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 illinoinensis*) 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 2.2–88.9% 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 consistency of ABS severity estimations, highlighting its potential as a practical resource for pecan producers and researchers.

Introduction

Pecan (*Carya illinoinensis*), 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 y 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 Argentine Pecan Cluster (CAPPECAN 2024), there are approximately 10,000 hectares dedicated to pecans in the country, yielding over 2,000 tons of production. High international market demand and its excellent nutritional value compared to other nuts make pecan a highly profitable crop (Atanasov et al. 2018; Castillo et al. 2018). In Catamarca Province, commercial pecan cultivation began in 2016, with the current planted area reaching approximately 458.4 hectares and plans for further expansion. Catamarca ranks as the second-largest province in planted area, following Entre Ríos, the leading producer province (National Institute of Statistics and Census 2018).

From a plant health perspective, pecan production faces significant challenges due to fungal diseases, which are among the most damaging threats. Key diseases affecting pecan health include pecan scab (*Venturia effusa*, syn. *Cladosporium caryigenum*), vein spot (*Gnomia nerviseda*), downy spot (*Mycosphaerella caryigena*), brown leaf spot (*Ragnhildiana diffusa*), shuck and kernel rot (*Phytophthora cactorum*), pink mold (*Trichothecium roseum*), cankers (*Phomopsis* sp.), anthracnose (*Colletotrichum gloeosporioides*), wilt (*Fusarium* spp.), and black spot disease (*Alternaria* spp.) (Sanderlin 1983; Reilly et al. 1998; Mantz et al. 2009; Mantz et al. 2009; Mantz et al. 2015; Samaniego-Gaxiola et al. 2015; Noelting et al. 2016; Madero et al. 2016; Poletto et al. 2021). These diseases can significantly impact on pecan production, reducing nut quality. Such is the case of Alternaria black spot (ABS), which has recently become an emerging foliar fungal disease of pecan in producing countries such as South Africa and China. This disease can induce premature defoliation and nut abortion. It can also retard growth and decrease tree vigor over time (Yan et al. 2022; Achilonu 2023; Fan et al. 2024).

In 2021 in pecan crops in the Central Valley of Catamarca, symptoms associated with ABS were observed with a high incidence rate. 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 to confirm the etiological agent. The samples were analyzed at the Plant Pathology Laboratory of the National University of Catamarca (FCA, UNCA). Morphological characterization of the pathogen on PDA medium and the completion of Koch's postulates identified *Alternaria* sp. as the causal agent.

In Argentina, limited studies have been conducted to assess or manage the impact of ABS on pecan production. Accurate quantification of disease severity is critical for conducting epidemiological studies, evaluating cultivar resistance, and developing effective management strategies, including fungicide applications (Bock et al. 2010; Pereira et al. 2020).

Although advanced methods like remote sensing and image analysis are available (Nomura and Oki 2021), visual estimation remains widely utilized. This traditional approach involves assigning a value (e.g., a score or percentage) to the severity of symptoms based on the rater's visual perception (Bock et al. 2021). Visual estimation is both time- and cost-efficient, requiring no specialized infrastructure or expensive equipment. However, this method has limitations, including errors that arise from the subjective nature of the rater's assessment abilities (Bock et al. 2020; Del Ponte et al. 2021).

Currently, two primary tools are used to enhance the accuracy of visual disease severity estimation. The first is training systems, which have proven effective in improving visual assessment skills. Sanabria-Velazquez et al. (2023) emphasized the value of training systems in enhancing the ability to estimate the severity of septoria leaf spot in stevia. These findings align with recent results reported by Cazón et al. (2025), highlighting the significant improvements in accuracy and precision for peanut late leaf spot assessments after just a few training sessions. Currently, various computer-based tools are available in online training interfaces. Notable examples include the *TraineR2* system (Del Ponte 2023), *SteviaScout* (Sanabria-Velazquez et al. 2023), and the *Field Crop Disease and Insect Defoliation Severity Training Tool* for field crops (Sisson and Mueller 2023).

The second tool, and likely the most commonly used in phytopathometry to enhance the rater's ability, is the standard area diagrams (SAD) set (Mazaro et al. 2006; Del Ponte et al. 2021; Bock et al. 2022; Lavilla et al. 2022). These consist of sets of illustrations representing incremental percentage severity values, designed to assist raters in accurately interpolating disease severity percentages (Nutter et al. 1993; Book et al. 2016; Del Ponte et al. 2017). Numerous studies have demonstrated the benefits of SAD set in reducing bias and improving accuracy (Nutter et al. 1998; Del Ponte et al. 2021; Bock et al. 2022). Despite being over a century old, SADs continue to be widely used. According to the SADBank, a curated repository of SAD studies (https://edelponte.shinyapps.io/sadbank/), more than 187 SADs for fungal, viral, bacterial, and oomycete diseases were published and validated between 1993 and 2025. This consistent frequency of publication highlights the enduring relevance and utility of these tools, even amidst advancements in modern technologies.

