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Development and Validation of a Standard Area Diagram Set for Assessment of Target Leaf Spot Severity on *Handroanthus serratifolius*

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ABSTRACT

Target leaf spot, caused by *Corynespora cassiicola*, is a common disease on *Handroanthus serratifolius* (yellow ipe) in Brazil. This study aimed to develop and validate a standard area diagram (SAD) set to estimate the severity of target spot on *H. serratifolius* leaves. For this purpose, a SAD set with 10 levels of severity (1%; 3%; 5%; 10%; 20%; 30%; 40%; 55%; 70% and 85%) was developed. The SAD set was validated by 20 raters with no experience in evaluating plant diseases. Both accuracy and precision improved when the proposed SAD set was employed. The statistical parameters evaluated were bias coefficient factor - C_b (no SAD set = 0.898, with SAD set = 0.994); correlation coefficient - r (no SAD set = 0.926, with SAD set = 0.979); and Lin's concordance correlation coefficient - ρ_c (no SAD set = 0.835, with SAD set = 0.974). In addition, estimates were more reliable as showed by the intra-class correlation coefficient - ρ (no SAD set = 0.792, with SAD set = 0.962). The SAD set proposed here is a useful tool for improving visual assessments of target spot severity on *H. serratifolius* leaves.

Ipes are tree or shrub species belonging to the Bignoniaceae family, with three genera recognised by molecular phylogeny: *Handroanthus* Mattos, *Roseodendron* Miranda, and *Tabebuia* Gomes ex DC. (Moraes Neto 2021). Among the species, *H. serratifolius* (Vahl) S. Grose is native to Brazil and has both ecological and economic importance (Lorenzi 2020). Its honey-producing flowers provide food for insects and birds. The species is widely used in landscaping due to its striking yellow inflorescences and in timber production for both conventional carpentry and civil construction (Ferreira et al. 2004). Additionally, it contains compounds of medicinal interest, with bactericidal, antiviral, antifungal, diuretic, and astringent properties, as well as being used in the phytoremediation of environments contaminated with anthropogenic wastes (Moraes Neto 2021).

Despite its hardiness, *H. serratifolius* is affected by several diseases recorded in urban, natural, and nursery conditions (Auer 2001; Ferreira 1989). Among these, target leaf spot, caused by *Corynespora cassiicola*, is highly prevalent in Brazil (Auer 2001; Ferreira 1989). Symptoms are brown to black leaf spots on the adaxial surface of the leaves, often with whitish central areas (Figure S1). The tonality of the spot and the

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central areas are much weaker on the abaxial surfaces of the leaves. The lesions vary in size, with diameters between 1 and 20 mm. Generally, several spots are formed per leaf, and they can coalesce and damage a large part of the blade. Under heavy disease pressure on seedlings, a high level of defoliation of the plants can occur. This reduces the plant's photosynthetic capacity, negatively impacting its growth and vigour (Ferreira 1989).

The growing conditions of *H. serratifolius* in nurseries, including high seedling density and frequent irrigation, favour pathogen dissemination and disease development (Ferreira 1989). Integrated management of target leaf spot in nurseries includes measures such as adequate spacing of plants, regular monitoring and removal of affected leaves, and spraying with fungicides (Auer 2001; Ferreira 1989).

Assessment of disease intensity in plants is necessary for many purposes, including monitoring epidemics, comparing phenotypes for disease resistance, and assessing the effects of treatments (chemical, biological, agronomic, or environmental factors) on disease. In all of these applications, visual disease estimates are used to draw conclusions and/or take action and, therefore, must be as accurate as possible (Bock et al. 2022).

