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Standard Area Diagram Set for Scab Evaluation in Fruits of sour Passion Fruit

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Abstract

Scab (*Cladosporium* spp.) significantly comprises the commercial acceptance of sour passion fruit (*Passiflora edulis*) because of the deformed and atrophied fruit appearance resulting from the development of the lesions. Therefore, the objective of this study was to elaborate and validate a standard area diagram set (SADs) for the severity evaluation of scab in fruits of sour passion fruit. The SADs comprised eight severity levels (0.6; 1; 2; 4; 8; 16; 37; and 46%) and was validated by 20 raters (G1 and G3, inexperienced; G2 and G4, experienced). Initially, all raters performed a non-aided SADs evaluation of the scab severity. Afterward, G1 and G2 completed the second evaluation without the proposed SADs, whereas G3 and G4 performed a SADs-aided assessment of the disease severity. The accuracy and precision of the evaluations were determined by simple linear regression and by the Lin’s concordance correlation coefficient. Constant and systematic errors decreased with the use of the SADs, demonstrating an approximation between the estimated and the actual values. Precision increased with an increase in the coefficient of determination. Also, the absolute error reduced by 66% (G3) and 47% (G4). Therefore, 94.4% (G3) and 98.8% (G4) of the estimates had up to ±10% of errors, which corresponds to a 20.4% (G3) and 5.6% (G4) increment in the estimates with errors within this variation range. As a result, accuracy and precision were higher in the SADs-aided groups. Inexperienced raters were the most benefited by the use of the SADs. The accuracy and precision of the non-aided groups had a slight or no increase when compared with the SADs-aided groups.

Keywords: *Passiflora edulis* Sims, *Cladosporium* spp., phytopathometry

1. Introduction

Sour passion fruit (*Passiflora edulis* Sims) is a tropical crop widely consumed and grown in different tropical regions (Freitas et al., 2016). The species is mainly intended for the market of fresh fruit and juices but is also used in food, cosmetics, and medicinal products (Faleiro, Junqueira, & Costa, 2016). Brazil is the largest sour passion fruit producer in the world, totaling approximately 703,500 tons per year (Brazilian Institute of Geography and Statistics [IBGE], 2016). However, Brazilian production has been oscillating, mainly due to the occurrence of diseases, such as scab (*Cladosporium* spp.). Scab affects the young growing tissues and may lead to significant losses if not controlled (Sussel, 2015). The peel of sour passion fruits infected with scab exhibit 3-5mm round, slightly deep, dark spots that later become rough and protuberant, resembling warts (Pio-Ribeiro & Mariano, 1997). The lesions are superficial and do not affect the pulp quality but may cause fruit drop (Junqueira, Sussel, Junqueira, Zacaroni, & Braga, 2016). However, the deformed and atrophied appearance impairs the acceptance of these fruits by the consumer market (Pio-Ribeiro & Mariano, 1997; Santos Filho & Santos, 2003).

Diseases quantification or pathometry is the process by which symptoms are evaluated and expressed in units that allow objective comparisons (Laranjeira, 2005). Disease severity is usually the most common variable in evaluations; it is mainly assessed by visual estimates of the percentage of the injured area in relation to the total area (Duarte et al., 2013). Visual estimates are highly subjective and may vary according to the innate ability of each rater. Thus, several strategies have been proposed to standardize estimates, such as the use of SADs (Madden, Hughes, & Van Den Bosch, 2007).

SADs are graphical representations of plant parts showing disease symptoms at distinct levels of severity (Alves et al., 2015). These SADs are known for providing more accurate, precise and reproducible evaluations (Del Ponte et al., 2017). The accuracy is characterized by the proximity between the estimated and the actual values and is measured by the intercepts and slope coefficients of the regression lines between these two values (Nutter, Teng, & Shokes, 1991). The precision measures the reliability and/or repeatability of the severity estimates (Nutter et al., 1991) and can be quantified by the coefficient of determination of the linear regressions established between the actual and the estimated severities, absolute errors, and the reproducibility of the estimates between pairs of raters (Nutter & Schultz, 1995). The estimated value should be as close as possible to the actual value, and these estimates should be consistent with each other (Vieira et al., 2014). In this context, the concept of agreement is determined as the product of precision (variability in the estimates) and accuracy, and it is used to compare the estimated with the actual values (Madden et al., 2007).