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) to improve the accuracy and reliability of visual disease severity estimation. This tool will contribute to a better understanding and management of this emerging pathology within Argentina's pecan production system.

Materials and Methods

SAD development and validation. 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 images were then processed using *Adobe Photoshop software* (2011) to eliminate shadows cast by leaf edges on the background which could affect severity measurement by the software. Each image was saved in JPG format at a resolution of 300 dpi. 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). This function automates image analysis by applying segmentation and quantification techniques to distinguish diseases from healthy tissue. The SAD set was designed in *Microsoft PowerPoint* (Microsoft 2024) considering the minimum and maximum severities observed in the samples collected. The diagrams were distributed at linear intervals, and an additional diagram was included at low severity levels to reduce overestimation (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/traineR2/) (Del Ponte 2023). This application, developed in the *R* environment (R Core Team 2022) with the *Shiny* package (Chang et al. 2023), incorporates real photographs of diseased leaves, each annotated with their corresponding severity percentage. On its homepage, the right-hand panel contains three tabs. The first tab (**Instruction**) provides usage guidelines; the second tab (**Estimates**) displays the actual severity values and those estimated during the training; and the third tab (**Plot and Accuracy**) presents key statistical parameters during training, such as Lin CCC, Bias Coefficient, Precision, and training time. This tab also provides a real-time graph plotting actual versus estimated severity. To ensure unbiased results during the validation process, the 'Estimate' and 'Plot and Accuracy' tabs remained hidden at all times, preventing evaluators from accessing statistical parameters or results during their assessments.

Thirty-five representative images of ABS-affected leaves were included in November 2024 in the *TraineR2* database. The validation process involved 34 raters with no prior experience using SADs. Among the raters, 19 were affiliated with the Centro de Investigaciones Agropecuarias (CIAP-INTA), located in Córdoba province, while the remaining 15 were from the Facultad de Ciencias Agropecuarias at Universidad Nacional de Catamarca (FCA-UNCA). The methodology followed the protocol described by Cazón et al. (2025) and to maintain impartiality in the evaluations, all participants in the study had no prior exposure to this online database. Raters performed the task on their personal computers, without time restrictions, and received a brief tutorial on system usage before beginning the assessment. The validation process was conducted in two phases: first, the raters assessed disease severity without SAD assistance. Upon completion, one of the study's authors downloaded the spreadsheets containing accuracy metrics from *TraineR2* to prevent raters from accessing their own results. After a ten-minute interval, the second phase started, during which raters performed the same task, but with SAD assistance this time. For raters affiliated with CIAP-INTA, the SAD was provided as a full-color printed A4 sheet, while for the FCA-UNCA raters, the SAD was projected onto a screen.

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 (ρc), generalized bias coefficient (Cb), and precision (Pearson's r), were calculated and displayed in *TraineR2* after each evaluation round. 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). The ICC was computed using the "ICC" function from the *psych R* package (Revelle 2020), with the "lmer" parameter set to "TRUE" to estimate confidence intervals and reliability scores (Cazón et al. 2025; Pereira et al. 2020). The OCCC was calculated with the "epi.occc" function from the *epiR* package in R (Stevenson et al. 2019).

SAD effect on accuracy. Accuracy (ρc), precision (Pearson's r), and bias coefficient (Cb) during the SAD 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. The quality of the model fit was checked via simulation of the residuals provided by the *DHARMa* R package, which provides p-values for assessing the normality of residuals, overdispersion, and outliers (Hartig 2021). Significant differences between the means resulting from the different stages of the validation and training processes were compared using Tukey's mean comparison test.

Results

Following the recommendations of Del Ponte et al. (2017), the developed SAD set consists of eight true-color representative images, ranging from 2.2–88.9% severity (Fig. 1). As previously described, approximately 15% intervals were defined between diagrams, with an additional diagram at 9.1% severity inserted between the minimum (2.2%) and 15.2% severity, resulting in the "amended-linear" percent scale as suggested by Liu et al. (2019).

No statistically significant interaction was observed between the laboratory affiliation and the aided results during the second phase of the SAD validation process. Thus, all raters were treated as a single group.

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

Table 1
Effect of the standard area diagram (SAD) to improve the visual severity estimation of ABS on the LCCC (c), precision (r), bias coefficient (Cb), error, 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.

