. More than 86% estimates in G3 and G4 presented errors within the 5% range (-5 to +5), indicating that the raters' estimates were closer to the actual severity value when they were aided by the SADs (Table 3).

The increments and reductions in the values of the SADs non-aided estimates were less expressive than those observed in the SADs-aided estimates. According to Nutter & Schultz (1995), values below 5% are considered excellent in SADs evaluation, based on the absolute errors, while values up to 10% are considered good. The presence of absolute errors, even minimal, are common in disease severity measurements

**Tabela 2.** Absolute errors (estimated severity minus actual severity) from the severity estimates of septoriosis (*Septoria passiflorae*) in fruit of sour passion fruit (*Passiflora edulis* Sims). Brasília, DF, Brazil, 2018.

Raters	E1	E2				
Unexperienced	Non-aided	Non-aided				
G1						
1	14.7 b	6.7 a				
2	3.9b 1.8a					
3	1.3 а 1.7 а					
4	5.9 a	8.3 b				
5	11.4 b	5.7 a				
Mean	7. 5 b	4.9 a				
Experienced	Non-aided	Non-aided				
G2						
6	2.2 b	1.3 a				
7	13.7 b	11.1 a				
8	4.4 a	4.2 a				
9	10.0 b	4.8 a				
10	4.6 a	3.2 a				
Mean	7.0 b	4.9 a				
Unexperienced	Non-aided	SADs-aided				
	G3					
11	8.5 b	1.6 a				
12	11.5 b	2.9 a				
13	13.1 b	2.8 a				
14	12.2 b	2.4 a				
15	3.9 a	2.9 a				
Mean	9.8 b	2.5 a				
Experienced	Non-aided	SADs-aided				
G4						
16	14.5 b	3.6 a				
17	3.9 b	2.4 a				
18	1.9 a	1.2 a				
19	12.6 b	1.9 a				
20	14. 6 b	2.3 a				
Mean	9.6 b	2.3 a				

E1 = evaluation 1; E2 = evaluation 2.

Means followed by the same letter in the lines does not differ between themselves by the Student t-test (P  $\leq$  0.05).

but can be compensated by the rapidity and standardization that the SADs aid provides (Stonehouse et al., 1994).

The reproducibility of the estimates among the raters was also used as an indicator of the SADs precision analysis. According to Nutter et al. (1993), different raters using the same scale and evaluating the same material, should estimate the same severity values, whose significance is verified by linear regressions between the severities estimated by the raters, in pairs. Without the aid of the SADs, the  $R^2$  values of determination of the estimates regressions between the pairs of raters in G3 and G4 ranged from 0.57 to 0.83 (0.70 of mean) and 0.62 to 0.81 (0.71), respectively. Using the SADs,  $R^2$  values ranged from 0.67 to 0.95 (0.78) in G3, and from 0.61 to 0.93 (0.81) in G4.

These results reinforce the precision increase in the estimates with the aid of the SADs and show the variability among the raters when using the same SADs, as reported by other studies (Bardsley & Ngugi, 2013; Venturini et al., 2015;



**Figure 6.** Distribution of the absolute errors (estimated severity minus actual severity) of the septoriosis (Septoria passiflorae) estimates in fruit of sour passion fruit (Passiflora edulis Sims) in the first evaluation, without the aid of the standard area diagram set (SADs), in groups 1 (A), 2 (B), 3 (C) and 4 (D); and in the second evaluation, without of the aid of the SADs, in groups 1 (E) and 2 (F), and with the aid of the SADs, in groups 3 (G) and 4 (H). Brasília, DF, Brazil, 2018.

**Table 3.** Mean maximum error (MEAV), in absolute value, at the 10% (values =  $x \pm 10$ ) and 5% (values =  $x \pm 5$ ) range of the estimates in relation to the actual severity value of septoriosis (*Septoria passiflorae*) in fruit of sour passion fruit (*Passiflora edulis* Sims). Brasília, DF, Brazil, 2018.

Evoluation	Parameters -	Groups				
Evaluation		1	2	3	4	
	MEAV	23.0	22.0	37.9	28.2	
1	% values = x ± 5	49.6	51.2	36.8	42.0	
	% values = $x \pm 10$	72.4	73.6	62.8	66.8	
	EMVA	14.5	15.9	15.3	10.6	
2	% values = x ± 5	58.8	63.6	86.4	86.8	
	% values = $x \pm 10$	87.2	84.0	96.0	98.4	

Correia et al., 2017). This variability is due to the ability of each rater to estimate the severity of the disease, and can be influenced by several factors, such as training, experience, disease complexity, different formats and distribution of the lesions (Nutter & Schultz, 1995; Bock et al., 2016).

