VERÔNICA VIEIRA BRÁS

STANDARD AREA DIAGRAM SET TO ASSESS SCAB SEVERITY IN ENTIRE-MARGINED LEAVES OF SOUR PASSION FRUIT

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Plant Pathology, to obtain the title of *Magister Scientiae*.

Advisor: Fabrício Ávila Rodrigues

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APPROVED: February 20, 2020

Assent:

Verônica Vieira Brás
Author

Fabrício Ávila Rodrigues
Advisor

To God,

To my parents Leny and Sidinei,

To my siblings Paloma and Júnior,

To my fiance Leandro.

I dedicate.

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First of all, I thank God for bless me and guide my ways.

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“In the great battles of life, the first step to victory is the desire to win.”

(Mahatma Gandhi)

BIOGRAPHY

VERÔNICA VIEIRA BRÁS, daughter of Leny Lopes Vieira Brás and Sidinei Luciano Brás, was born on May 15, 1995, in Viçosa, Minas Gerais State.

In May 2013, she started to study Agronomy at Universidade Federal de Viçosa (UFV). In 2014, she was intern at the Laboratory of Population Biology of Plant Pathogens at UFV. In 2016, she started the internship at the Laboratory of Host-Pathogen Interaction at UFV. From January to July of 2017, she received training at the Mycology Laboratory at the Louisiana State University in United States of America. In July of 2018, she obtained the title of Agronomist.

In August of 2018, she started the Master's Degree program in the Graduate Program of Plant Pathology at UFV under the guidance of Professor Fabrício Ávila Rodrigues. She defended her Dissertation on February 20, 2020.

ABSTRACT

BRÁS, Verônica Vieira, M.Sc., Universidade Federal de Viçosa, February, 2020. **Standard area diagram set to assess scab severity in entire-margined leaves of sour passion fruit**. Advisor: Fabrício Ávila Rodrigues.

Scab, caused by *Cladosporium cladosporioides* complex, is one of the most important diseases affecting passion fruit yield and fruit quality. The assessment of plant diseases is important to determine their correct management and the standard area diagrams (SADs) are useful tools to achieve this goal. This study aimed to develop and validate a SAD to quantify the severity of scab in entire-margined leaves of sour passion fruit. The SAD obtained has ten images of scab severity values (0.4, 3.5, 5.4, 8.0, 11.1, 16.2, 26.1, 38.2, 47.9, and 58.9%). To evaluate the SAD, twenty raters were involved (ten experienced (EP) and ten inexperienced (IP)) who estimated the same set of fifty images twice being first without and after with the use of the SAD. The accuracy was significantly improved by using SAD. The coefficient of bias (C_b) were 0.75 and 0.98 for IP and 0.96 and 0.98 for EP without and with SAD, respectively. The correlation coefficient (r) was 0.89 and 0.97 for IP and 0.95 and 0.97 for EP without and with SAD, respectively. Lin's concordance correlation coefficient (ρ_c) were 0.66 and 0.95 for IP and 0.91 and 0.95 for EP without and with SAD, respectively. Moreover, the coefficient of determination (R^2) was significantly improved by using SAD. The SAD proposed will improve the accuracy and precision during the evaluation of scab severity in entire-margined leaves of sour passion fruit.

Keywords: Accuracy. Epidemiology. Foliar disease. Phytopatometry. Scab quantification.

RESUMO

BRÁS, Verônica Vieira, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Escala diagramática para avaliar a severidade da verrugose em folhas de margens inteiras de maracujazeiro azedo**. Orientador: Fabrício Ávila Rodrigues.

A verrugose, causada por fungos do complexo *Cladosporium cladosporioides*, é uma das doenças mais importantes afetando a produção do maracujazeiro e a qualidade dos frutos. A avaliação das doenças de plantas é importante para realizar o manejo correto e as escalas diagramáticas são ferramentas úteis para isso. Este estudo teve como objetivo elaborar e validar uma escala diagramática para quantificar a severidade da verrugose em folhas com margens inteiras de maracujazeiro. A escala obtida contém dez imagens com valores de severidade (0,4; 3,5; 5,4; 8,0; 11,1; 16,2; 26,1; 38,2; 47,9 e 58,9%). Para avaliar a escala, vinte avaliadores foram envolvidos (dez experientes (EP) e dez inexperientes (IP)), os quais estimaram o mesmo conjunto de cinquenta imagens, duas vezes, primeiro sem e depois com a escala. A acurácia aumentou significativamente usando a escala. O coeficiente de viés (C_b) foi 0,75 e 0,98 para IP e 0,96 e 0,98 para EP, sem e com a escala, respectivamente. O coeficiente de correlação (r) foi 0,89 e 0,97 para IP e 0,95 e 0,97 para EP, sem e com a escala respectivamente. O coeficiente de correlação concordante de Lin foi 0,66 e 0,95 para IP e 0,91 e 0,95 para EP, sem e com a escala, respectivamente. Além disso, o coeficiente de determinação (R^2) aumentou significativamente usando a escala. A escala proposta aumentará a acurácia e a precisão durante a avaliação da severidade da verrugose em folhas com margens inteiras de maracujazeiro.

Palavras-chave: Acurácia. Doença foliar. Epidemiologia. Fitopatometria. Quantificação da verrugose.

SUMMARY

INTRODUCTION

The passion fruit (*Passiflora edulis* Sims) is a tropical fruit native from Brazil known to be the largest producer and consumer worldwide (FAO, 2017). The fruit is quite appreciated by its flavor, leaves extracts are used to obtain soothing and cosmetics besides being used as an ornamental plant (Faleiro and Junqueira, 2016). The scab, caused by fungi species of the *Cladosporium cladosporioides* complex, affects young leaves, branches, flower buds, flowers, and fruits from passion fruit plants (Fischer and Rezende, 2008; Rosado et al., 2019). The symptoms are small round spots on the leaves, which firstly translucent and afterward become necrotic, displaying greenish-grey centers, leading to leaf abscission (Joy and Sherin, 2012). On fruits, the lesions develop and become corklike, prominent, and brown (Ribeiro and Mariano, 1997).

