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Development and validation of a standard area diagram set to assess corn grey leaf spot severity and foliar fungicide control efficacy

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Abstract

Grey leaf spot (GLS), caused by *Cercospora* spp., has become a very important foliar disease for second-crop corn season, mainly in South America. Therefore, this study aimed to develop and validate a standard area diagram set (SADs) to estimate the severity of GLS and to apply the SADs established in this study to evaluate the efficacy of fungicides for GLS control in field trials. The SADs with nine levels of severity (0.5; 2; 5; 10; 20; 30; 40; 50 and 60%) improved accuracy, precision and reliability. To evaluate the control of GLS in corn, two experiments spraying chemical fungicides were conducted, one in Rio Verde (GO) (experiment A) and another in Chapadão do Sul (MS) (experiment B). Both experiments were conducted in randomized block design, with 10 treatments and four replicates. For the severity assessment, the SADs elaborated in this study was used. From this severity data over time, disease progress curves, area under the disease progress curve (AUDPC), and yield were obtained. Based on the AUDPC and yield values, all treatments differed from the control. The fungicide fluxapyroxad + pyraclostrobin + mefentrifluconazole had the highest value, giving 72.8% control efficacy and the highest yield maintenance of 43.5%. The other treatments had an intermediate control efficacy and yield. The SADs proposed here is a useful tool for improving visual assessments of GLS severity on corn leaves and fungicides can be used for integrated disease management.

KEYWORDS
chemical control, corn pathology, epidemiology, foliar disease, phytopathometry

1 | INTRODUCTION

Corn (*Zea mays* L.) is one of the most cultivated cereals worldwide (Da Silva Timm et al., 2023), considered an important crop, and it is used in several fields, including the food industry, animal production and agricultural production (Wang et al., 2020). In 2022, global production of this grain was 1.227 billion tons, making it the second most produced crop in the world, only behind sugar cane. Brazil was responsible for the production of 109.4 million tons of corn in 2022, occupying third place in the ranking of producing countries, behind the leaders United States and China (FAO, 2022).

The yield potential of corn crops is influenced by the occurrence of epidemics of multiple foliar diseases (Munkvold & White, 2016). Due to the increase in consecutive crops, notably the second season, epidemics of grey leaf spot (GLS) have become frequent, with a more widespread distribution, limiting the sustainable production of corn (Custódio et al., 2020).

GLS was first identified in the United States of America in 1924 (Munkvold & White, 2016), and the losses caused by this foliar disease can reach up to 80% (Casela et al., 2006; Cota et al., 2018; Custódio et al., 2020).

The first report of the disease in Brazil dates back to 1934, but the first epidemic records occurred in the southwest of Goiás in the first crop season of 1999/2000 and in the second crop season of 2000 (Reis et al., 2004). The high severity of this disease, at epidemic levels, occurs in the main Brazilian corn-producing regions (Brito et al., 2008; Juliatti et al., 2004), mainly in monoculture areas in no-tillage system (Reis et al., 2004).

In Brazil, GLS is predominantly caused by the fungi *Cercospora zeina* Crous & U. Braun 2006 and *Cercospora zeae-maydis* Tehon & E.Y. Daniels 1925 (Neves et al., 2015), a representative of the Ascomycota phylum. It is a facultative parasite, and during the saprophytic phase the pathogen survives in cultural remains or in soil organic matter (Munkvold & White, 2016).

Normally, the first symptoms are observed two to three weeks before the phenological vegetative tassel (VT) emergence stage in leaves of the lower third of plants close to the source of pathogen inoculum in cultural remains (Ward et al., 1999; Wise et al., 2016). After tassel emergence, in susceptible hybrids, the disease can develop rapidly and advance to the middle and upper third of the plants. Symptoms may vary depending on the host genotype. In some cases, the lesions may have dark edges or a yellow halo. Initial lesions are difficult to identify, but old lesions are easier, as they typically have a rectangular appearance, delimited by the veins of the leaf (Munkvold & White, 2016). These lesions are brown or light grey in colour and in highly susceptible hybrids they present extensive necrotic areas (Custódio et al., 2019; Latterell & Rossi, 1983). Highly susceptible hybrids may present symptoms covering more than 50% of the plant's leaf tissue area and can even be observed on the cob bracts. In the field, the best time to inspect symptoms is during the pre-tassel vegetative stage with 10 leaves (V10) or before and the dent reproductive stage (R5) (Paul & Munkvold, 2005; Ward et al., 1999).