For a leaf spot disease such as target spot of *H. serratifolius*, disease intensity is best expressed by determining severity (Kranz 1988), which can be defined as the percentage or proportion of the area or volume of plant tissue covered by disease symptoms (Madden et al. 2007). Visual estimates are the most commonly used methods for assessing plant disease severity. Still, they are subject to subjectivity and can cause serious errors in accuracy, precision, and reliability (Bock et al. 2022). Standard area diagram (SAD) sets have been valuable tools for minimising subjectivity and errors in disease severity estimates (Bock et al. 2022; Del Ponte et al. 2022). There are 187 validated SADs for quantifying the severity of diseases caused by 136 pathogens in 72 host plant species (Del Ponte and Cazón 2025). There are SAD sets for target spot caused by *C. cassiicola* on other host species, including Barbados cherry (Celoto and Papa 2010), cotton (Fantin et al. 2018), cucumber (Teramoto et al. 2011), roselle (Ortega-Acosta et al. 2016), and soybean (Soares et al. 2009), but none for target leaf spot of *H. serratifolius*. Developing a SAD set to assess target spot severity on *H. serratifolius* leaves is essential for monitoring disease progression over time, evaluating its impact under different environmental or seasonal conditions, determining the effectiveness of control treatments (fungicides or biologicals), and assessing the resistance of host genotypes—all within the nursery context. This study aimed to develop and validate a SAD set to improve and standardise the assessment of target spot severity on *H. serratifolius* leaves and analyse the accuracy, precision, and reliability of the estimates with its use.

To develop the SAD set, 200 leaves of *H. serratifolius* with different levels of target spot severity were collected in a forest nursery in São Lourenço da Mata (State of Pernambuco, Brazil; 8°00′07″ S and 35°01′04″ W). The leaves were scanned using a Canon multifunction printer (Model L3250) at 300 dpi, and the actual severity of the disease in each image was determined using ASSESS v. 2.0 (Lamari 2008). The SAD set illustration was produced in colour from a standard *H. serratifolius* leaf with the aid of graphical software (Krita v. 5.2.3), according to the symptoms of the disease, aiming to represent the form, frequency, and standard of distribution of disease lesions. The severity intervals followed the linear distribution for the SAD set development as suggested by Del Ponte et al. (2017).

To validate the SAD set, 20 raters with no experience in disease quantification and a set of 50 images (leaves) were used. The leaves ranged from 1% (minimum severity) to 85% (maximum severity) and the other leaves were selected to obtain the most varied levels of disease severity homogeneously. The raters were instructed to recognise and delineate the symptoms, use the SAD set, and interpolate their estimates when necessary. The individual images were projected using a datashow and simultaneously presented to all participants in the same room. First, disease severity estimates were carried out without the SAD set, using an interval of 30 s between each leaf. After 1 week, the same raters estimated the severity of the same set of 50 images in a different sequence, but this time with the help of the SAD set.

Lin's concordance correlation (LCC) was used to determine agreement between estimates and actual values. Accuracy (υ-scale bias, u-location bias, C_b-bias coefficient factor), precision (r-correlation coefficient), and overall agreement (ρc-LCC) were calculated. Inter-rater reliability (R^2-coefficient of determination and ρ-intra-class correlation) of the estimates without and with the SAD set was also calculated. Assessment of rater accuracy, precision, and inter-rater reliability were analysed as previously described (Dolinski et al. 2017). The LCC statistics (u, υ, C_b, r, and ρ_c) were estimated by the epi.ccc function of the epiR package (Stevenson et al. 2025). For each statistic analysed (u, υ, C_b, r, ρ_c and R^2), the difference between the means (i.e., with SAD set minus without SAD set) was calculated, and an equivalence test was used to test for significance. 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. All analyses were based on 10,000 balanced bootstrap samples. If the CIs embraced zero, the difference was considered non-significant ($\alpha = 0.05$). The built-in boot.sample R function was used for the equivalence test. ρ was estimated using the icc function of the irr R package (Gamer et al. 2022). If the CIs values overlapped, the difference was considered non-significant ($\alpha = 0.05$). The statistical analyses were performed using R (R Core Team 2025).