Fungi belonging to the *Cladosporium* genus are widely dispersed in nature (Bensch et al., 2012; Crous et al., 2007). The scab on passion fruits has been reported in Australia, Brazil, Venezuela, and Zimbabwe (Fischer and Rezende, 2008). In Brazil, this disease occurs in all passion fruit-producing areas resulting in great economic losses to the producers (Fischer and Rezende, 2008). The management of scab includes the use of low plant density in the field and pruning the infected parts of the plants (Joy and Sherin, 2012). The spray of plants with fungicides must be used when they are blooming and at their intensive growth stage (Joy and Sherin, 2012).

Quantifying plant diseases is an important step to accomplish experiment assessments; hence, it is primordial to propose management strategies (Madden et al., 2007). Severity, which is obtained as the proportion of infected tissue related to whole tissue, is used to quantify many diseases on plants (Amorim, 1995). Determining the intensity is useful because it allows comparing the efficiency of fungicides, biological control agents, and determine the level of resistance of a particular cultivar (Vale et al., 2004). Standard area diagrams (SADs) are images with affected parts of the plant; this tool is used to aid in plant disease assessments (Bergamin Filho and Amorim, 1996).

The assessment of diseases occurring in sour passion fruits in breeding programs aiming to obtain resistant cultivars has been performed through descriptive scales (Batistti et al., 2013; Viana et al., 2014; Kudo et al., 2012). This type of evaluation is subjective and does not enable fitting the visual acuity when assessing the damaged area (Campbell and Madden, 1990; Santos et al., 2017). Therefore, a tool of reliability is essential for obtain reproducible data, accuracy and precision of the disease quantification. Accuracy is the closeness of quantified values in a sample in relation to the actual values whereas the precision refers to the repetibility of the estimates of values associated with the sample, containing the minimum variation possible between it, the redrodutiblitity is the variability of estimates between raters (Vale et al., 2004).

In general, SADs improve the accuracy and precision of disease severity assessment (Bock et al., 2010). Appropriate SADs must be easy to visualize, provide reliable values, be used in a wide of different conditions, possess representative severity intervals, and allows a quick evaluation (Berger, 1980). Therefore, in their development, the upper and under value of the SAD should be correspondent to the maximum and minimum levels of disease found on plants exposed to the disease under field conditions (Horsfall and Barrat, 1945; Nutter and Schultz, 1995). After the SAD is elaborated, it is mandatory its validation before use to improve accuracy and precision during disease severity assessments (Capucho et al., 2011).

Some SADs were developed to assess diseases on passion fruit including anthracnose on fruit (Fischer et al., 2009); bacterial spots on fruits (Costa et al., 2018), on entire-margined leaves (Costa et al., 2019a) and on leaves (Monzani et al., 2018); scab on fruits (Costa et al., 2019b).

To date, to the best of our knowledge, a SAD has not been developed and validated to assess scab in entire-margined leaves of sour passion fruit. Therefore, two issues were addressed in the present study: i) to develop a SAD as an assessment aid for estimating scab severity on entire-margined leaves of sour passion fruit and ii) to evaluate the effect of the SAD and rater experience on the accuracy, precision, and reliability of the scab estimates.

MATERIAL AND METHODS

Plant growth

Plants of passion fruit from cultivar “UFV M0515” (75-days old) were obtained from the “Departamento de Agronomia” at “Universidade Federal de Viçosa” (UFV). The plants were kept in a greenhouse (temperature of 25 ± 2ºC and relative humidity of 65 ± 5%) before being inoculated with *C. passiflorae*. Plants were fertilized weekly (50 mL per pot) with a nutrient solution containing: KCl (192 mg/L), K_2SO_4 (104.42 mg/L), $MgSO_4.7H_2O$ (150.35 mg/L[1]), urea (61 mg/L), NH_4NO_3 (100 mg/L), $NH_4MO_7O_{24}.4H_2O$ (0.27 mg/L), H_3BO_3 (6.67 mg/L), $ZnSO4.7H_2O$ (1.61 mg/L), $CuSO_4.5H_2O$ (1.74 mg/L), $MnCl_2.4H_2O$ (4.10 mg/L), $FeSO_4.7H_2O$ (4.08 mg/L), and disodium-EDTA (5 mg/L) (Rodrigues et al., 2001). Plants were watered as needed.

Inoculum production and plant inoculation

Entire-margined leaves of sour passion fruit plants cultivar “UFV M0515” exhibiting typical symptoms of scab were collected in the orchard at UFV. The fungus *Cladosporium* sp. was directly isolated from the diseased leaves and grown in Petri dishes containing potato-dextrose-agar (PDA), which were kept in a growth chamber (temperature of 25ºC and 12 h photoperiod) for ten days. The fungal isolate was preserved in silica gel and deposited in the Fungi Collections “Coleção Octávio Almeida” in the Department of Plant Pathology at UFV receiving the identification code of DOA 1263-COAD 2244. The identification of the species of *Cladosporium* was made by sequencing target sequences of the rRNA-ITS region (including internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2), partial actin, and the partial translation elongation factor. The PCR conditions, electrophoresis analysis, sequencing, and obtaining consensus sequences were performed as described by Rosado et al. (2019). Based on a phylogenetic analysis, the isolate of *Cladosporium* was confirmed to belong to *Cladosporium passiflorae*, which is a new specie recently described by Rosado et al. (2019).

The isolate DOA 1263-COAD 2244 of *C. passiflorae* was used to inoculate the plants. This isolate of *C. passiflorae* was grown on Petri dishes containing PDA and kept in a growth chamber (temperature of 25°C and 12h photoperiod). After seven days, plugs of PDA medium containing fungal mycelia were transferred to new Petri dishes containing the same medium. The Petri dishes were incubated in a growth chamber at 25°C under photoperiod of 12h for 12 days. After this period, conidia were carefully removed from the media using a soft bristle brush with water-containing gelatin (0.5% wt/vol). The conidial suspension was calibrated with a hemocytometer to obtain a concentration of 3 × 10^6 conidia mL^{-1}. The conidial suspension was sprayed on the leaves of each plant (25 mL per pot) with an atomizer (Paasche Airbrush Co., Chicago, IL, USA). A total of 50 plants were inoculated with *C. passiflorae*. After inoculation, plants were kept in a mist chamber (temperature of 25 ± 2°C and relative humidity of 90 ± 5%) in the dark during 12 h. A misting system with nozzles (model NEB-100; KGF Company, São Paulo, Brazil) spraying mist every 30 min above the plant canopy kept the relative humidity of 90 ± 5%. Plant canopy received a natural photon flux density of ≈ 900 µmol m^{-2} s^{-1}.