Despite the reduced yield and depreciation of fruit quality (Junqueira et al., 2016) caused by scab, no studies have developed nor validated SADs for disease severity in sour passion fruit. This study aimed at (1) elaborating and validating a SADs to asses the scab severity in sour passion fruit; (2) comparing accuracy, precision, and agreement of scab severity estimates with and without the aid of the SADs; and (3) comparing accuracy, precision, and agreement of the estimates from inexperienced and experienced raters.

2. Methods

For SADs elaboration, 50 fruits of sour passion fruit (BRS Gigante Amarelo and Yellow Master FB200 commercial cultivars) presenting scab disease symptoms were harvested at Paraná Farm commercial orchard, located in Nucleo Rural Pipiripau, Planaltina, DF, Brazil (lat. 47°29′56.92″ S; long. 15°30′15.08″ W, and 955 m asl). The image obtention process, SADs elaboration and validation procedures, and data analyses were performed according to previously described by Costa, Pires, Peixoto, Blum, and Faleiro (2018).

Fruit surface was photographed, and total fruit and diseased area (necrotic + chlorotic) were determined using the IMAGE J software (Schneider, Rasband, & Eliceiri, 2012), thereby obtaining the percent of fruit area affected by scab, which was considered as the actual (true) disease severity. These values were then used as a reference for assessing the accuracy and precision of rater estimates when aided or not aided by the SADs. The SADs' lower limit reproduced the minimum value of scab severity found in the image analysis of the 50 fruits, whereas the SADs' upper limit reflected the maximum value of disease severity registered. Intermediate levels were determined according to logarithmic increments (Nutter & Schultz, 1995).

The validation of the SADs was performed by 20 raters and used 50 images of fruits showing different scab severity intensities. In the first evaluation (Evaluation 1), all raters assessed the disease severity without the aid of the SADs (non-aided evaluation). In a second moment, the raters were divided into four groups of five raters per group (G1 and G3, inexperienced; G2 and G4, experienced). G1 and G2 completed another non-aided evaluation whereas G3 and G4 performed 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), as well as by the Lin's concordance correlation coefficient (LCCC; ρ_c) (Costa et al., 2018). The most accurate raters were those whose estimates provided linear regression equations with intercept ("*a*") not significantly different from 0 (constant error-free estimates); and slope coefficient ("*b*") not significantly different from 1 (systematic error-free estimates), based on the t-test applied to "*a*" and "*b*" (Nutter & Schultz, 1995). Raters with higher values of R^2 and absolute errors (the difference between estimated and actual severities) of lower magnitudes were considered as of higher precision (Kranz, 1988). The reproducibility was measured using the R^2 values for each pair of raters. The closer to 1.0 is the R^2 value of the comparison between two raters, the higher is the reproducibility (Nutter & Schultz, 1995). Additionally, raters with the highest ρ_c values presented the most considerable agreement between estimated and actual severity values (Lin, 1989; Bock, Poole, Parker, & Gottwald, 2010). The accuracy, precision, absolute errors, agreement, and inter-reliability of estimates were compared without and with the use of the SADs for both inexperienced and experienced raters.

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).

3. Results and Discussion

The SADs proposed in this work for scab quantification in sour passion fruit included eight severity levels (0.6%, 1%, 2%, 4%, 8%, 16%, 37%, and 46%) (Figure 1). The mean severity was 6%, and 86% and 96% of the fruits exhibited severity levels lower than 10% and 20%, respectively.

Considering the SADs-aided evaluation, 40% of the raters in G3 had intercept values equal to 0 ($P \leq 0.05$), without constant errors and with no increase in relation to the first evaluation. Conversely, 80% of the raters showed slope coefficients of the line equal to 1 ($P \leq 0.05$), without systematic errors. In G4, 60% of the raters had intercept values equal to 0 ($P \leq 0.05$), while only 20% showed slope coefficients equal to 1 ($P \leq 0.05$) (Table 1).

The use of the SADs improved the accuracy in G3 when compared with G1 and G2. The SADs provided an intercept value and slope coefficient closer to 0 and 1, respectively, concerning the first evaluation. Also, the mean values were lower than those of the double non-aided evaluation (Table 1). This result indicates that despite the constant and/or systematic errors committed by some raters, the SADs-aided evaluation showed fewer errors than the non-aided evaluation, which thus reduces the overestimation trend observed in the first evaluation.

In the non-aided evaluation, the R^2 values varied between 0.79 and 0.95 (mean 0.89) in G3, and between 0.92 and 0.97 (0.94) in G4. In the SADs-aided evaluation, these values ranged from 0.85 to 0.95 (0.90) and from 0.94 to 0.97 (0.95) for inexperienced and experienced raters, respectively, indicating a slight increase (1%) in the R^2 values. Conversely, precision was slightly reduced in Evaluation 2 of G1 and G2 (Table 2).

