



Development and validation of a standard area diagram (SAD) set for assessing *Alternaria* black spot severity in pecan leaves

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Abstract

Pecan [*Carya illinoensis* (Wangenh.) C. Koch] cultivation is expanding in Argentina, with Catamarca Province emerging as a significant production region. However, fungal diseases such as *Alternaria* black spot (ABS), caused by *Alternaria* spp., pose an increasing threat to crop yield and health. Considering that disease quantification is crucial for epidemiological studies and management, this study aimed to design and validate a standard area diagram (SAD) set to improve the visual estimation of ABS severity on pecan leaves. Using 255 diseased leaves, an eight-image SAD set with severity levels that linearly ranged from 2.2% to 88.9% was designed. Thirty-four raters participated in the validation process using the online platform *TraineR2* in two phases: unaided and aided assessments. The use of the SAD set significantly improved accuracy metrics. Lin's concordance correlation coefficient (CCC) increased from 0.93 to 0.97, while precision (r) rose from 0.92 to 0.97. Additionally, inter-rater reliability, measured using the intraclass correlation coefficient (ICC), improved from 0.86 to 0.93. This study demonstrates the effectiveness of the SAD set tool in enhancing the accuracy and reliability of ABS severity estimations, highlighting its potential as a practical resource for pecan producers and researchers.

Keywords *Carya Illinoensis* · Foliar disease · Phytopathometry · Epidemiology

Pecan [*Carya illinoensis* (Wangenh.) C. Koch], a member of the family *Juglandaceae*, is a tree native to the south-central and southeastern United States and northeast Mexico (Sparks 2005; Nadler et al. 2017). It is the most commercially cultivated species within the genus *Carya*, recognized

as an important nut and woody oil tree worldwide (Sun and He 1982; Grauke et al. 2016; Huang et al. 2019). In Argentina, pecan cultivation is an emerging and expanding fruit production system. According to data from the Argentina Pecan Cluster (CAPPECAN 2024), there are approximately 10,000 hectares dedicated to pecans in the country, yielding over 2,000 tons of production.

From a plant health perspective, pecan production faces significant challenges due to fungal diseases. Among these, *Alternaria* black spot (ABS) has recently emerged as an important foliar disease in several producing countries such as South Africa and China. This disease can cause premature defoliation, nut abortion, reduced tree vigor, and overall yield losses (Yan et al. 2022; Achilonu et al. 2023; Fan et al. 2024).

In 2021, symptoms associated with ABS were observed with high incidence in pecan orchards in the Central Valley of Catamarca, Argentina. Symptoms included irregular necrotic lesions and dark brown to black spots on the leaf blades and fruit surfaces. Leaves and fruit with symptoms associated with the disease were collected, and *Alternaria* sp. was identified as the causal agent.

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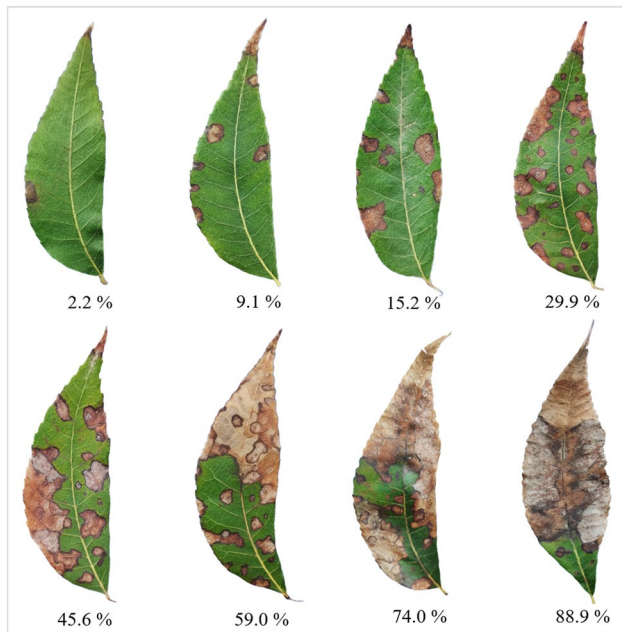