Development of the SAD

For the elaboration of the SAD, a total of 150 diseased entire-margined leaves with different levels of scab severity were collected from the inoculated plants. The leaves were individually digitalized using a scanner at a resolution of 600 dpi. After that, each leaf had the proportion of diseased area measured using the QUANT software (Vale et al., 2003). Considering the minimum and the maximum level of disease severity obtained, eight intermediate levels with linear tendency composed the SAD illustration. A total of ten leaves were chosen to create the SAD image after determining the highest and the lower severity values.

Validation of the SAD

A total of twenty raters (ten inexperienced and ten experienced) were involved in the validation of the SAD. Fifty diseased leaves images processed by QUANT with a range of severity were prepared as PowerPoint slides. First, the raters assessed a set of fifty images without the SAD and assessed with the SAD after that. To assess each diseased leaf with the SAD, the rater compared the leaf image to the SAD and then estimated the severity.

The data obtained from each rater were used to determine accuracy and precision by Lin's concordance correlation coefficient (ρ_c) (Lin, 1989). For this experiment, the Lin's coefficient represents the most suitable analysis because it combines the measures of precision and accuracy beyond being based in the relational fit of the data with the line of concordance (45º): $\rho_c = C_b \times r$ where: C_b is a bias correction factor that measures how far the best fit line is from line of concordance; therefore it is an accuracy measurement, r is the correlation coefficient between estimated severity (Y) and actual severity (X), which is a precision measurement. C_b is the bias correction factor and is derived from $C_b = 2/[(υ + 1/υ + u^2)]$, where $υ = σy / σx$, and σy and σx are the standard deviations of Y and X, respectively; and $u = (μy - μx) / \sqrt{(σy . σx)}$, where μy and μx are the mean values of Y and X, respectively. The term υ measures the SAD difference between actual and estimated values, which is defined by the difference in slope of the two lines. Equal slopes would have a $υ = 1$. The term u is a reflection of a location shift relative to the SAD, primarily reflecting height differences in the lines. Equal heights would have $u = 0$. A perfectly accurate measurement occurs when the points are on the concordance line (*e.g.*, $r = 1$, $C_b = 1$ [$υ = 1$, $u = 0$], and thus, $ρ_c = 1$) (Bock et al., 2010; Nita et al., 2003).

For all parameters analyzed (r, C_b, υ, u, and $ρ_c$) and for the inter-rater reliability, the differences between means (*e.g.*, with SAD minus without SAD) was calculated and an equivalence test used to test their significance (Yi et al., 2008; Bardsley and Ngugi, 2012; Yadav et al., 2013). The equivalence test was used to calculate the 95% confidence intervals (CIs) for each statistic (the difference between the means) by bootstrapping using the percentile method (with an equivalence test, the null hypothesis is the converse of H0; *e.g.*, the null hypothesis is nonequivalence). All analyses were based on 2000 balanced bootstrap samples using PROC SURVEY SELECT/PROC UNIVARIATE (SAS Institute Inc., Cary, NC, USA). The 95% CIs were calculated on the difference between the means of the groups. If the CIs embrace zero, the difference was considered non-significant ($P = 0.05$). Precision was also determined with an analysis of the absolute error (estimated severity minus actual severity). The reliability of the estimates was determined by linear regression analysis of the inter-rater estimates for each leaf and using the R^2 of each pair of the rater's estimates to judge reliability (Nutter and Schultz, 1995). Regression analysis was performed with MiniTab V17 (Minitab Inc., Pennsylvania, United States).

RESULTS

The SAD developed in the present study has ten images of scab-diseased entire-margined leaves being each one comprising a distinct severity values that ranged from 0.4 to 59.8% (Fig. 1). Lin's concordance analysis revealed that the raters had estimates of scab that approached the actual severity when they used the SAD irrespective of their experience (Figs. 2 and 3). There was a linear relationship between estimated and actual scab for all raters. The assessment with SAD aid resulted in a significant improvement for all bias and accuracy component statistics of Lin's concordance correlation coefficient (r, C_b, υ, u, and ρ_c) for the inexperienced raters and r, C_b, and ρ_c for the experienced ones (Table 1 and Fig. 4).

The use of the SAD significantly improved the scale shift (υ) only for the inexperienced raters (Table 1). The υ ranged from 0.22 to 1.54 (mean of 1.12) and from 0.87 to 1.10 (mean of 1.01) without and with the use of the SAD, respectively. The frequency of the raters who had υ values between 0.90 and 1.20 increased from 30 to 90% with the use of the SAD indicating that the slope of the best fitting line was closer to the concordance line when the SAD was used (Fig. 4a).

The location shift (u) significantly improved by using the SAD for the inexperienced raters (Table 1 and Fig. 4). The scab estimates made without the SAD resulted in u values that were positive for most inexperienced raters ranging from -1.01 to 0.90 (mean of 0.33) indicating, therefore, an overestimate of scab severity. In contrast, the u values ranged from -0.10 to 0.29 (mean of 0.10) when the SAD was used. For 30 and 100% of the raters that did not use and used the SAD, respectively, the u values were between -0.10 and 0.35 indicating that the best fitting line was closer to the concordance line by using SAD (Fig. 4b).

The agreement (ρ_c) was significantly improved for all raters regardless of their experience with the aid of the SAD (Table 1 and Fig. 2). In the absence of the SAD, ρ_c ranged from 0.35 to 0.97 (mean of 0.75) for the inexperienced raters and from 0.91 to 0.99 (mean of 0.96) for the experienced ones. In contrast, the inexperienced raters had a ρ_c range from 0.96 to 0.99 (mean of 0.98), and the experienced ones had a range from 0.97 to 0.99 (mean of 0.98) when the SAD was used. These improvements were further confirmed by the increase in the frequency of raters who had ρ_c values above 0.90 (20 and 100% without and with the SAD aid, respectively) (Fig. 4c).

Without the use of the SAD, the precision (r) ranged from 0.83 to 0.93 (mean of 0.89) and from 0.91 to 0.97 (mean of 0.95) for the inexperienced and experienced raters, respectively (Table 1). The use of the SAD increased these ranges from 0.95 to 1.00 (mean of 0.97) and from 0.95 to 0.99 (0.97) for inexperienced and experienced raters, respectively. The increase in precision was also confirmed by the reduction in absolute errors when the SAD was used, which was particularly evident for the inexperienced raters (Figs. 3). The frequencies of the raters who had r values above 0.95 were 50 and 100% without and with the SAD aid, respectively, indicating an increase in precision with the use of the SAD (Fig. 4d).

