A standard area diagram set for severity assessment of botrytis leaf blight of onion

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A standard area diagram set for severity assessment of botrytis leaf blight of onion

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Abstract This work aimed to develop and validate a standard area diagram set (SADs) to estimate the severity of botrytis leaf blight of onion caused by *Botrytis squamosa*. For this purpose, a SADs with nine levels of severity (0.3; 1.0; 2.5; 5.0; 10; 20; 30; 40; and 57.7%) was developed from 193 symptomatic leaves collected in the field. The SADs was validated by 16 raters with no experience in evaluating plant diseases. Both precision and accuracy improved when they used the SADs. The statistical parameters for the 16 raters were: coefficient of bias - C_b (no SADs = 0.592, with SADs = 0.988); correlation coefficient – r (no SADs = 0.864, with SADs = 0.921); and Lin's concordance correlation coefficient – ρc (no SADs = 0.513, with SADs = 0.909). In addition, estimates were more reliable: coefficient of determination - R^2 (no SADs = 0.681, with SAD = 0.796); intra-class correlation coefficient - ρ (no SADs = 0.729, with SADs = 0.876). The SADs proposed here is a useful tool for improving visual assessments of severity of botrytis leaf blight of onion.

Keywords *Allium cepa* · *Botrytis squamosa* · Disease assessment · Phytopathometry

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Onion (*Allium cepa* L.) is the most economically important *Allium* species in Brazil and worldwide. Among the vegetables produced in Brazil, onion ranks third in production volume, with approximately 1.7 million tons per year (IBGE 2017). Botrytis leaf blight of onion, caused by *Botrytis squamosa* J.C. Walker, is a widespread foliar disease, and it has been reported in almost all continents (Carisse et al. 2011). However, the disease is more severe in countries with temperate climate (Lorbeer et al. 2007). In Southern region of Brazil, which has a subtropical climate and is responsible for 47% of Brazilian production, botrytis leaf blight can cause serious damage, especially during seedling growth (April to July).

According to Carisse et al. (2011), botrytis leaf blight develops in two stages: a leaf spotting phase followed by a leaf blighting one. During the first stage, the leaf lesions are whitish, with 1 to 5 mm in length. Sometimes, a slit oriented lengthwise along the lesion can be observed. In the second phase, leaf blight, which starts at the leaves tip, occurs within 5 to 12 days after initial lesion development under optimum conditions. *Botrytis cinerea* can also cause onion leaf flecks, when artificially inoculated under controlled environmental conditions, however, only *B. squamosa* can develop the leaf blighting phase (Lorbeer et al. 2007).

The use of standard area diagram set (SADs) is a way of visually estimating, not measuring, plant disease severity (Bock et al. 2010). Besides, evaluations using SADs are fast, do not require equipment, and often improve the precision and accuracy of the raters. Despite the relevance of botrytis leaf blight of onion, there is no SADs published to assess the disease.

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The aim of this study was to develop and validate a SADs to estimate the severity of botrytis leaf blight of onion, providing a useful tool for the evaluation of the disease.

One hundred ninety-three leaves of onion with symptoms of botrytis leaf blight were sampled at the experimental area of Epagri, Ituporanga, State of Santa Catarina, Brazil, during the 2017/18 season. The symptomatic leaves were randomly collected from trials of cultivars resistance and/or fungicide efficiency, which allowed the manifestation of the disease in different levels of severity. The disease is endemic in the region, which favours the natural occurrence of the pathogen (Araújo et al. 2018).

The symptomatic leaves were individually digitized at a resolution of 300 dpi, using a scanner (RICOH®, Aficio MP 201SPF). For each leaf, the proportion of diseased area was determined using the QUANT software (Vale et al. 2003). The SAD illustrations were chosen from these samples with adjustments made by the PhotoImpression® (ArcSoft) image editing program, establishing a set of nine disease severity levels, in a linear distribution (Nutter Jr. and Esker 2006; Bock et al. 2010). Whitish spots and leaf necrosis were quantified as diseased area. Foliar chlorosis was not considered.

The SADs was validated by 16 raters with no previous experience in plant disease severity assessments. Fifty images of diseased leaves were displayed as a slide show in PowerPoint to the raters. Each image was displayed for one minute and the raters were then asked to write down the estimated percentage of diseased area on a form. For the second assessment, they evaluated the same 50 images again in a different sequence, but with the aid of the SADs. The accuracy, precision and inter-rater reliability of the estimates with and without the SADs were calculated as previously described (Dolinski et al. 2017), using the R software (R Core Team 2017).