Fig. 1 Standard area diagrams (SAD) set for visual estimation of the severity of *Alternaria* black spot (ABS), caused by *Alternaria* sp. in Pecan (*Carya illinoensis*)

As an emerging disease in Argentina, limited research has been conducted to assess or manage the impact of ABS on pecan production. Accurate quantification of disease severity is essential for conducting epidemiological studies, evaluating cultivar resistance, and designing effective management strategies, including fungicide applications (Bock et al. 2010; Pereira et al. 2020).

Fig. 2 Scatter plot representing the relationship between the rater's absolute errors and the actual severity percentage before using the SAD set (Unaided) and after using the SAD set (Aided). Dots represent the individual error. The red line is a smooth conditional mean produced as default using the `geom_smooth` function in the `ggplot2` package of R

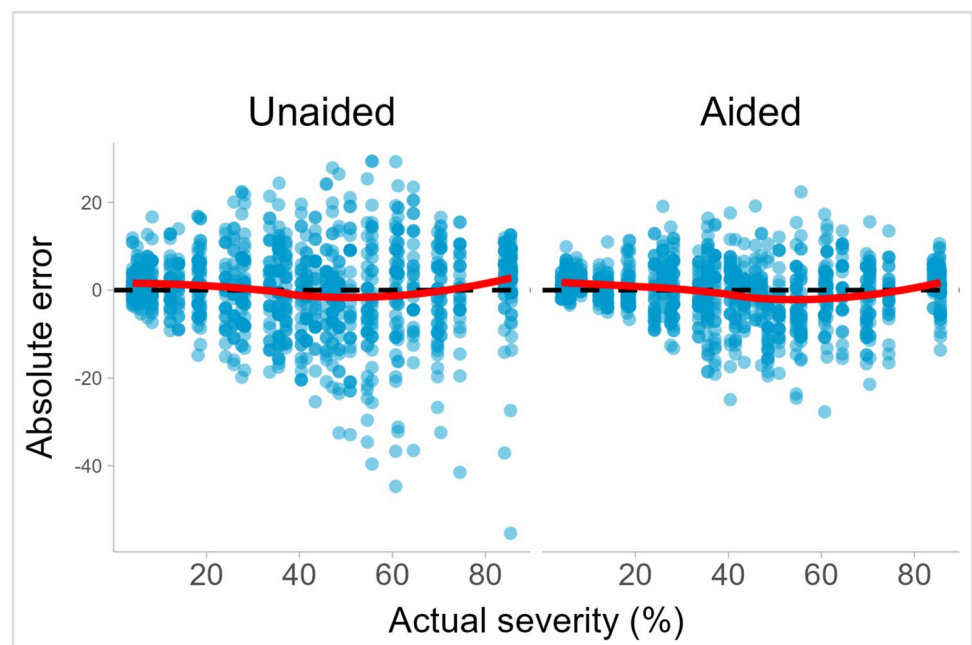


Table 1 Effect of the SAD set to improve the visual severity estimation of ABS on the LCCC (ρ_c), precision (r), bias coefficient (C_b), location bias (u), scale bias (v) standard deviation (Sd), agreement between raters described by the intraclass correlation coefficient (ICC) at 95% of the confidence interval (95% CI) and meantime of severity estimation per image

	Unaided ¹	Aided ¹
ρ_c	0.93a	0.97b
r	0.92a	0.97b
C_b	0.97a	0.99b
Sd	9.66	6.25
u	0.09	-0.22
v (95% CI)	0.99	0.97
ICC (95% CI)	0.86	0.93

¹ Means followed by a common letter are not significantly different by the Tukey-test at the 5% significance level

Although advanced methods such as remote sensing and image analysis are available (Nomura and Oki 2021), visual estimation remains the most widely used method due to its simplicity, low cost, and speed (Bock et al. 2021). However, visual assessments are prone to bias and error due to their subjective nature (Bock et al. 2020; Del Ponte et al. 2021).