ρ		
	Unaided[1]	Aided[1]
ρ_c	0.93a	0.97b
r	0.92a	0.97b
C_b	0.97a	0.99b
Error	0.09	-0.22
Sd	9.66	6.25
ICC (95% CI)	0.86 (0.80–0.92)	0.93 (0.90–0.96)

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

In terms of accuracy, Lins´CCC increased from 0.93 (unaided) to 0.97 (aided), representing a notable gain of 0.04 points. Precision, measured by Pearson’s r also improved by 0.05, rising from 0.92 (unaided) to 0.97 (aided). These results underscore the effectiveness of SAD in enhancing the alignment between estimated and actual severity values.

Among the 34 evaluators who participated in the validation process, 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, indicating the robustness of the SAD tool implementation.

After the validation process, both the ICC and OCCC parameters showed similar values, increasing from 0.86 (unaided) to 0.93 (aided). For that reason, Table 1 only provides information on the ICC value. These results highlight the tool's ability to reduce variability among evaluators and improve agreement in severity assessments.

Discussion

The nut sector is one of the most promising economies in Argentina. Pecan production demonstrates capabilities to boost the sector by the availability of cultivars adapted to different soil and climatic conditions in the country, which drives the production of this crop in several regions, such as Northeast, Central, Patagonia, and Northwest (Bischoff et al. 2020). In Catamarca province, pecan farming began less than a decade ago, leaving many production-related challenges yet to be addressed, including plant health issues and their impact on yield. Among these issues, ABS has recently been identified as a concern.

As previously mentioned, identifying and quantifying diseases are crucial for understanding their impact on crops and determining the appropriate management strategies (Del Ponte et al. 2024). In the case of ABS, its presence was initially overlooked following the crop's introduction due to the striking similarity of its symptoms to those caused by zinc deficiency. Because the pecan crop has a greater need for Zn than most other fruit and nut trees (Ojeda-Barrios et al. 2012; Heerema 2013), and the availability of this microelement is low in soils such as those of the central valley of Catamarca (ph > 7.0). However, clear distinguishing features exist between these two conditions. Lesions caused by zinc deficiency are typically small, necrotic, and uniformly distributed across the entire leaf, reflecting a generalized physiological imbalance in the plant. In contrast, lesions caused by *Alternaria* sp. are generally larger, irregular, and exhibit an aggregated pattern, with spots concentrated in specific areas of the leaf due to the localized activity of the pathogen (Fig. 5). These symptomatic patterns are well defined in our SAD, so in addition to providing support to quantify the disease, it allows us to identify it more clearly.

While there is a SAD for estimating the severity of brown leaf spots (Poletto et al., 2020), we identified several reasons why the development of a new one was necessary for this pathosystem. First, the SAD designed by Poletto et al. (2020) was specifically developed to assist in severity assessments of brown leaf spot caused by *Ragnhildiana diffusa* in pecan. In this case, the diagrams do 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 incorporates images of actual lesions caused by a different pathogen (*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. Although both SADs contain eight diagrams, our SAD 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 diagrammatic scales, as recommended by Del Ponte et al. (2017). Overall, we believe the new SAD offers a more accurate and standardized approach for assessing the severity of Alternaria leaf spot in pecan. The inclusion of real lesion images, well-defined linear severity intervals between diagrams, and its specific design for evaluating a disease caused by a different pathogen are key features that justify the development and adoption of this tool for disease assessments.

Regarding the methodology used in this work, the validation process of our SAD was conducted using the *TraineR2* system, which proved to be highly effective for this purpose (Cazón et al. 2025). Initially, 35 images were included in the system for training disease severity estimation, and these same images were consistently employed throughout the validation process. Although Del Ponte et al. (2017) recommend using 40 to 50 images for this purpose, we opted to work with the 35 images available. This limitation was mitigated by including a substantial cohort of 34 evaluators from two different locations, ensuring robust validation of the SAD.

Despite the differences in the second stage of the validation process between laboratories, no statistically significant variations were detected in the parameters related to accuracy. For this pathosystem, the size of the SAD set and its presentation format to raters (whether as a printed scale on A4 sheets or projected on a screen) did not impact the results obtained. However, in a previous study, Pethibridge and Nelson (2018), suggest that the size and type of device used can influence the accuracy of evaluators, particularly when employing mobile devices. For this reason, the use of mobile phones was not considered during our validation process to ensure consistency and minimize potential biases. It is worth noting that the way a SAD set is presented may influence visual perception, depending on factors such as screen resolution, lighting conditions, time of assessment, and viewing distance (Sanabria-Velazquez et al. 2023). Although our findings did not show an impact on this specific system, future studies should explore these variables further by using both digital or printed images of the SAD under various environmental conditions. This approach could help us gain insights into the reliability and reproducibility of this tool.