Nine illustrations, covering the minimum (0.3%) and the maximum (57.7%) of botrytis leaf blight severity observed in the field, comprised the SADs (Fig. 1).

All statistical parameters (υ, u, C_b, r, and ρc) of Lin's concordance correlation (LCCC) were significantly improved when the raters used the SADs to estimate disease severity, demonstrating that both the accuracy and precision of the estimated values were improved (Table 1). Using the SADs, the mean of LCCC (ρc), for the 16 raters, improved significantly (0.513 without SADs, and 0.909 with SADs). Based on estimated and actual severity, assessments made by the raters were closer to the actual values using the SADs (Fig. 2a-b). The absolute error of the estimates reduced significantly when the raters used the SADs (Fig. 2c-d).

Fig. 1 Standard area diagram set to assess severity of botrytis leaf blight of onion (*Botrytis squamosa*). The numbers below each picture represent the actual percentage (%) of leaf area showing symptoms (whitish spots and leaf necrosis). Foliar chlorosis was not considered

Inter-rater reliability of assessments by 16 raters was significantly improved. Without the SADs, the intra-class correlation coefficient mean (ρ) was 0.729 (confidence intervals = 0.629–0.817), while using the SADs, this value was 0.876 (confidence intervals = 0.827–0.918). In turn, the mean of inter-rater coefficient of determination (R^2) of the pairwise comparisons was 0.681 (minimum = 0.354, maximum = 0.881) and 0.796 (minimum = 0.562, maximum = 0.932) without and with SADs, respectively. The 95% confidence interval (CI) of this mean was 0.093 to 0.135. As the CI

Table 1 Effect of using a standard area diagram set (SADs) as an assessment aid on the bias, accuracy, precision and agreement of severity assessments of botrytis leaf blight of onion (*Botrytis squamosa*), on 50 leaves as estimated by16 raters

Variables	Means[a]		Difference between means[b]	95% CIs of the difference[c]
	No SADs	With SADs		
Scale (υ)[d]	1.779 (0.302)	1.046 (0.081)	−0.732 (0.075)	**−0.879 - 0.583**
Location (u)[e]	1.036 (0.445)	0.038 (0.125)	−0.998 (0.112)	**−1.216 - 0.793**
Coefficient of bias (C_b)[f]	0.592 (0.140)	0.988 (0.010)	0.395 (0.034)	**0.328–0.463**
Correlation coefficient (r)[g]	0.864 (0.045)	0.921 (0.026)	0.056 (0.012)	**0.031–0.081**
LCCC (ρ_c)[h]	0.513 (0.127)	0.909 (0.029)	0.396 (0.001)	**0.335–0.459**

[a] The values for standard deviation are in parentheses

[b] Mean of the difference between each rating. The values for standard errors are in parentheses (bootstrap calculated values)

[c] 10,000 bootstrap samples were used to obtain the confidence intervals (CIs). If the CIs embrace zero, the difference was not significant ($\alpha = 0.05$). Bold numbers represent significance of the difference

[d] Scale bias or slope shift (υ, 1 = no bias relative to the concordance line)

[e] Location bias or height shift (u, 0 = no bias relative to the concordance line)

[f] The correction factor (C_b) measures how far the best-fit line deviates from 45^0 and is a way to measure accuracy

[g] The precision is measured by the correlation coefficient (r)

[h] Lin's concordance correlation coefficient (LCCC) combines both measures of precision (r) and accuracy (C_b) to measure agreement with the true value