The LCCC ( $\rho_c$ ) combines elements of accuracy and precision in order to determine the agreement between estimated and actual values (Lin, 1989) and has been used with great success in recent studies of SADs validation (Schwanck & Del Ponte, 2014, Braido et al., 2015, Dolinski et al., 2017, Sachet et al., 2017). The LCCC confirmed the previously presented results, demonstrating that the accuracy and precision of the raters were improved when aided by the SADs in comparison to the non-aided evaluations (Table 4). **Table 4.** Correlation values between estimated severity and actual severity (*r*), bias correction factor ( $C_b$ ) and Lin's concordance correlation coefficient ( $\rho_c$ ) for septoriosis (*Septoria passiflorae*) severity estimates in fruit of sour passion fruit (*Passiflora edulis* Sims). Brasília. DF. Brazil. 2018.

Raters	E1			E2			
Unexperienced	Non-aided			Non-aided			
	r	C <sub>b</sub>	ρ	r	C <sub>b</sub>	ρ	
		G1					
1	0.94	0.43	0.41	0.95	0.71	0.68	
2	0.90	0.88	0.79	0.94	0.89	0.84	
3	0.97	0.97	0.94	0.97	0.98	0.95	
4	0.92	0.77	0.71	0.75	0.70	0.52	
5	0.86	0.51	0.44	0.86	0.79	0.67	
Mean	0.92	0.71	0.66	0.89	0.81	0.73	
Experienced	Non-aided			Non-aided			
		G2					
6	0.96	0.96	0.92	0.95	0.99	0.95	
7	0.88	0.44	0.39	0.88	0.47	0.42	
8	0.96	0.84	0.80	0.96	0.83	0.80	
9	0.92	0.57	0.52	0.93	0.83	0.77	
10	0.86	0.84	0.72	0.93	0.91	0.84	
Mean	0.92	0.73	0.67	0.93	0.81	0.76	
Unexperienced	Non-aided			SADs-aided			
		G3					
11	0.85	0.57	0.49	0.93	0.99	0.92	
12	0.92	0.52	0.47	0.98	0.88	0.86	
13	0.94	0.48	0.45	0.91	0.94	0.85	
14	0.92	0.50	0.46	0.96	0.94	0.90	
15	0.89	0.84	0.75	0.93	0.91	0.84	
Mean	0.90	0.58	0.52	0.94	0.93	0.88	
Experienced	Non-aided			SADs-aided			
G4							
16	0.91	0.43	0.39	0.90	0.90	0.81	
17	0.86	0.89	0.76	0.95	0.95	0.91	
18	0.98	0.88	0.86	0.98	0.99	0.97	
19	0.92	0.49	0.45	0.92	0.98	0.90	
20	0.82	0.41	0.34	0.95	0.96	0.91	
Mean	0.90	0.62	0.56	0.94	0.96	0.90	

E1 = evaluation 1; E2 = evaluation 2.

The accuracy, measured by the bias correction factor ( $C_b$ ), improved from 0.58 to 0.93 in G3 and from 0.62 to 0.96 in G4 when the SADs was used. The precision, measured by the correlation coefficient (r), increased from 0.90 to 0.94, with and without use of the SADs, respectively, for the two groups. The agreement ( $\rho_c$ ) increased in 100% of the raters who used the SADs, with increases ranging from 0.52 to 0.88 (G3) and from 0.56 to 0.90 (G4) (Table 4). Such increments were significant in the SADs-aided groups when compared to those who performed the second non-aided evaluation, and can be confirmed by the greater proximity between the generated lines between actual and estimated severities, and the 1:1 line in G3 (Figure 4) and G4 (Figure 5) in relation to the proximity degree between the lines observed in G1 (Figure 2) and G2 (Figure 3).

SADs have improved the accuracy and precision of estimates of septoriosis severity in some crops, including *S. glycines* in soybean (Polizel & Juliatti, 2010), *S. helianthi* in sunflower (Lenz et al., 2009) and *S. apicola* in mandioquinha-

salsa (Mesquini et al., 2009). However, this study is the very first record of a SADs used to estimate the septoriosis severity in sour passion fruit. The results reported here showed that the evaluations performed by all raters were closer to the actual value when the proposed scale was used. Therefore, this SADs may be applied in breeding programs for the study of resistance among genotypes and selection of superior materials, under field and protected cultivation.