In this study, we developed a SAD set to assess the severity of target leaf spot on *H. serratifolius* leaves. Ten illustrations, covering the minimum (1%) and the maximum (85%) of disease observed in the field, comprised the SAD set (Figure 1). At severity levels above 5%, the representation of symptoms included necrotic tissues and coalesced lesions, as adopted in other SAD sets proposed for foliar diseases (Araújo et al. 2019; Conforto et al. 2024; Correia et al. 2017; Rocha et al. 2024). Coalescence of lesions is a common feature of symptoms in *H. serratifolius* as the spots expand, causing extensive areas of necrotic tissues on the leaf blades (Ferreira 1989). The severity of target spot observed in *H. serratifolius* leaves collected from the nursery (up to 85%) contradicts the report by Kranz (1977) that the severities of many plant diseases are most frequently below 30% due to defoliation. In target leaf spot on *H. serratifolius*, plants can display severely injured foliage without defoliation.

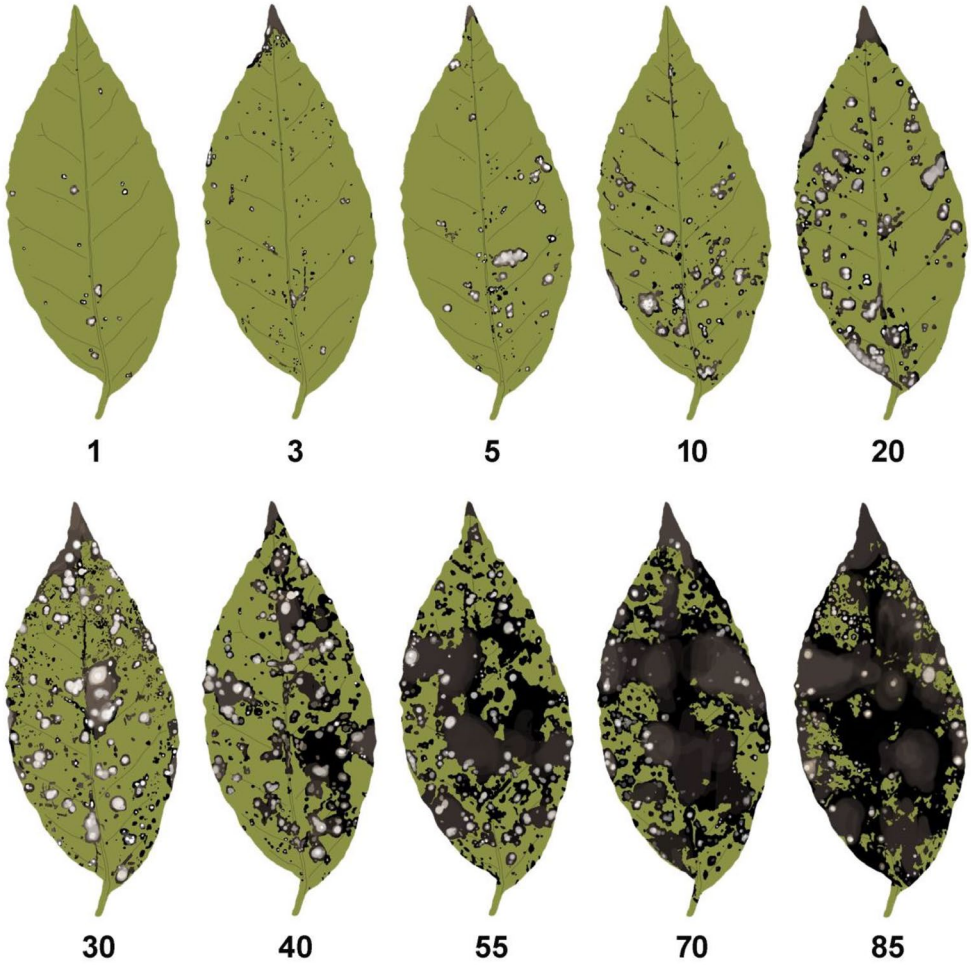


FIGURE 1 | Standard area diagram (SAD) set to assess to severity of target spot (caused by *Corynespora cassiicola*) on *Handroanthus serratifolius* leaves. Numbers represent the percentage (%) of diseased leaf area corresponding to the typical necrotic lesions.