As shown in Fig. 2, the dispersion of absolute errors is significantly reduced with the use of SAD, as evidenced by the decrease in the standard deviation from 9.66 (unaided) to 6.25 (aided) (Table 1). Regarding the absolute error values, the mean reveals negative values in aided evaluations (-0.22), indicating a slight systematic bias introduced by the scale. This bias tends to underestimate severity values, particularly within the mid-range (40–70% severity), as illustrated by the red smooth line in Figure XX. Given the characteristics of this pathosystem, along with recommendations from Liu et al. (2019) to include additional diagrams for lower severity levels, it may also be advisable to incorporate another diagram for mid-range severity values to address this slight bias. Nevertheless, considering the significant improvements observed in other parameters (LCCC, r, standard deviation, ICC), this bias can be considered negligible.

The SAD developed in this study demonstrated a significant improvement in raters' abilities. Among the 34 raters who participated in the assessment process, only one experienced a slight decrease in performance. 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 SADs (Del Ponte et al. 2021; Cazón et al. 2025). In this study, the rater in question initially achieved an LCCC of 0.96 without assistance, which slightly declined to 0.93 when using the SAD (Fig. XX). Regarding precision (Pearson’s r), we observed a statistically significant increase from 0.92 (unaided) to 0.97 (aided). While this improvement of

0.05 may seem modest, it aligns with data reported by Del Ponte et al. (2021). Their meta-analytical approach revealed that symptom patterns influence disease severity assessments. According to their findings, pathosystems characterized by a low number of large, coalescent lesions are easier to evaluate than those exhibiting more complex symptom patterns. As a result, severity assessments of pathosystems with simpler lesion patterns tend to have a higher baseline precision than those with numerous small lesions. This explains the benefits of using our SAD for diseases similar to Alternaria black spot.

Conclusion

This study highlights the effectiveness of the SAD developed for assessing Alternaria black spot in pecan leaves. The tool significantly improved evaluator accuracy and precision, underscoring its potential to enhance disease severity assessments in epidemiological studies and management strategies. Evaluating the adaptation of this tool under different environmental conditions would further improve its applicability and robustness, contributing to more effective disease management in pecan cultivation and beyond.

Declarations

Author's contribution

MVC conceptualized the study, collected leaf samples, contributed to the SAD validation process, conducted the bibliographic research, and wrote the manuscript. **FDC, RMK, and JAP** contributed to the SAD 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 validation process and data analysis, and co-wrote the manuscript.

Data availability

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

Conflict of interest

All authors declare that they have no conflicts of interest.