### **Conclusions**

The SADs elaborated and validated in this study increased the accuracy, precision and agreement of the septoriosis severity estimates in passion fruit, approaching the estimated values to the actual severity values of the disease, also contributing to a greater standardization of the estimates among raters.

## Acknowledgements

The present study was accomplished with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001. The authors express their thanks to all the raters who participated in the validation steps of the diagrammatic SADs.

## **Literature Cited**

- Aktar, M.; Shamsi, S. Incidence and severity of Blight Disease of *Tagetes erecta* and *T. patula*. Bioresearch Communications, v.4, n.1, p.464-469, 2018. http://www.bioresearchcommunications. com/pdf/BRC18V4I1074.pdf. 10 Jan. 2018.
- Bardsley, S.J.; Ngugi, H.K. Reliability and accuracy of visual methods used to quantify severity of foliar bacterial spot symptoms on peach and nectarine. Plant Pathology, v.62, n.2, p.460-74, 2013. https://doi.org/10.1111/j.1365-3059.2012.02651.x.
- Bock, C.H.; Chiang, K.S.; Del Ponte, E.M. Accuracy of plant specimen disease severity estimates: concepts, history, methods, ramifications and challenges for the future. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, v.11, n.39, p.1-13, 2016. https://doi. org/10.1079/PAVSNNR201611032.
- Bock, C.H.; Parker, P.E.; Cook, A.Z.; Gottwald, T.R. Characteristics of the perception of different severity measures of citrus canker and the relations between the various symptom types. Plant Disease, v.92, n.6, p.927-939, 2008. https://doi.org/10.1094/ PDIS-92-6-0927.
- Bock, C.H.; Poole, G.; Parker, P.E.; Gottwald, T.R. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Critical Reviews in Plant Sciences, v.29, n.2, p.59-107, 2010. https://doi. org/10.1080/07352681003617285.
- Braido, R.; Gonçalves-Zuliani, A.M.O.; Nocchi, P.T.R.; Belasque Jr., J.; Janeiro, V.; Bock, C.H.; Nunes, W.M.C. A standard area diagram set to aid estimation of the severity of Asiatic citrus canker on ripe sweet orange fruit. European Journal of Plant Pathology, v.141, n.2, p.327–337, 2015. https://doi.org/10.1007/s10658-014-0544-0.

Campbell, C.L.; Madden, L.V. Introduction to plant disease epidemiology. 1.ed. New York: John Wiley, 1990. 532p.