The parameter C_b was also significantly improved by using the SAD for both inexperienced and experienced raters (Table 1, Fig 4). The C_b values without the SAD ranged from 0.35 to 0.97 (mean of 0.75) and from 0.91 to 1.00 (mean of 0.96) for inexperienced and experienced raters, respectively. In contrast, the inexperienced and experienced raters had C_b values that ranged from 0.96 to 1.00 (mean of 0.98) and from 0.97 to 1.00 (mean of 0.98), respectively, when the SAD was used. The use of the SAD also increased the frequency of the raters who had C_b values above 0.90 from 50 to 100% (Fig. 4e).

Without the SAD, the experienced raters had values that were significantly better for r, C_b, and ρ_c compared to the inexperienced raters (Table 2). However, when the SAD was used, none of the parameters estimated were significantly higher for the experienced raters than for the inexperienced ones. Although the estimates of severity had improved for the raters regardless of their experience, the biggest gains in agreement, bias, and precision were obtained for the inexperienced raters (Fig. 5).

In addition to accuracy and precision, the inter-rater reliability of the estimates was used as another indicator of the value of the SAD in assessing scab severity. The equivalence test revealed that the coefficient of determination (R^2) was significantly improved when the raters used the SAD (Table 1). The mean of R^2 by using the SAD increased from 0.70 to 0.88 for the inexperienced raters and from 0.84 to 0.91 for the experienced ones. In the absence of the SAD, 82,5% of the pairwise comparisons had an R^2 lower than 0.85, but when the SAD was employed, the R^2 was lower than 0.85 only for 17.5% of the pairwise comparisons (Fig. 4f).

DISCUSSION

The evaluation of diseases severities is of essential value in any study involving plant disease control and epidemiology. The SADs are useful tools to assess plant disease severity because they aid raters to estimate the severity with high precision and accuracy. Several studies have demonstrated the use of SADs increases accuracy, precision, and reliability in the assessments including blast (Rios et al., 2013) and spot blotch (Domiciano et al., 2014) on wheat, Glomerella leaf spot on apple (Moreira et al., 2019), target spot on cotton (Fantin et al., 2018), frogeye leaf spot (Debona et al., 2015), and rust on soybean (Godoy et al., 2006), rust on coffee (Capucho et al., 2011), leaf blight on eggplant (Correia et al., 2017), and bacterial blight on passion fruit (Monzani et al., 2018).

Although the scab causes the death of passion fruit plants in the nursery besides bloom delay and yield losses as well as decreasing fruit quality (Torres, 1983; Joy and Sherin, 2012), there are no appropriate methods to assay the severity of this disease on entire-margined leaves with great reliability. In the present study, the SAD was developed only for entire-margined leaves because of the occurrence of this disease mainly on seedlings in the nurseries. The SAD developed in the present study to evaluate scab severity on the entire-margined leaves of passion fruit plants demonstrated large accuracy, precision, and reproducibility of the disease estimative considering that both real and estimated severities became closer to each other.

The SAD developed in the present study has ten color images of entire-margined leaves with severity levels ranging from 0.4 to 58.9%. These severity levels were sufficient to include the range of severities observed on the leaves of plants grown under controlled conditions. Moreover, severity below 0.4% is very difficult to detect on the leaves, whereas severity above 58.9% may cause plant defoliation. Therefore, the ten levels proposed for the SAD set to evaluate scab severity on the leaves of passion fruit plants are suitable and sufficient for its assessment.

The severity levels for scab obtained in the present study have a linear tendency and agree with the findings of other researchers (Rios et al., 2013; Sachet et al., 2017; Gonzaléz-Domíngues et al., 2014; Duan et al., 2015; Debona et al., 2015), whereas some other SADs have a logarithmic tendency (Horsfall and Barrat 1945; Godoy et al., 2006; Lenz et al., 2010). This tendency obeys the Weber-Fechner law, which uses a logarithmic arrangement between real and estimates severity. However, this law is not substantiated and studies have questioned its use (Nita et al., 2003; Nutter and Esker, 2006; Bock et al., 2010).

Overestimation (positive values of *u*) of severity by raters is well-reported in the literature (Rios et al., 2013; Costa Lage et al., 2015; Andrade et al., 2019; Santos and Spósito, 2018). Despite some studies, underestimation (negative values of *u*) is also documented (Gomes et al., 2004; Michereff et al., 2000). In the present study, the severity of scab was overestimated by inexperienced raters regardless of the use of SAD. However, the experienced raters tended to underestimate scab severity when they did not use SAD. In general, the severity is overestimated by raters when the range is between 0 and 10%, and leaves exhibit small lesions (Del Ponte et al., 2017). Disease distribution, leaves shape, and lesion size might influence the tendency to over or underestimate plant diseases severities (Bock et al., 2011; Sherwood et al., 1983; Spolti et al., 2011).

The equivalence test is a useful tool for agreement studies (Yi et al., 2008). In the present study, the equivalence test was used to determine the precision, accuracy, and reliability of the SAD set to evaluate scab severity on the entire-margined leaves of passion fruit plants. In other studies, the equivalence test has been used in the statistical analysis of the SADs, and it affords a statistical test to judge improved reliability and agreements (Bardsley and Ngugi, 2013; Yadav et al., 2012; Duarte et al., 2013). Based on 95% confidence intervals (CIs) by bootstrapping of means difference, the equivalence test showed that all five parameters and the determination coefficient (R^2) were significantly improved with the use of the SAD by inexperienced raters. In contrast, for experienced raters, the precision (r), accuracy (C_b), determination coefficient (R^2) and agreement (ρ_c) were improved by using SAD.

The inter-rater reliability, determined by the determination coefficient (R^2), was used as another indicator of the relevance of the SAD use. The estimates of scab by using SAD resulted in less variability between severity estimates for all pairs of raters. Thus, the determination coefficient was improved for all raters when the SAD was used. In other studies, the use of the SAD increased the reliability of estimates (Bock et al., 2016; Debona et al., 2015; Monzani et al., 2018; Dolinski et al., 2017).