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Figures

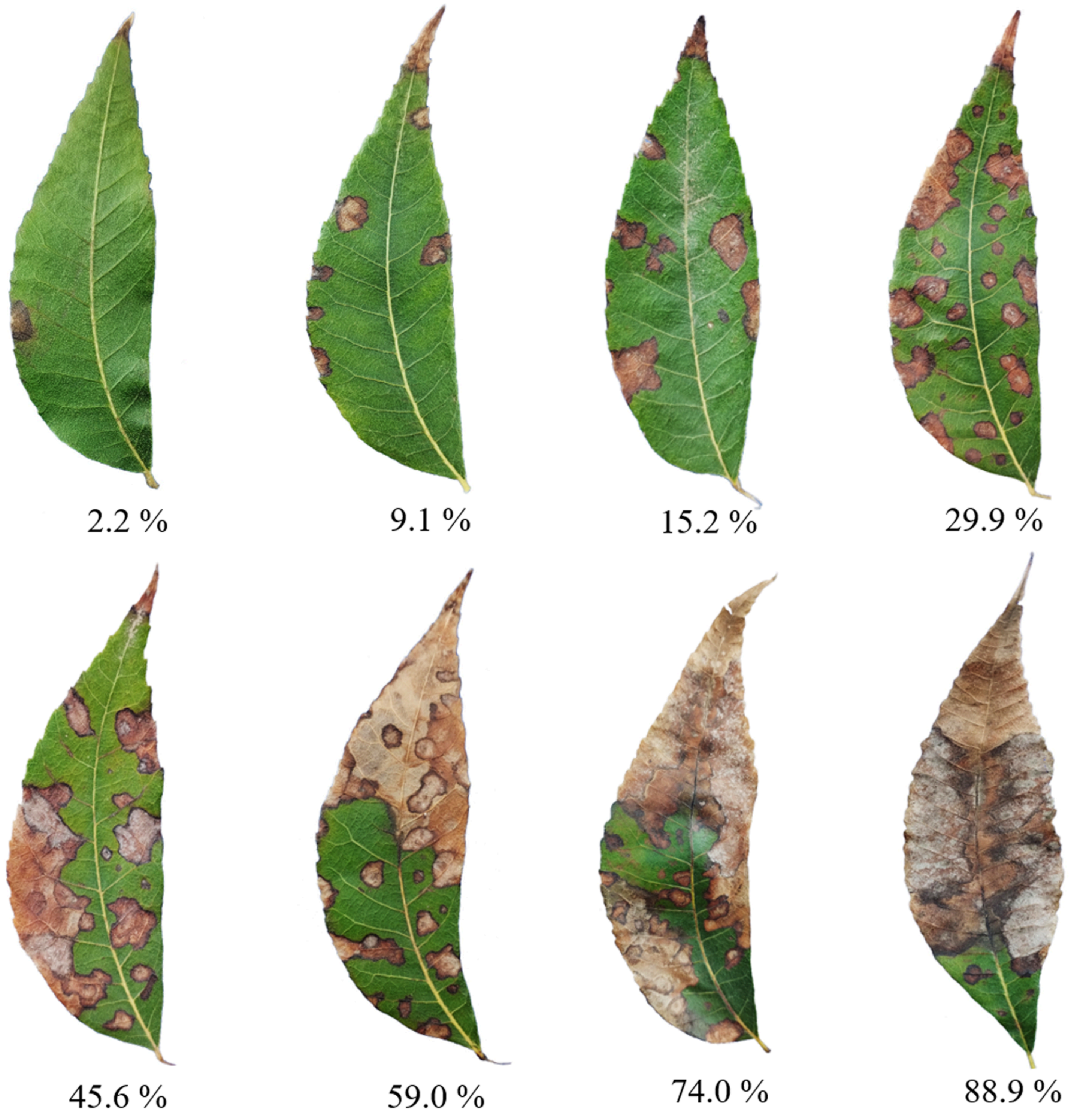

Figure 1

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

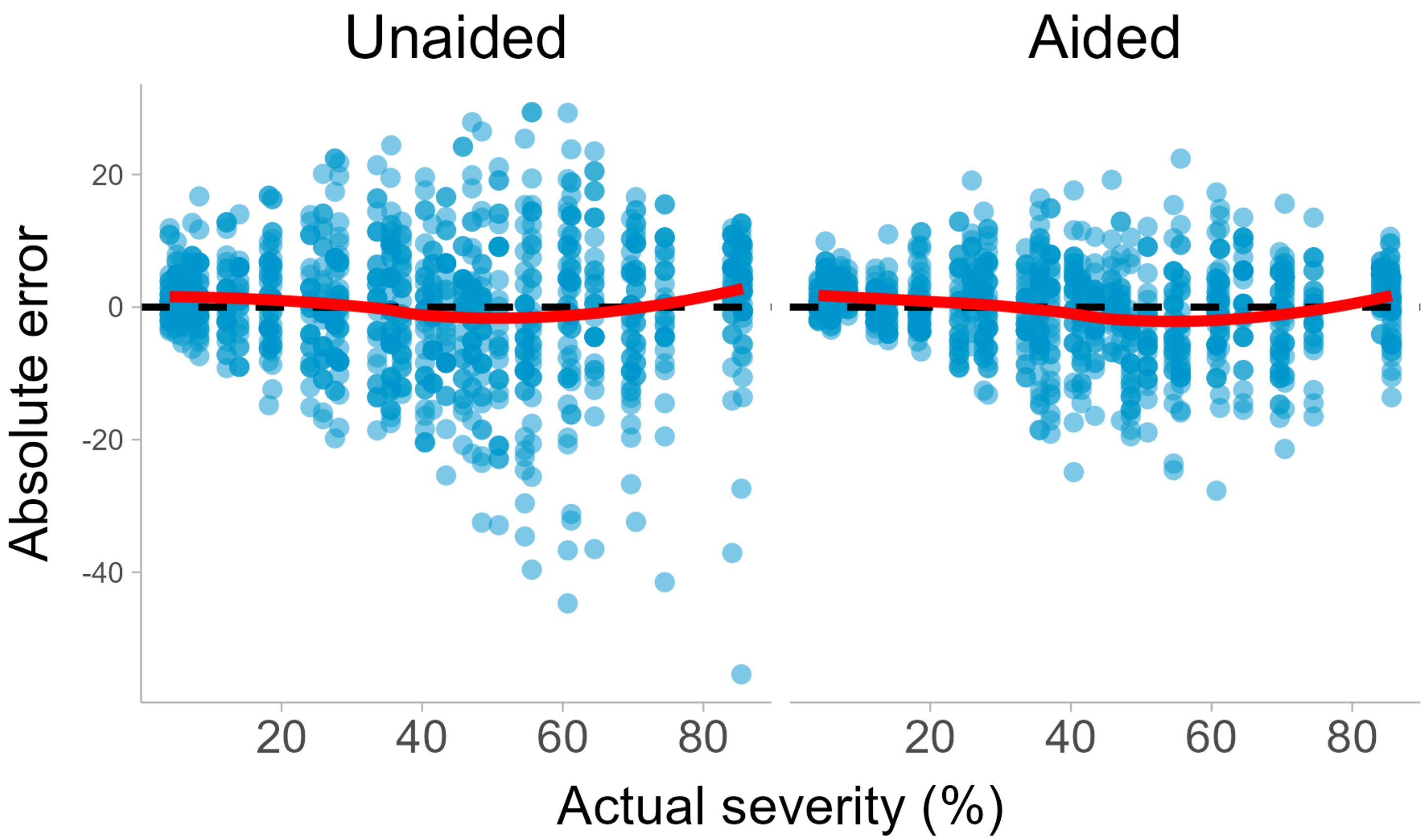

Figure 2

Scatter plot representing the relationship between the rater's absolute errors and the actual severity percentage before using SAD (Unaided) and after using SAD (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.