To improve the accuracy of visual estimations, two main tools are commonly used: training systems and standard area diagrams (SAD) sets. The former has proven effective in enhancing assessment skills through various computer-based tools available on online platforms, such as *TraineR2* (Del Ponte 2023), *SteviaScout* (Sanabria-Velazquez et al. 2023), and the *Defoliation Training Tool* (Sisson and Mueller 2023).

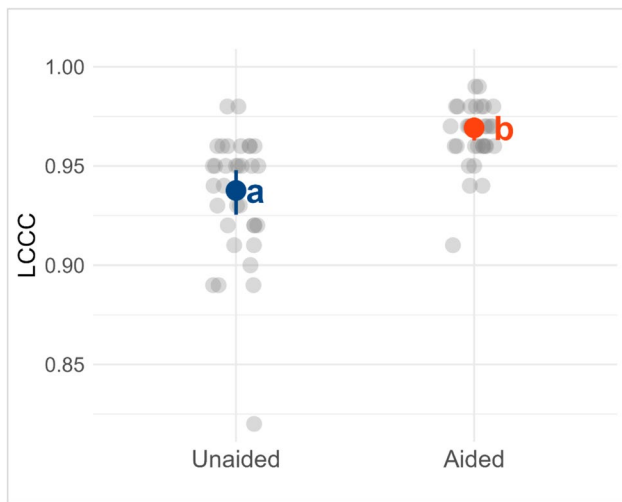


Fig. 3 The overall accuracy of the rater's estimates of *Alternaria* black spot (ABS) severity before (Unaided) and after (Aided) using the SAD set. Dots represent raters' individual Lin's concordance correlation coefficient (LCCC). Error bars represent the 95% confidence interval for the mean LCCC. Letters represent the Tukey mean comparison test groupings (5% probability). Different letters represent different groups

SAD sets, likely the most widely used tool in phytopathometry, consist of reference illustrations representing incremental levels of disease severity. These aids help raters interpolate severity values more accurately (Nutter et al. 1993; Bock et al. 2016; Del Ponte et al. 2017). Numerous studies have confirmed the ability of SAD sets to reduce bias and improve accuracy (Del Ponte et al. 2021; Bock et al. 2022). Despite

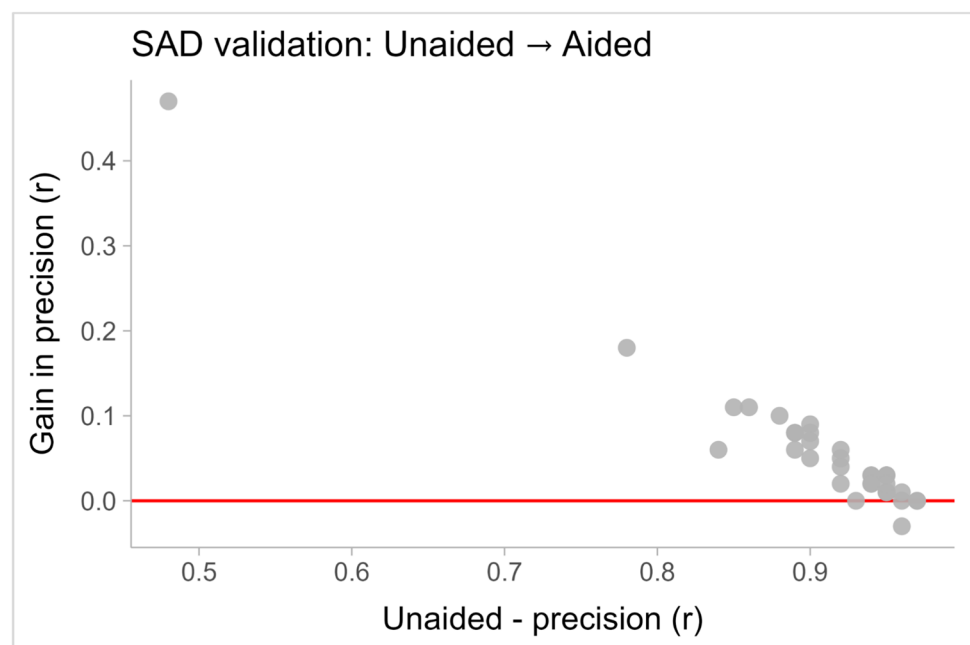
being over a century old, SAD sets remain a fundamental resource in plant disease assessment.