Especially the inexperienced raters obtained absolute errors of the estimates quite reduced by using SAD. When using SAD, most of the raters received values of scale shift (υ) and location shift (u) closer to 1 and 0, respectively, indicating that the fitted line and the line of concordance were closer. Regardless of the rater experience, other parameters used to obtain r, C_b, and ρ_c were improved by using SAD showing that, in general, the estimates of scab severity on the entire-margined leaves of passion fruit plants were more accurate and precise when the raters used the SAD. These results suggest that the SAD proposed in the present study was essential to improve the assessments of the raters during scab severity estimates even for the experienced raters.

Several studies have demonstrated that rater experience, training in disease assessments and familiarity with disease symptoms influence the accuracy and reliability of the disease estimates (Bardsley and Ngugi, 2013). In general, inexperienced raters produced estimates less precise and accurate than experienced raters as reported by several researchers (Debona et al., 2015; Yadav et al., 2013; González-Domínguez et al., 2014). Inexperienced raters tend to deviate more from the actual severity than experienced raters, though an inexperienced rater may inherently have a high degree of precision and accuracy (Yadav et al., 2013). Thereby, the use of SADs overall benefits more not experienced raters, which also improves reliability between raters (Yadav et al., 2013).

In conclusion, the SAD developed in the present study increased the precision, accuracy and reliability in the assessment of scab severity on the entire-margined leaves of passion fruit plants. It is expected that this SAD could be used to compare the efficiency of chemicals and inducers of host resistance for scab management, in the screening of genotypes with high level of basal resistance in passion fruit breeding programs, to perform epidemiological studies for this disease as well as to monitor scab development in decision making of the more appropriated method for a successful disease control.

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TABLES AND FIGURES

Table 1. Effect of the use of a standard area diagram set (SAD) as an assessment aid on the bias, precision, and overall agreement of estimates of scab in entire-margined leaves of sour passion fruit made by raters with or without experience in assessment.

Experiences	LCC statistic	Means		95% CI[a] of the difference between means
		No SADS	With SADS	
Inexperienced	Scale shift (v)[b]	1.12	1.01	**0.322 - 0.143**
	Location shift (u)[c]	0.33	0.10	**0.550 – 0.156**
	Bias correction factor (C_b)[d]	0.75	0.98	**0.060 – 0.101**
	Correlation coefficient (r)[e]	0.89	0.97	**0.415 - 0.348**
	Concordance coefficient (ρ_c)[f]	0.66	0.95	**0.211 - 0.385**
	Determination coefficient (R^2)	0.70	0.88	**0.149 - 0.204**
Experienced	Scale shift (v)	1.02	1.05	-0.244 – 0.105
	Location shift (u)	-0.02	0.01	-0.189 – 0.139
	Bias correction factor (C_b)	0.96	0.98	**0.008 – 0.04**
	Correlation coefficient (r)	0.95	0.97	**0.011 – 0.032**
	Concordance coefficient (ρ_c)	0.91	0.95	**0.028 – 0.062**
	Determination coefficient (R^2)	0.84	0.91	**0.053– 0.077**

[a] Bootstrap calculated difference between means and confidence intervals (CIs). If the CIs embrace zero, difference is **not significant** at the 5% level. Bold numbers represent significance of the difference.
[b] Scale or slope shift relative to the perfect relationship (1 = perfect relation between x and y).
[c] Location or height shift relative to the perfect relationship (0 = perfect relation between x and y)
[d] Bias correction factor that measures how far the best-fit line deviates from a line at 45°. No deviation from the 45° line occurs when $C_b = 1$. C_b is calculated from v and u and is a measure of accuracy.
[e] Correlation coefficient (r) that measures precision.
[f] Lin's concordance correlation coefficient (ρ_c) combines both precision (r) and accuracy (C_b) ($\rho_c = r.C_b$) to measure agreement with the true value (Lin, 1989).

Table 2. Effect of rater experience on the bias, precision and overall agreement of estimates of scab severity made by ten raters either unaided or aided by a standard area diagram set (SAD).

Assessments	LCC statistic	Means		95% CI[a] of the difference between means
		Inexperienced	Experienced	
No SADS	Scale shift (υ)[b]	1.12	1.02	-0.32 – 0.147
	Location shift (u)[c]	0.33	-0.02	-0.721 – 0.076
	Bias correction factor (C_b)[d]	0.75	0.96	**0.117 – 0.317**
	Correlation coefficient (r)[e]	0.89	0.95	**0.036 – 0.086**
	Concordance coefficient (ρ_c)[f]	0.66	0.91	**0.162 – 0.35**
	Determination coefficient (R^2)	0.70	0.84	**0.109 – 0.171**
With SADS	Scale shift (υ)[b]	1.01	1.06	-0.025 – 0.078
	Location shift (u)[c]	0.10	0.18	-0.045 – 0.145
	Bias correction factor (C_b)[d]	0.98	0.98	-0.014 – 0.012
	Correlation coefficient (r)[e]	0.97	0.97	-0.009 – 0.016
	Concordance coefficient (ρ_c)[f]	0.95	0.95	-0.021 – 0.026
	Determination coefficient (R^2)	0.88	0.91	**0.016 – 0.038**

[a] Bootstrap calculated difference between means and confidence intervals (CIs). If the CIs embrace zero, difference is not significant at the 5% level. Bold numbers represent significance of the difference.
[b] Scale or slope shift relative to the perfect relationship (1 = perfect relation between x and y).
[c] Location or height shift relative to the perfect relationship (0 = perfect relation between x and y).
[d] Bias correction factor that measures how far the best-fit line deviates from a line at 45°. No deviation from the 45° line occurs when $C_b = 1$. C_b is calculated from υ and u and is a measure of accuracy.
[e] Correlation coefficient (r) that measures precision.
[f] Lin's concordance correlation coefficient (ρ_c) combines both precision (r) and accuracy (C_b) ($\rho_c = r.C_b$) to measure agreement with the true value (Lin, 1989).