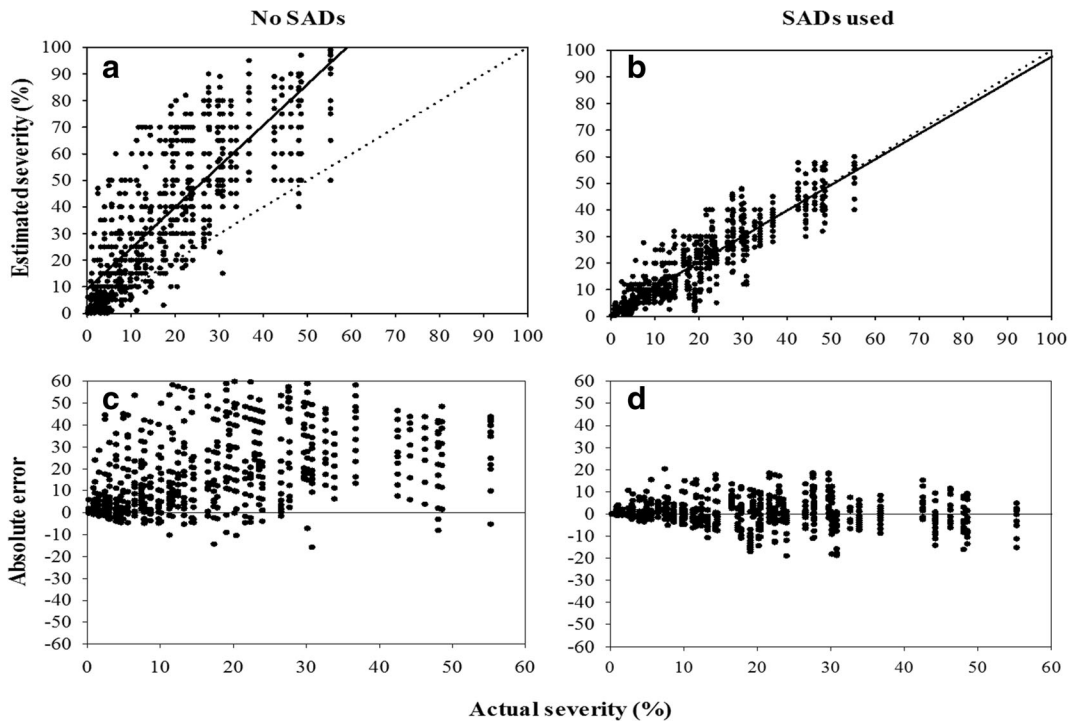

Fig. 2 Relationship between actual and estimated severity of botrytis leaf blight of onion (*Botrytis squamosa*) without (**a**) and with (**b**) the use of a standard area diagram set (SADs) for 50 diseased leaves by 16 raters. The solid line represents the best-fitting line, whereas the dotted line is the concordance line, which represents perfect agreement between actual and estimated severity (slope of 1, intercept of 0). Absolute error (estimated severity minus actual severity) of the estimates without SADs (**c**) and with SADs (**d**) for the 50 diseased leaves. Low absolute errors indicate that the estimated severity was more accurate

did not embrace zero, the difference was significant ($\alpha = 0.05$).

The SADs developed in this study improved accuracy, precision, and reliability of the estimations of botrytis leaf blight severity. The onion leaf has a cylindrical shape (3D surface) and the SADs shows illustrations on 2D surface. Although the symptom of leaf blight is uniform on both sides, the whitish spots may have different arrangements and/or quantities on the different sides of the leaf. Therefore, to estimate the average severity of the leaf, we recommend that the evaluations be performed in the abaxial and adaxial part of the leaf. As well as the SADs developed to estimate the severity of sooty blotch and flyspeck on apple fruit (Spolti et al. 2011), the present SADs proposes a simplified form of evaluation, allowing improvement in the severity estimates, without the need of complex methodologies for evaluating 3D surfaces.

Despite the availability of software with high accuracy and precision in the quantification of severity, such as Quant (Vale et al. 2003), APS Assess 2.0: Image Analysis Software for Plant Disease Quantification, and Leaf Doctor (Pethybridge and Nelson 2015), standard area diagram sets are still a powerful tool to improve the accuracy and precision of experienced or inexperienced raters (Lage et al. 2015; Nuñez et al. 2017). In addition, a SADs does not require specific conditions of image acquisition, nor people with specific training, and it is practical for assessments of large amounts of samples.

According to Del Ponte et al. (2017), there is no SADs for any onion disease. Therefore, this work developed and validated the first SADs for this vegetable. The SADs for botrytis leaf blight of onion may assist in more accurate, precise and reliable estimates of disease severity in epidemiological, management and resistance studies.

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Compliance with ethical standards

Ethical statement The authors ensure that:
- This manuscript has not been submitted to more than one journal for simultaneous consideration.
- This manuscript has not been published previously.
- This work is not split up into several parts to increase the quantity of submissions.
- No data have been fabricated or manipulated to support our conclusions.
- No data, text, or theories by others are presented as if they were the author's own.
- Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities at the institute/organization where the work has been carried out.
- Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.
- Authors are strongly advised to ensure the correct author group, corresponding author, and order of authors at submission.
- There is no conflict of interest in the accomplishment and submission of this work.

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