The precision of the raters can also be quantified by the distribution of errors or absolute errors, in which the error is the dependent variable, and the actual severity is the independent variable (Nutter & Schultz, 1995). Mean absolute errors equal to 6.8 (G1), 3.7 (G2), 8.0 (G3), and 3.5 (G4) were observed in evaluation 1. In the second evaluation, G1 and G2 had mean absolute errors of 6.8 and 3.5, respectively, which did not differ from the values identified in the first evaluation. However, the use of the SADs reduced the mean absolute errors of the estimates by 66.1% in G3 (2.7) and 47% in G4 (1.8). As a consequence, differences were detected between SADs-aided and non-aided assessments within the same group ($P \leq 0.05$).

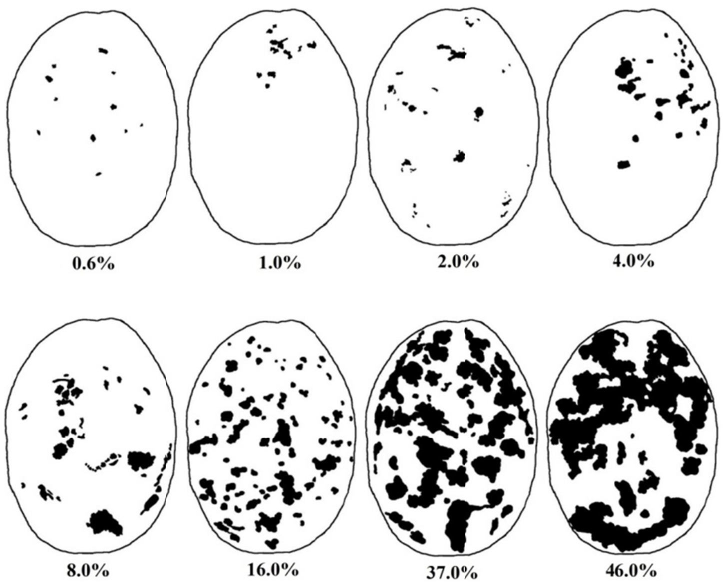

Figure 1. Standard area diagram set (SADs) for scab (*Cladosporium* spp.) evaluation in fruits of sour passion fruit (*Passiflora edulis* Sims)

The error distribution of non-aided evaluations varied from -5.0 to +39.0 (G1), from -9.0 to +24.0 (G2), from -8.0 to +46.0 (G3), and from -12.0 to +35.0 (G4). In the second non-aided evaluation, the errors varied between -9.0 and +34.0 (G1) and between -7.0 and +22.2 (G2). In the SADs-aided evaluation, error intervals ranged from -12.0 to +21.0 for inexperienced raters and from -11.0 to +15.0 for experienced raters, with reductions of 60.4% (G3) and 43% (G4) in the mean maximum errors (Table 2). The decrease in the absolute errors resulted in 94.4% (G3) and 98.8% (G4) of the estimates within the 10% variation (-10 to +10) (Table 2), which is considered satisfactory, according to criteria used to validate SADs (Nutter & Schultz, 1995). The SADs-aided evaluation reduced by 74% and 81.3% (G3 and G4, respectively) the number of estimates outside this variation range. Based on Nutter and Schultz (1995), raters whose errors do not exceed 5% (-5 to +5) are classified as excellent. The use of the SADs increased by 36.4% and 16.1% the number of estimates within the range of 5% in G3 and

G4, respectively. These values are higher than the 3% increase observed in the second evaluation in G2, indicating that the SADs-aided evaluation reduced the absolute errors in relation to the non-aided evaluation (Table 2).