Based on estimated and actual severity, assessments made by the raters were closer to the actual values using the SAD set (Figure 2). The absolute error of the estimates decreased when the raters used the SAD set (Figure 3). The LCC statistical parameters (υ, u, C_b, r and ρ_c) were significantly improved when the raters used the SAD set as an aid to estimate disease severity. The scale bias (υ) decreased from 1.195 to 1.072 (CI = −0.182 to −0.033), without and with the aid of the SAD set, respectively. Using the SAD set, the location bias (u) decreased from 0.267 to 0.018 (CI = −0.419 to −0.079). The correction factor (C_b) approached 1 using the SAD set. The accuracy (C_b) was 0.898 and 0.994 without and using the SAD set, respectively (CI = 0.051 to 0.140). In addition, using the SAD set helped the evaluators to more precisely estimate the severity of the disease. Without using the SAD set, the correlation coefficient (r) was 0.926, and with the SAD set, it increased to 0.979 (CI = 0.033 to 0.074). The LCC (ρ_c) without the aid of the SAD set was 0.835, and with the SAD, it improved to 0.974 (CI = 0.088 to 0.189). This shows that the accuracy and precision of the disease severity estimates were improved with SAD set use. As the CI did not include zero, the differences were statistically significant ($\alpha = 0.05$).

Inter-rater reliability is measured by the coefficient of determination (R^2) and intra-class correlation coefficient (ρ), and the assessments by the 20 raters were significantly improved with SAD set use. Without the SAD set, the R^2 mean between evaluators in paired comparisons was 0.779 (minimum = 0.436, maximum = 0.972) and 0.934 (minimum = 0.859, maximum = 0.977), without and with the SAD set, respectively. The 95% CIs of this mean were 0.240 to 0.169. As the CIs did not embrace zero, the difference was significant ($\alpha = 0.05$). Similarly, the ρ mean was 0.792 (CIs = 0.703 to 0.864), while using the SAD set, this value was 0.962 (CIs = 0.945 to 0.975). As the CIs did not overlap, the difference was considered significant ($\alpha = 0.05$).

Raters overestimated disease severity without SAD set, as evidenced by positive absolute errors and a positive location bias ($u = 0.267$). With SAD set, there was a slight tendency to overestimate severity, indicated by predominantly positive absolute errors and a positive and close to zero location bias ($u = 0.018$).

Disease assessment is essential for epidemiological and management studies of target leaf spot on *H. serratifolius*. It was demonstrated that the proposed SAD set improved the accuracy, precision, and reliability of the severity estimates of this disease as observed for other leaf diseases caused by *C. cassiicola* on Barbados cherry (Celoto and Papa 2010), cotton (Fantin et al. 2018), cucumber (Teramoto et al. 2011), roselle (Ortega-Acosta et al. 2016), and soybean (Soares et al. 2009). Based on the results obtained, the use of the SAD set developed in this