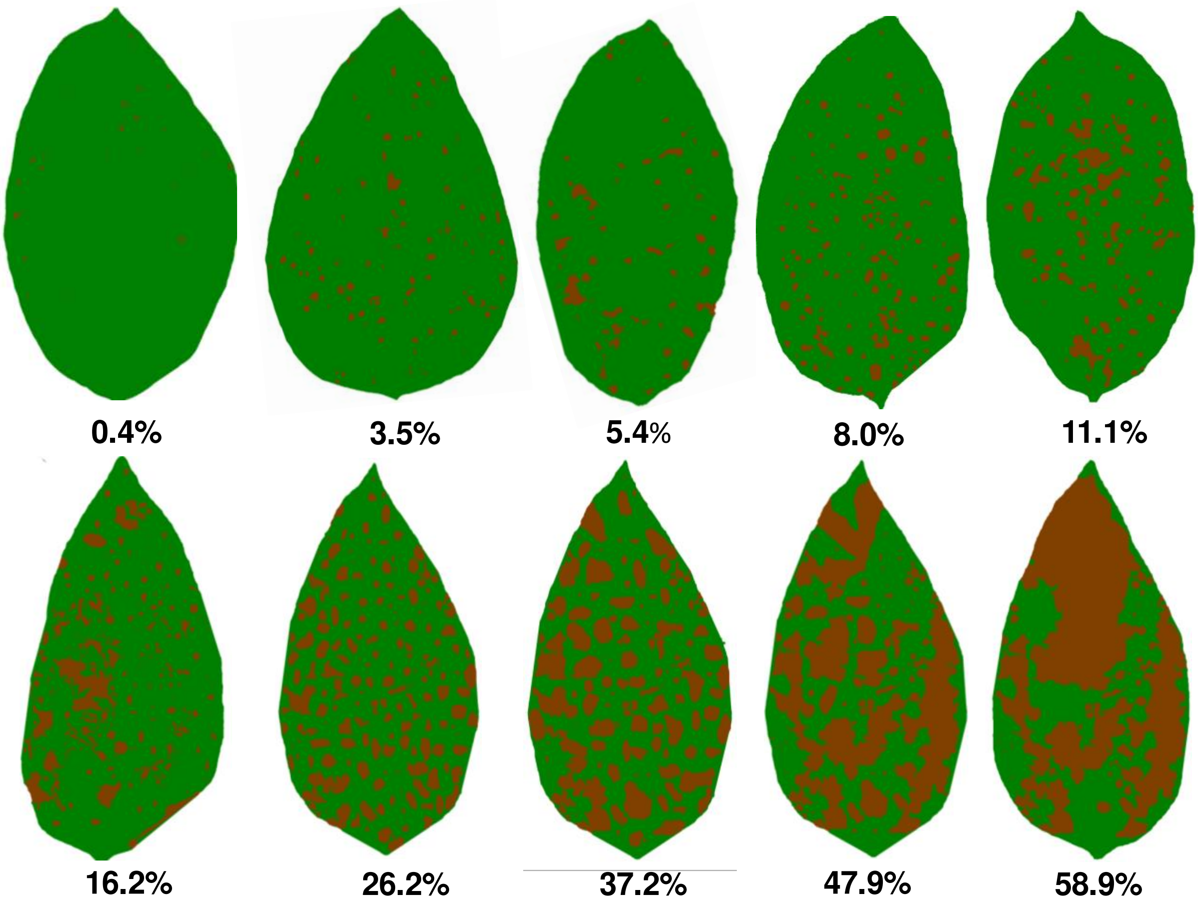

Figure 1. Standard area diagram set (SAD) developed as an aid for scab assessment on the entire-margined leaves of passion fruit plants. The values shown represent the percentage (%) of the leaf area with scab symptoms.

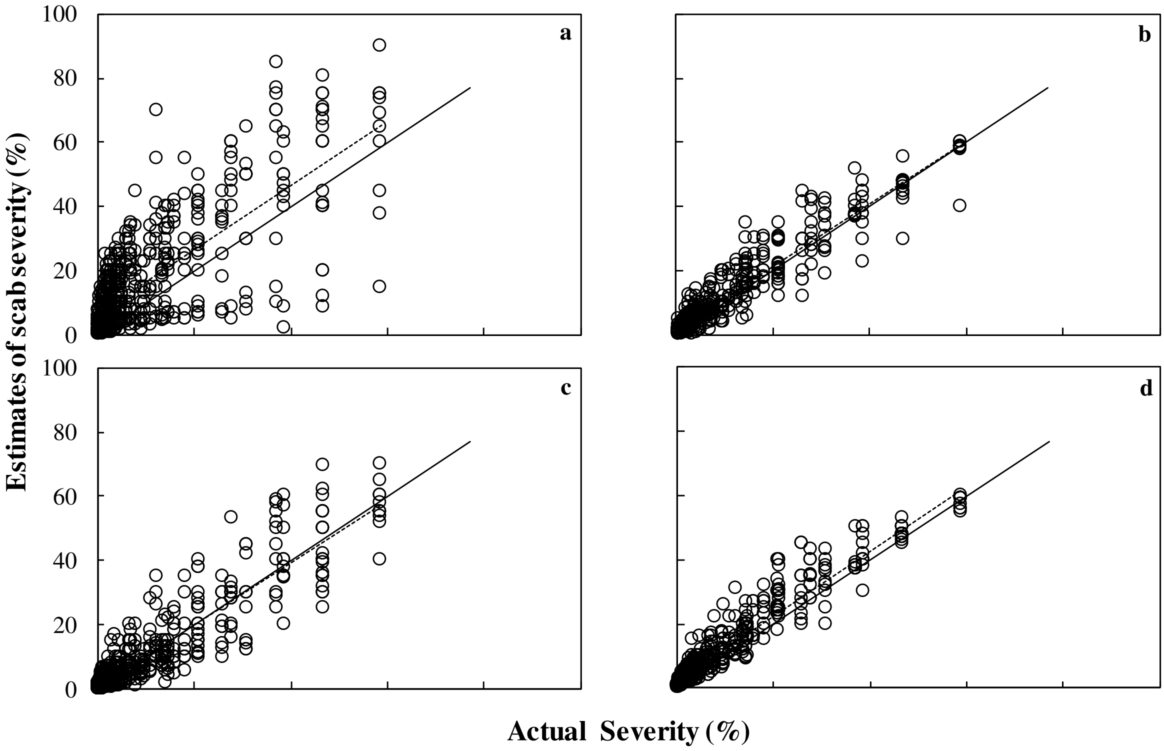

Figure 2. Relationship between the actual and the estimated scab severity assessed on 50 diseased entire-margined leaves of passion fruit plants by ten inexperienced (a and b) and ten experienced (c and d) raters without (a and c) and with (b and d) the use of a standard area diagram set (SAD). In each chart, the dotted line represents the best-fit linear regression line, whereas the solid line is the concordance line that represents a perfect agreement between actual and estimated severity (slope of 1 and intercept of 0). The agreement was determined according to Lin's concordance correlation coefficient (ρ_c) calculated as the product of the correlation coefficient (r) and the bias correction factor (C_b). C_b is a product of location shift (u) and scale shift (v), indicating changes in line-height and slope, respectively.