The reproducibility of the non-aided evaluation was average to high; the linear regressions between the severity levels estimated by the five raters resulted in coefficients of determination ranging from 0.69 to 0.95 (mean 0.83) in G3 and from 0.85 to 0.97 (0.91) in G4. However, the SADs-aided evaluation reduced the mean R^2 values of the raters' estimates (0.80 for G3 and 0.89 for G4). The increase in the reproducibility in the SADs-aided evaluation is desirable as it allows different raters to find similar results for their disease severity estimates while evaluating different experiments (Nutter & Schultz, 1995). However, the present results revealed higher reproducibility in the non-aided evaluations when compared with the SADs-aided evaluations. SADs, as well as most of the methods of disease severity quantification, is prone to subjectivity since raters have distinct abilities to discriminate disease severity degrees (Venturini, Santos, & Oliveira, 2015; Correia et al., 2017). Even with the use of the SADs, the accuracy and precision of estimates may be affected by other factors, such as the complexity of the disease severity, the size, the color, and the number of lesions (Kranz, 1988; Bock et al., 2010). Factors inherent to each individual, such as fatigue, mood, and experience may also affect the estimates (Nutter & Schultz, 1995; Bock, Chiang, & Del Ponte, 2016). However, training raters may result in a standardized evaluation, reducing the observed discrepancies and consequently increasing the reliability of the estimates among pairs of raters. Various studies have demonstrated the relevance of training to increase performance, especially for the less experienced raters (González-Dominguez et al., 2014; Bock et al., 2016; Sachet, Citadin, Danner, Guerrezi, & Pertille, 2017).

Table 1. Intercepts (*a*), slope coefficients (*b*), and coefficients of determination (R^2) of linear regression for actual severity versus estimated severity of scab (*Cladosporium* spp.) in fruits of sour passion fruit (*Passiflora edulis* Sims)

	Raters	**Evaluation 1**			**Evaluation 2**		
	Inexperienced	**No SADs**			**No SADs**		
		a	*b*	R^2	*a*	*b*	R^2
G1	1	1.06*	0.90*	0.97	0.63	1.05	0.97
	2	6.63*	0.80*	0.94	7.75*	0.86*	0.87
	3	0.49	1.76*	0.94	-1.70	1.65*	0.92
	4	9.59*	1.61*	0.85	12.01*	1.82*	0.88
	5	1.28	1.63*	0.96	0.44	1.27*	0.95
	Mean	3.81	1.34	0.93	3.83	1.33	0.92
	Experienced	**No SADs**			**No SADs**		
G2	6	1.31*	1.13*	0.96	1.32*	1.10*	0.96
	7	2.53*	1.43*	0.94	2.73*	1.38*	0.93
	8	3.64*	0.80*	0.92	4.24*	0.88*	0.91
	9	1.48*	1.13*	0.96	1.02*	0.98	0.95
	10	2.42*	1.13*	0.87	-0.11	1.48*	0.83
	Mean	2.28	1.13	0.93	1.84	1.17	0.92
	Inexperienced	**No SADs**			**With SADs**		
G3	11	0.88	1.49*	0.95	1.39*	0.98	0.91
	12	8.79*	2.31*	0.79	3.20*	1.05	0.85
	13	1.32*	1.10	0.91	1.06	0.94	0.89
	14	1.15	2.13*	0.89	1.31*	1.05	0.90
	15	-2.32*	1.52*	0.91	0.16	1.22*	0.95
	Mean	1.97	1.71	0.89	1.43	1.05	0.90
	Experienced	**No SADs**			**With SADs**		
G4	16	-0.44	0.84*	0.97	-0.33	1.19*	0.97
	17	1.22*	0.96	0.92	-0.33	0.97	0.95
	18	1.88*	0.80*	0.94	0.57	0.85*	0.95
	19	4.68*	1.53*	0.90	1.69*	0.90*	0.90
	20	-0.44	1.31*	0.95	0.87*	0.76*	0.95
	Mean	1.38	1.09	0.94	0.50	0.93	0.95

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

Table 2. Mean maximum error in absolute value (MEAV), at 10% (x±10) and 5% (x±5) error range of the severity estimates in relation to the actual severity of scab (*Cladosporium* spp.) in fruits of sour passion fruit (*Passiflora edulis* Sims)

Evaluation	Parameters	Groups			
		1	2	3	4
1	**MEAV**	25.6	15.5	39.4	14.4
	% x±5	56.8	78.8	64.8	79.6
	% x±10	84.0	94.8	78.4	93.6
2	**MEAV**	26.9	19.7	15.6	8.2
	% x±5	63.2	81.2	88.4	92.4
	% x±10	78.4	95.2	94.4	98.8

According to the Lin's concordance correlation coefficient (ρ_c), severity estimates were closer to the actual values in the SADs-aided evaluations (Table 3). Considering the SADs-aided evaluations, the precision of the estimates, measured by the *r* value, slightly increased in G3; it did not change in G2 and G4; and decreased in G1. The C*b* value, which measures accuracy, slightly increased in G1 and decreased in G2. Conversely, with the use of the SADs, 100% of the raters improved their accuracy levels, with increases of 27.3% and 5.4% in G3 and G4, respectively, in relation to the first evaluation (Table 3). Thus, the Lin's concordance correlation coefficient detected a more significant increase in the raters' accuracy levels when compared with the parameters of the linear regression analysis.