Given the lack of standardized tools for quantifying the impact of ABS on pecan leaves in Argentina, this study aims to design and validate a standard area diagram (SAD) set to improve the accuracy and reliability of visual disease severity estimation.

To this end, a total of 255 leaves displaying various levels of ABS severity were collected in May 2024 from 15-year-old pecan trees in Capayán, Catamarca, Argentina (28° 41' 43" S, 65° 56' 09" W). These leaves were taken to the laboratory of Plant Pathology of the Catamarca National University and photographed using a 50mp smartphone on a blue background. The percentage of leaf area affected by *Alternaria* sp. was quantified using the "analyze objects" function of the *Pliman* package (Olivoto 2022) in *R* (R Core Team 2022). The SAD set was designed in *Microsoft PowerPoint* (Microsoft 2024), considering the minimum and maximum severities observed in the samples collected (Del Ponte et al. 2017; Liu et al. 2019; Cazón et al. 2025).

SAD set validation was performed using the online training platform *TraineR2* (<https://delponte.shinyapps.io/train-eR2/>) (Del Ponte 2023) on 35 representative images of ABS-affected leaves, following the methodology used by Cazón et al. (2025). Thirty-four raters with no prior SAD experience participated in the study using their personal computers, without time restrictions. The validation process was conducted in two phases: first, the raters assessed disease severity without SAD set assistance. Upon completion, one of the study's authors downloaded the spreadsheets containing accuracy metrics from *TraineR2* to prevent raters from

Fig. 4 Relationship between unaided precision (r) and the gain in precision achieved through the use of SAD set. Each dot represents the accuracy gain (calculated as the difference between SAD-aided and SAD-unaided accuracy) for an individual rater. The red line marks the threshold separating accuracy gains (above the line) from accuracy losses (below the line)



accessing their own results. After a ten-minute interval, the second phase started, during which raters performed the same task, but with SAD set assistance this time.

To assess accuracy and reliability, the absolute error (the difference between the estimated and actual severity) and its standard deviation (indicating error variability) were calculated for each rater across different assessment rounds (Del Ponte 2023). The agreement level between the actual and estimated severity by raters was determined by calculating Lin's concordance correlation coefficient (Lin's CCC) (Lin 1989; Madden et al. 2007; Del Ponte et al. 2017). Key accuracy metrics, including overall accuracy (ρ_c), generalized bias coefficient (Cb), and precision (Pearson's r), were calculated and displayed in *TraineR2* after each evaluation round. Scale bias (v) and location bias (u) were calculated as previously described (Dolinski et al. 2017). The inter-rater reliability was evaluated using the intraclass correlation coefficient (ICC) (Shoukri and Pause 1999) based on the 95% confidence interval, and the overall concordance correlation coefficient (OCCC), an improved Lin's CCC method for multiple raters (Barnhart et al. 2002).

Accuracy (ρ_c) and precision (Pearson's r) during the SAD set validation were modeled using generalized linear mixed-effects models, where the interaction between aid (Unaided, Aided) was included as a fixed effect, and the raters as random intercepts. Model fit was verified using *DHARMA* residual simulations (Hartig 2021), and Tukey's test was used to compare mean differences.