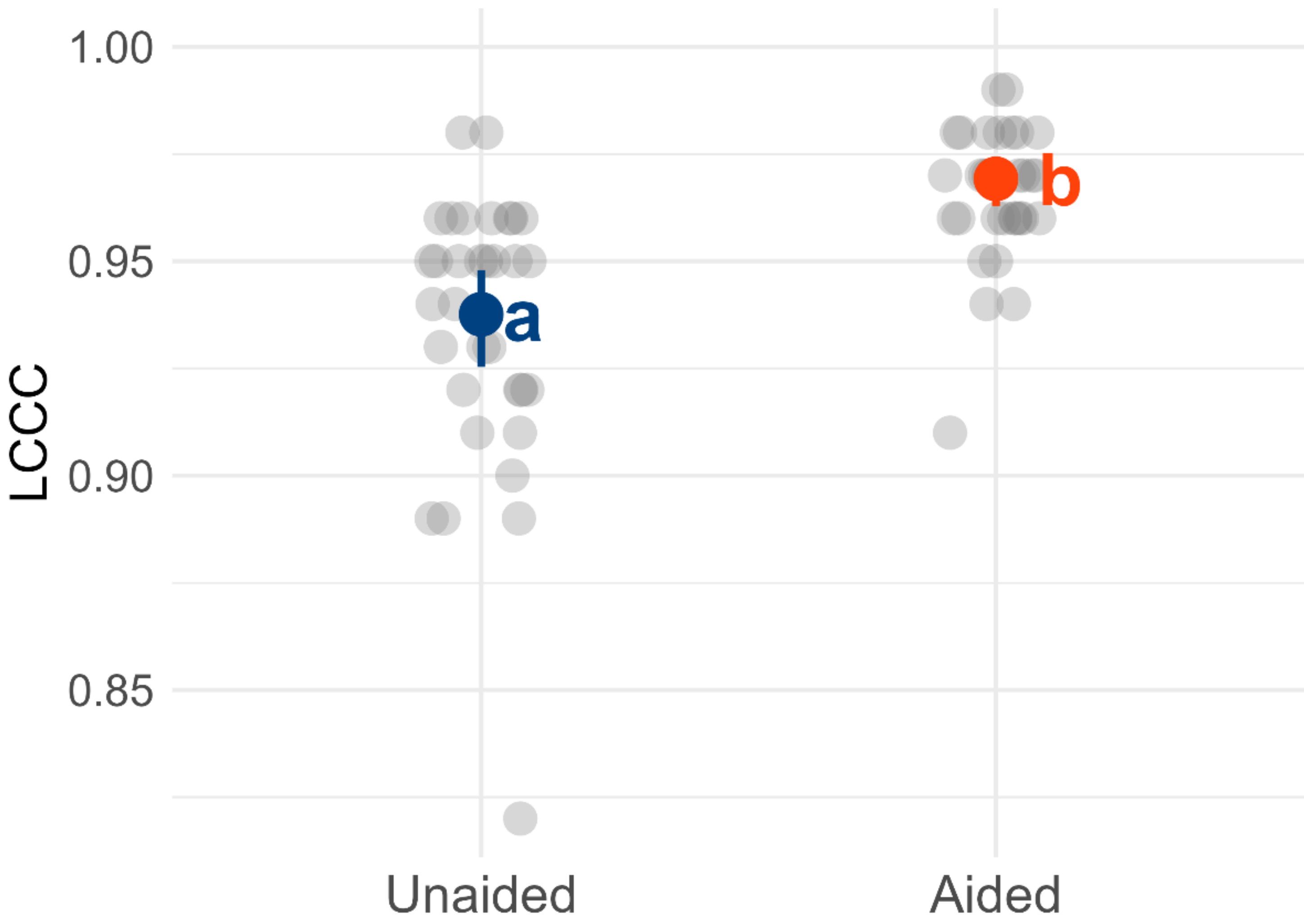

Figure 3

The overall accuracy of the rater's estimates of Alternaria black spot (ABS) severity before (Unaided) and after (Aided) using the SAD. 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.