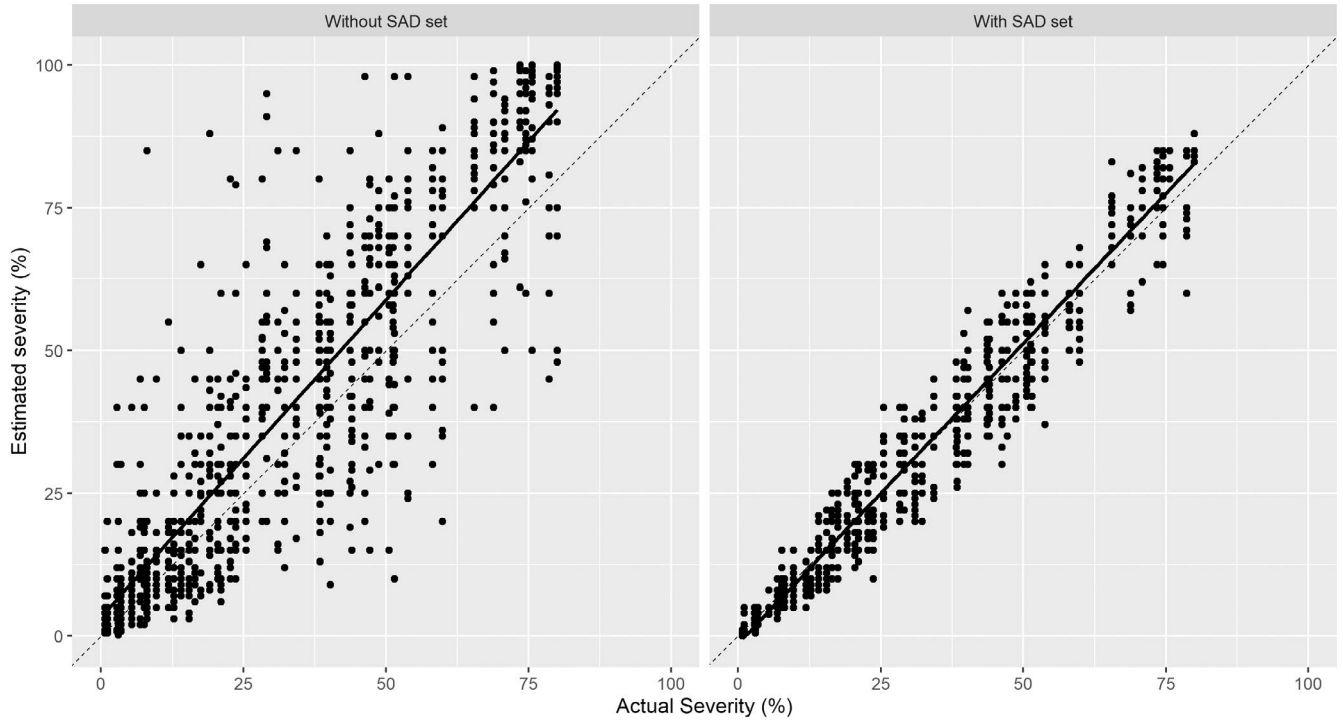


FIGURE 2 | Relationship between actual and estimated severity of target spot (caused by *Corynespora cassiicola*) on *Handroanthus serratifolius* leaves without and with the use of a standard area diagram (SAD) set for 50 diseased leaves obtained from 20 raters. The dotted line represents the 1:1 relationship.