The resulting SAD set comprises eight true-color images representing severities from 2.2% to 88.9% (Fig. 1), with 15% intervals and an extra diagram at 9.1% to minimize early-stage overestimation, following Liu et al. (2019).

The use of the SAD set significantly reduced the absolute error ($P < 0.05$) and its variability (Fig. 2). Specifically, the standard deviation decreased from 9.66 (unaided) to 6.25 (aided) (Table 1), improving consistency in severity estimation across raters.

In terms of accuracy, Lin's CCC increased from 0.93 (unaided) to 0.97 (aided), representing a notable gain of 0.04 points. Precision (Pearson's r) also improved by 0.05, rising from 0.92 to 0.97. Additionally, scale bias (v) slightly decreased from 0.99 to 0.97, and location bias (u) shifted from 0.09 to -0.22 , indicating an improvement in overall agreement (Table 1). While changes in Lin's CCC and Pearson's r may appear modest, they are consistent with the data presented by Del Ponte et al. (2021) for pathosystems with ABS-like features (Figs. 3 and 4).

Regarding the 35 images used in the validation process (fewer than the 40–50 suggested by Del Ponte et al. 2017), to ensure robust validation, a large number of evaluators were included (34). Among them, 29 exhibited improvements in precision after using the SAD tool. Four evaluators maintained their initial levels of precision, while only one evaluator experienced a slight decrease in performance (LCCC of 0.96 without

assistance, which slightly declined to 0.93 when using the SAD set). This outcome is consistent with findings in the literature, which suggest that raters with high baseline accuracy levels may exhibit minimal or negative changes when using SAD sets (Del Ponte et al. 2021; Cazón et al. 2025).

After the validation process, both the ICC and OCCC increased from 0.86 (unaided) to 0.93 (aided) (Only ICC values are shown in Table 1), indicating reduced inter-rater variability and improved agreement in severity assessments.

To date, two SAD sets have been developed for assessing disease severity in pecan diseases. The first was proposed by Yadav et al. (2013) for evaluating pecan scab symptoms on fruit, while the second was developed by Poletto et al. (2021) to estimate the severity of brown leaf spot on leaves. Although the latter has been considered as a potential reference for assessing other foliar diseases in pecan, we identified several reasons why the development of a new SAD set was necessary for this specific pathosystem. First, the SAD set designed by Poletto et al. (2021) does not allow for precise visualization of symptoms, as brown-colored markings represent them. Additionally, the highest severity levels include chlorotic areas around the lesions, which may introduce difficulties in symptom recognition. In contrast, our SAD set incorporates images of actual lesions caused by *Alternaria* sp., with clearly defined boundaries between diseased and healthy leaf tissue. Other considerations are related to the severity intervals between diagrams representing each SAD set. Although both SAD sets contain eight diagrams, our SAD set employs linear severity intervals and includes an additional diagram for lower severity levels to minimize overestimation at these early disease stages (Del Ponte et al. 2017; Liu et al. 2019; Cazón et al. 2025). This feature aligns with the best practices for developing SAD sets, as recommended by Del Ponte et al. (2017).

In conclusion, this study highlights the effectiveness of the SAD set developed for assessing ABS in pecan leaves. The tool significantly improved evaluator accuracy and reliability, underscoring its potential to enhance disease severity assessments in epidemiological studies and management strategies.

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Author's contribution MVC conceptualized the study, collected leaf samples, contributed to the SAD set validation process, conducted the bibliographic research, and wrote the manuscript. FDC, RMK, and JAP contributed to the SAD set validation process, participated in data analysis, and co-wrote the manuscript. SM and CC provided critical feedback and contributed to manuscript writing. NBL and LIC conceptualized the study, coordinated the SAD set validation process and data analysis, and co-wrote the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available at the following repository link: <https://repositorio.inta.gov.ar/xmlui/handle/20.500.12123/22231>

Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

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