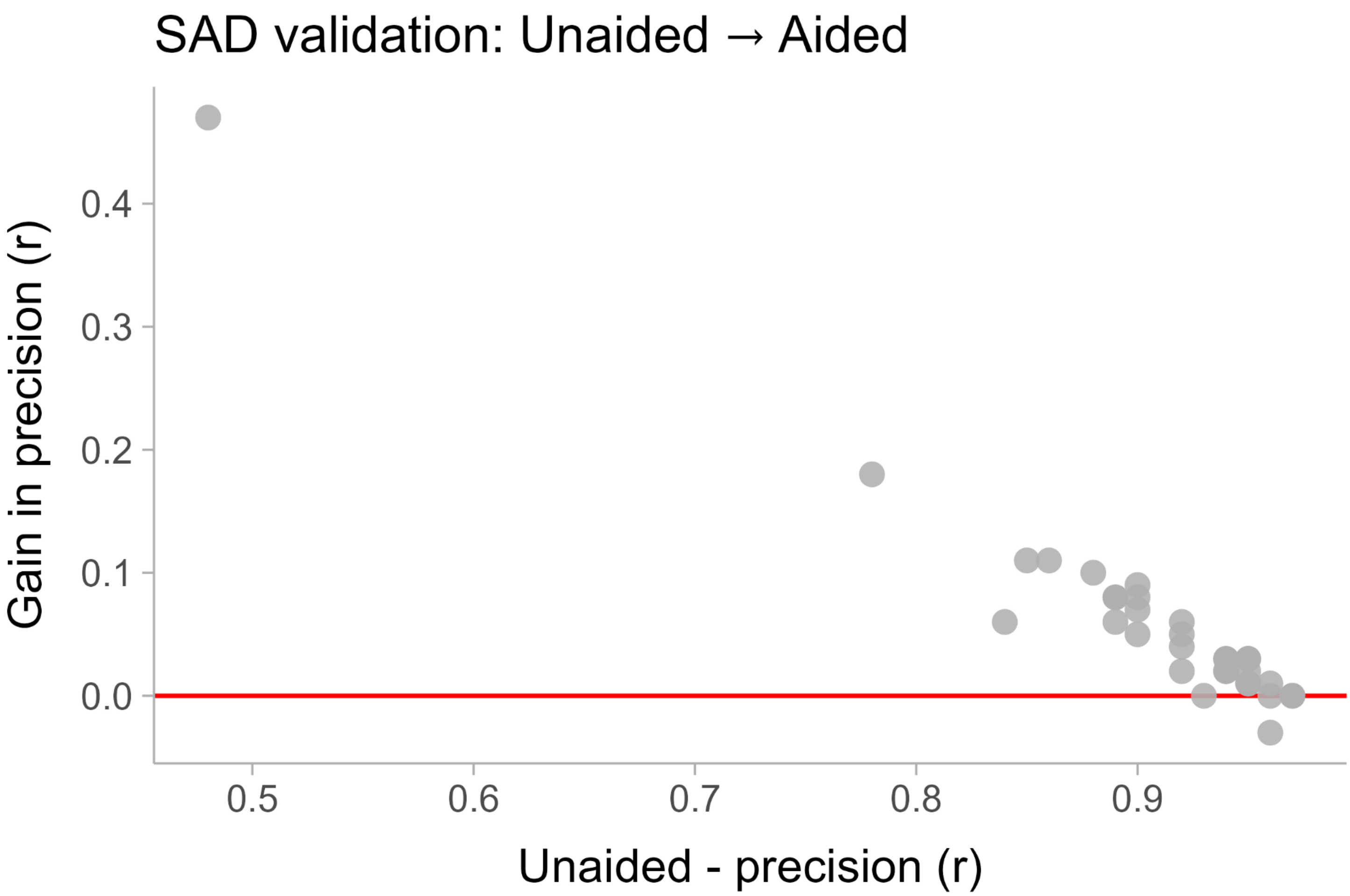

Figure 4

Relationship between unaided precision (r) and the gain in precision achieved through the use of SAD. 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).