- Castro, A.P.G. Desempenho agronômico, diversidade genética e avaliação de doenças em progênies de maracujazeiro-azedo. Brasília: Universidade de Brasília, 2015. 204p. Tese Doutorado. http://repositorio.unb.br/handle/10482/19082. 18 Jan. 2018.
- Correia, K.C.; Queiroz, J.V.J.; Martins, R.B.; Nicoli, A.; Del Ponte, E.M.; Michereff, S.J. Development and evaluation of a standard area diagram set for the severity of phomopsis leaf blight on eggplant. European Journal of Plant Pathology, v.149, n.2, p.269-276, 2017. https://doi.org/10.1007/s10658-017-1184-y.
- Damasceno, V.F.F.; Furtado, E.L.; Ferreira Filho, P.J. Comparação de dois métodos de elaboração e validação de escala diagramática para a quantificação da severidade da mancha de *Cylindrocladium* em eucalipto. Summa Phytopathologica, v.40, n.3, p.248-255, 2014. https://doi.org/10.1590/0100-5405/1960.
- De Paula, P.V.A.A.; Pozza, E.A.; Santos, L.A.; Chaves, E.; Maciel, M.P.; Paula, J.C.A. Diagrammatic scales for assessing brown eye spot (*Cercospora coffeicola*) in red and yellow coffee cherries. Journal of Phytophatology, v.164, n.10, p.791-800, 2016. https://doi. org/10.1111/jph.12499.
- Dias, M.S.C. Principais doenças fúngicas e bacterianas do maracujazeiro. Informe Agropecuário, v.21, n.206, p.34-38, 2000. http://www.epamig.br/download/ia\_206\_a-cultura-domaracujazeiro\_set\_out\_2000. 21 Jan. 2019.
- Dolinski, M.A.; Duarte, H.S.S.; Silva, J.B.; De Mio, L.L.M. Development and validation of a standard area diagram set for assessment of peach rust. European Journal of Plant Pathology, v.148, n.4, p.817-824, 2017. https://doi.org/10.1007/s10658-016-1138-9.
- Faleiro, F.G.; Junqueira, N.T.V.; Braga, M.F.; Oliveira, E.J.; Peixoto, J.R.; Costa, A.M. Germoplasma e melhoramento genético do maracujazeiro: histórico e perspectivas. Planaltina: Embrapa Cerrados, 2011. 36p.
- Instituto Brasileiro de Geografia e Estatística IBGE. Produção Agrícola Municipal – Culturas temporárias e permanentes. Rio de Janeiro: IBGE, 2016. 62p.
- Kranz, J. Measuring plant disease. In: Kranz, J.; Rotem, J. (Ed.). Experimental techniques in plant disease epidemiology. Heidelberg: Springer, 1988. p.35-50.
- Kudo, A.S.; Peixoto, J.R.; Junqueira, N.T.V.; Blum, L.E.B. Suscetibilidade de genótipos de maracujazeiro-azedo à septoriose em casa de vegetação. Revista Brasileira de Fruticultura, v.34, n.1, p.200-205, 2012. https://doi.org/10.1590/S0100-29452012000100027.
- Lenz, G.; Costa, I.D.; Balardini, R.S.; Stefanelo, M.S.; Marques, L.N.; Arrué, A. Escala diagramática para avaliação de severidade de mancha-de-septoria em girassol. Ciência Rural, v.39, n.8, p.2527-2530, 2009. https://doi.org/10.1590/S0103-84782009000800040.
- Librelon, S.S.; Souza, E.A.; Pereira, R.; Pozza, E.A.; Abreu, A.F.B. Diagrammatic scale to evaluate angular leaf spot severity in primary leaves of common bean. Australasian Plant Pathology, v.44, n.4, p.385–395, 2015. https://doi.org/10.1007/s13313-015-0360-9.
- Lin, L.I. A concordance correlation coefficient to evaluate reproducibility. Biometrics, v.45, n.1, p.255-268, 1989. https:// doi.org/10.2307/2532051.