For 100% of the raters, the concordance between the actual severity values and the estimated severity values improved with the use of the SADs. In the non-aided evaluations, ρ_c values varied from 0.42 to 0.93, with a mean of 0.73 (G3), and from 0.71 to 0.96, with a mean of 0.90 (G4). However, in the SADs-aided evaluation, ρ_c values ranged from 0.87 to 0.95 (mean 0.93) and from 0.94 to 0.97 (0.96) in G3 and G4, respectively (Table 2.15). According to the classification proposed by McBride (2005), these values reflect a change in the magnitude of the concordance between the actual and estimated severity in G3, from poor to moderate, and in G4, from moderate to substantial.

Linear regression is one of the most frequently used strategies for the validation of disease estimate methods and the evaluation of the accuracy and precision of disease severity estimates in leaves, fruits, and pods (Del Ponte et al., 2017; Costa et al., 2018). Nevertheless, some authors suggest that statistical tests for estimate errors may be highly influenced by the precision of the raters in the linear regression (Lin, 1989; Shoukri & Pause, 1999). Therefore, several studies consider the Lin's concordance correlation the most acceptable method for this kind of experiment since this coefficient associates accuracy and precision measures to evaluate the agreement between the actual and estimated values (Madden et al., 2007; Capucho et al., 2011; Duarte et al., 2013).

Table 3. Correlation coefficient between estimated and actual severities (r), bias correction factor (C_b), and Lin's concordance correlation coefficient (ρ_c) for scab (*Cladosporium* spp.) severity estimates in fruits of sour passion fruit (*Passiflora edulis* Sims)

	Raters	**Evaluation 1**			**Evaluation 2**		
	Inexperienced	**No SADs**			**No SADs**		
		r	C_b	ρ_c	r	C_b	ρ_c
G1	1	0.99	1.00	0.98	0.99	0.99	0.98
	2	0.97	0.86	0.83	0.93	0.82	0.76
	3	0.97	0.78	0.76	0.96	0.85	0.81
	4	0.92	0.59	0.54	0.94	0.49	0.46
	5	0.98	0.81	0.79	0.98	0.95	0.92
	Mean	0.97	0.81	0.78	0.96	0.82	0.79
G2	**Experienced**	**No SADs**			**No SADs**		
	6	0.98	0.97	0.95	0.98	0.98	0.96
	7	0.97	0.84	0.82	0.97	0.86	0.83
	8	0.96	0.96	0.92	0.96	0.95	0.90
	9	0.98	0.97	0.95	0.97	1.00	0.97
	10	0.93	0.94	0.87	0.91	0.86	0.79
	Mean	0.96	0.94	0.90	0.96	0.93	0.89
G3	**Inexperienced**	**No SADs**			**With SADs**		
	11	0.97	0.87	0.84	0.95	0.99	0.95
	12	0.89	0.47	0.42	0.92	0.94	0.87
	13	0.95	0.97	0.93	0.94	1.00	0.94
	14	0.94	0.65	0.61	0.95	0.98	0.94
	15	0.95	0.89	0.85	0.97	0.96	0.94
	Mean	0.94	0.77	0.73	0.95	0.98	0.93
G4	**Experienced**	**No SADs**			**With SADs**		
	16	0.99	0.97	0.96	0.99	0.98	0.97
	17	0.96	1.00	0.95	0.97	1.00	0.97
	18	0.97	0.98	0.95	0.97	0.99	0.96
	19	0.95	0.75	0.71	0.95	0.99	0.94
	20	0.97	0.95	0.92	0.97	0.96	0.94
	Mean	0.97	0.93	0.90	0.97	0.98	0.96

4. Conclusion

The SADs proposed in this study enhanced the accuracy and precision of the scab severity estimates in sour passion fruit. The SADs benefited inexperienced raters to a greater extent. Only 5.6% (G3) and 1.2% (G4) of the estimates had errors higher than 10% in SADs-aided evaluations.

These results suggest that the use of the proposed SADs to quantify scab severity in fruits may provide more realistic information regarding the pathosystem *Cladosporium* spp. (sour passion fruit). A better evaluation could minimize yield losses related to disease severity, allowing comparisons of treatments and levels of genotype resistance in breeding programs, besides enabling epidemiological studies.

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