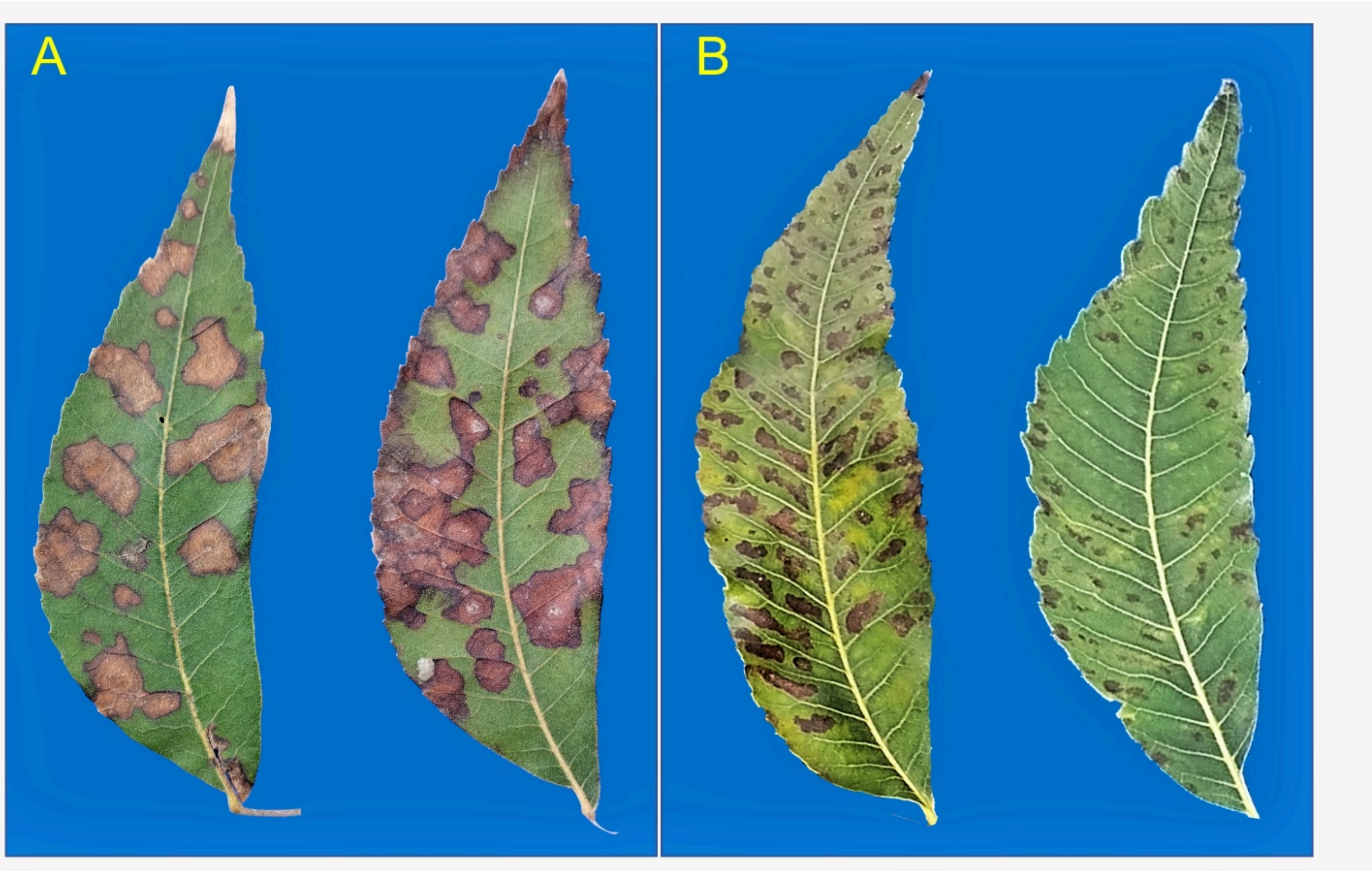

Figure 5

Difference between lesions caused by Alternaria black spot (**A**) and lesions caused by zinc deficiency (**B**) in pecan leaves.