- Marcuzzo, L.L; Duarte, T.S.; Rosa Neto, A.J.; Hoffmann, F. Efeito de fosfito de potássio e de fungicidas no controle da cercosporiose (Cercospora beticola) da beterraba. Summa Phytopathologica, v.42, n.2, p.186-187, 2016. https://doi.org/10.1590/0100-5405/213.
- Mesquini, R.M.; Schwan-Estrada, K.R.F.; Godoy, C.V.; Vieira, R.A.; Zarate, N.A. H.; Vieira, M.C. Escala diagramática para a quantificação de Septoria apiicola e Cercospora arracacina em mandioquinha-salsa. Tropical Plant Pathology, v.34, n.4, p.250-255, 2009. https://doi.org/10.1590/S1982-56762009000400008.
- Nuñez, A.M.P.; Monteiro, F.P.; Pacheco, L.P.; Rodríguez, G.A.A.; Nogueira, C.C. A.; Pinto, F.A.M.F.; Medeiros, F.A.V.; Souza, J.T. Development and validation of a diagrammatic scale to assess the severity of black rot of crucifers in Kale. Journal of Phytopathology, v.165, n.3, p.195-203, 2017. https://doi.org/10.1111/jph.12550.
- Nutter Jr., F.W.; Gleason, M.L.; Jenco, J.H.; Christians, N.C. Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. Phytopathology, v.83, n.8, p.806– 812, 1993. https://doi.org/10.1094/Phyto-83-806.
- Nutter Jr., F.W.; Schultz, P.M. Improving the accuracy and precision of disease assessment: selection of methods and use of computer-aided training programs. Canadian Journal of Plant Pathology, v.17, n.2, p.174-184, 1995. https://doi.org/10.1080/07060669509500709.
- Nutter Jr., F.W.; Worawitlikit, O. Disease.Pro: a computer program for evaluating and improving a person ability to assess disease proportion. Phytopathology, v.79, p.1135, 1989.
- Ortega-Acosta, S.Á.; Velasco-Cruz, C.; Hernández-Morales, J.; Ochoa-Martínez, D. L.; Hernández-Ruiz, J. Escalas logarítmicas diagramáticas para evaluar la severidad del manchado de hojas y cálices de jamaica. Revista Mexicana de Fitopatología, v.34, n.3, p.270-285, 2016. https://doi.org/10.18781/R.MEX.FIT.1606-6.
- Parreira, D.F.; Zambolim, L.; Costa, R.V.C.; Silva, D.D.; Marcondes, M.M.; Lanza, F.E.; Neves, W.S.; Figueiredo, J.E.F.; Souza, A.G.C.; Cota, L.V. A method for Colletotrichum graminicola inoculation in maize stalks. Revista Brasileira de Milho e Sorgo, v.15, n.1, p.53-64, 2016. https://doi.org/10.18512/1980-6477/rbms.v15n1p53-64.
- Polizel, A.C.; Juliatti, F.C. Quantificação de doenças foliares da soja por escalas diagramáticas. Enciclopédia Biosfera, v.6, n.11, p.1-9, 2010. http://www.conhecer.org.br/enciclop/2010c/ quantificacao%20de%20doencas.pdf 18 Jan. 2018.
- Poltronieri, T.P.; Silveira, S.F.; Vivas, M.; Santa Catarina, R.; Cortes, D.F.; Azevedo, A.O.; Pereira, M.G. Selecting black-spot resistant papaya genotypes derived from backcrossing and hybrids. Genetics and Molecular Research, v.16, n.1, gmr16019401, 2016. https://doi.org/10.4238/gmr16019401.
- Sachet, M.R.; Danner, M.A.; Citadin, I.; Pertille, R.H.; Guerrezi, M.T. Standard area diagram set for olive leaf spot assessment. Ciência Rural, v.47, n.6, e20160923, 2017. https://doi.org/10.1590/0103-8478cr20160923.
- Santos, P.H.D.; Mussi-Dias, V.; Freire, M.G.M.; Carvalho, B.M.; Silveira, S.F. Diagrammatic scale of severity for postharvest black rot (Ceratocystis paradoxa) in coconut palm fruits. Summa Phytopathologica, v.43, n.4, p.269-275, 2017. https://doi. org/10.1590/0100-5405/170792.
- Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. Nature Methods, v.9, p.671-675, 2012. https://doi.org/10.1038/nmeth.2089.

- Schwanck, A.A.; Del Ponte, E.M. Accuracy and reliability of severity estimates using linear or logarithmic disease diagram sets in true colour or black and white: A study case for rice brown spot. Journal of Phytopatholy, v.162, n.10, p.670-682, 2014. https:// doi.org/10.1111/jph.12246.
- Stonehouse, J. Assessment of Andean bean diseases using visual keys. Plant Pathology, v.43, n.3, p.519-527, 1994. https://doi. org/10.1111/j.1365-3059.1994.tb01586.x.
- Sussel, A.A.B. Manejo de doenças fúngicas em goiaba e maracujá. Planaltina: Embrapa Cerrados, 2010. 43p.
- Tomerlin, J.R.; Howell, T.A. Distrain: a computer program for training people to estimate disease severity on cereal leaves. Plant Disease, v.72, n.5, p.455-459, 1988. https://www.apsnet.org/ publications/plantdisease/backissues/Documents/1988Articles/ PlantDisease72n05\_455.PDF. 18 Jan. 2018.
- Valeriano, R.; Pozza, E.A.; Santos, L.A.; Chaves, E.; Barbosa Jr., M.P.; Ferreira, M. A. Escala diagramática e reação diferencial de clones para oídio do eucalipto. Scientia Forestalis, v.43, n.105, p.51-61, 2015. http://www.ipef.br/publicacoes/scientia/nr105/cap05. pdf. 22 Jan. 2018.
- Venturini, M.T.; Santos, L.R.; Oliveira, E.J. Development of a diagrammatic scale for the evaluation of postharvest physiological deterioration in cassava roots. Pesquisa Agropecuária Brasileira, v.50, n.8, p.658-668, 2015. https://doi.org/10.1590/S0100-204X2015000800004.
- Vilela, M.S. Diversidade genética, produtividade e reação de progênies de maracujazeiro às doenças sob condições de campo. Brasília: Universidade de Brasília, 2013. 181p. Tese Doutorado. http://repositorio.unb.br/handle/10482/13705. 10 Jan. 2018.