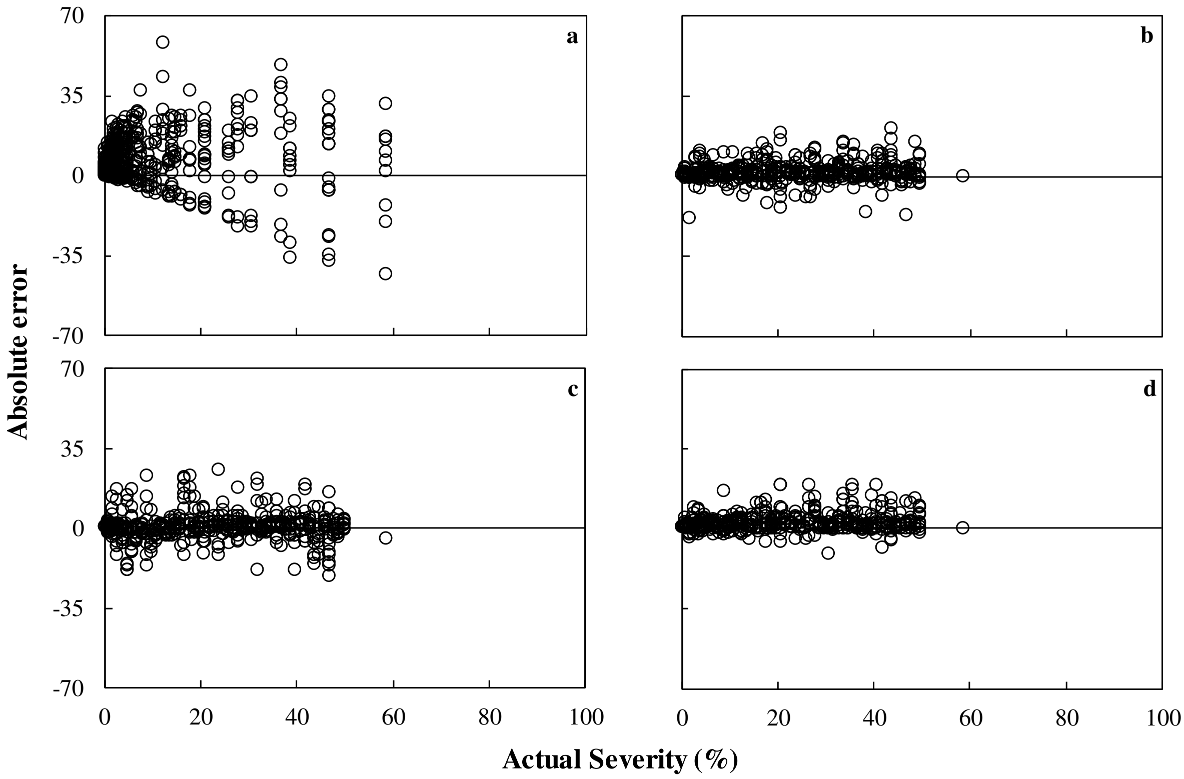

Figure 3. Absolute error (estimated severity minus actual severity) of estimates of scab severity on 50 diseased entire-margined leaves of passion fruit plants for each of ten inexperienced (a and b) and ten experienced (c and d) raters without (a and c) and with (b and d) the use of a standard area diagram set (SAD). Low absolute errors indicate that the estimated severity was similar to the actual severity.