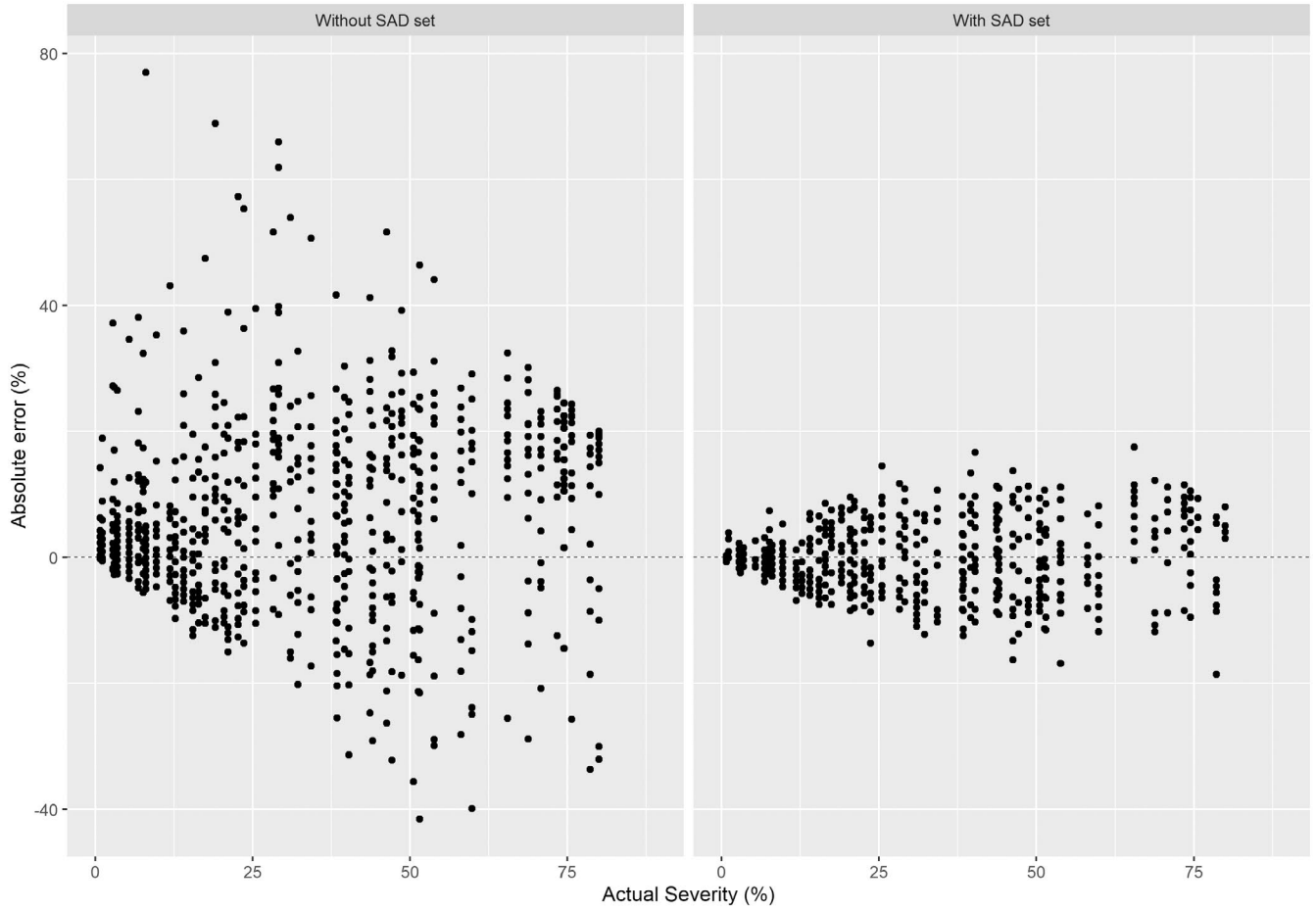


FIGURE 3 | Absolute error (estimated severity minus actual severity) of the severity estimates of target spot (caused by *Corynespora cassiicola*) on *Handroanthus serratifolius* leaves without standard area diagram (SAD) set and with SAD set for the 50 diseased leaves obtained from 20 raters.

study can be indicated to assess the severity of the target spot on *H. serratifolius* leaves.

Author Contributions

Project conceptualization: Ingryd Luzia de Farias Andrade, Humberson Rocha Silva, Marcos Paz Saraiva Câmara and Sami Jorge Michereff. Methodology: Henrique da Silva Silveira Duarte, Humberson Rocha Silva, Ricardo Brainer Martins and Sami Jorge Michereff. Investigation: Ingryd Luzia de Farias Andrade, Ana Gabriele Gurgel Amaral and Kamila Câmara Correia. Resources: Marcos Paz Saraiva Câmara; Data analysis: Henrique da Silva Silveira Duarte, Humberson Rocha Silva and Sami Jorge Michereff. Preparation of the draft manuscript: Ingryd Luzia de Farias Andrade. Review and editing manuscript: Henrique da Silva Silveira Duarte and Sami Jorge Michereff. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jph.70105.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.