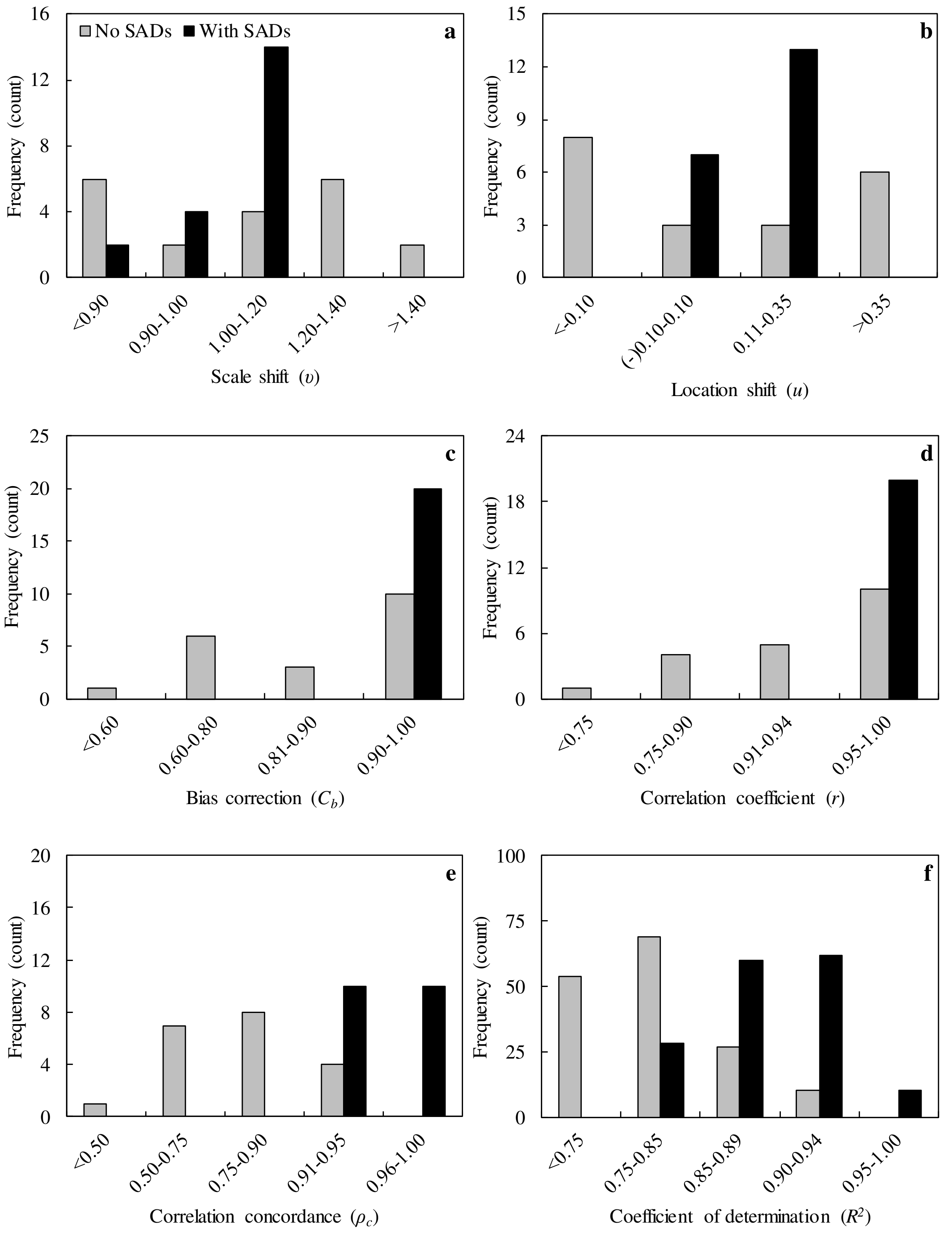

Figure 4. Frequency of bias, precision, and agreement, and inter-reliability without and with the use of a standard area diagram set (SAD) assessment aid by 20 raters who assessed 50 images of diseased entire-margined leaves of passion fruit plants. (a) scale shift (υ), (b) location shift (u), (c) bias correction factor (C_b), (d) correlation coefficient (r), (e) Lin's concordance correlation coefficient (ρ_c) and (f) coefficient of determination (R^2).

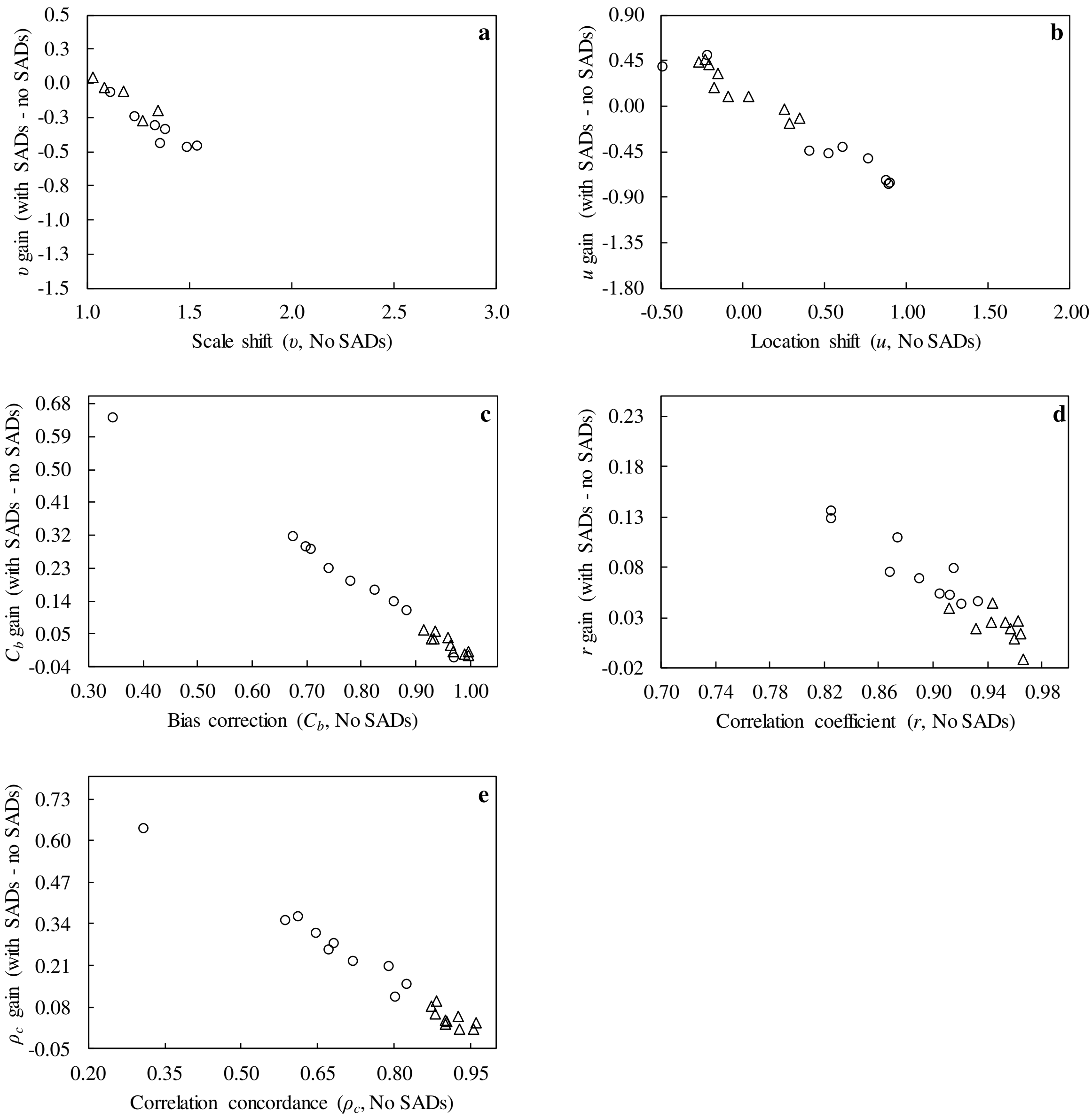

Figure 5. Relationship between gain (difference between the estimate with and without using a standard area diagram set (SADS)) for the determination of (a) scale shift (υ), (b) location shift (u), (c) bias correction factor (C_b), (d) correlation coefficient (r), and (e) Lin's concordance correlation coefficient (ρ_c) of visual severity estimates. The estimates were generated by 10 inexperienced (*circles*) and 10 experienced (*triangles*) raters using a set of 50 images of diseased entire-margined leaves of passion fruit plants.