To evaluate the effect of different control measures, we need to adopt efficient methods to quantify the disease. Thus, the intensity of the disease can be determined in three ways: incidence, severity, and prevalence. The choice for one of them will be based on the purpose of the study, as well as the pathosystem considered. For GLS, the intensity of the disease can be expressed by determining the severity, that is, by the proportion or percentage of diseased tissue in relation to the total tissue (Madden et al., 2007). As a strategy for quantifying diseases in this way, standard area diagram sets (SADs) stand out, helping to minimize errors during estimates by providing illustrated representations of the possible severity levels of the disease (Del Ponte et al., 2017, 2022). However, a SADs for the foliar assessment of grey leaf spot in corn pathology has not yet been developed, validated and used. Therefore, it is important that the developed and validated SADs is used to assist in estimating the severity of the disease and to evaluate the best control tool.

Genetic resistance is the most effective and widely used measure to control corn diseases (Munkvold & White, 2016). However, after the implementation of the crop, the use of foliar fungicides to control GLS in second-crop corn is necessary in South America. In Brazil, the high price in the corn market, higher levels of severity in the field caused by residues originating from no-tillage system and due to the availability of new fungicides in the last years justified chemical control such as one of the main control measures used to protect the yield potential of hybrids in the vegetative and reproductive phenological stages (Custódio et al., 2020; Pinto et al., 2004). Therefore, it is essential to determine the efficacy of control and yield maintenance provided by the fungicides currently recommended for the control of grey leaf spot.

The present work aimed to develop and validate a SADs to estimate the severity of GLS to evaluate the best control measures. Furthermore, the proposed SADs were used to evaluate the control efficacy of foliar fungicides in field trials and obtain important information for integrated disease control.

2 | MATERIALS AND METHODS

The study was conducted in Brazil, in two stages. Initially, a SADs was proposed to assess the severity of GLS in corn. Then, the control efficacy of nine fungicides over GLS was evaluated using the SADs previously developed. The development of the SADs was conducted at the epidemiology lab of Federal University of Paraná (UFPR), Curitiba, Paraná (PR), and Paraná Agricultural Development Institute (IDR-Paraná/IAPAR), Londrina, PR. The field study was conducted in two different areas: at the experimental station Campos Agricultural Research, in Rio Verde, Goiás (GO), and Chapadão Foundation in Chapadão do Sul, Mato Grosso do Sul (MS).

2.1 | Development and validation of a SADs for grey leaf spot severity assessment in leaves

To develop the SADs, 300 corn leaves with typical GLS symptoms, infected by natural inoculum, were collected and photographed from several fields and hybrids among the country to compose visible records of increasing lesions in the leaf tissue, so a wide range of severities was obtained to consider different disease intensities in the SADs.

The leaves were individually processed using a discriminant analysis on QUANT (Vale et al., 2003) software. Necrotic areas were considered diseased areas and green tissue and midvein as healthy areas. Based on the highest and lowest severity levels observed in these processed leaves, the SADs illustration was elaborated from a standard corn leaf with the aid of CorelDRAW Graphical Suite (2022) and PhotoImpression (ArcSoft, Inc., Califórnia, EUA) software to insert lesions and distribute them according to the pattern of disease development seen in the processed leaves. The severity intervals followed the linear distribution for the SADs development as suggested by Del Ponte et al. (2017).

For validation of the SADs, 50 images of corn leaves with different severities were projected on Microsoft PowerPoint for twenty inexperienced raters, previously instructed on how to assess disease severity. First, the raters had 30 s per leaf to assign severity values without the aid of the SADs. After 10 min of rest, the same images of the leaves were shown in another randomized order, and the raters estimated grey leaf spot severity with the aid of the SADs, also having 30 s per leaf.

The accuracy (υ – scale bias; u – location bias; C_b – bias coefficient factor), precision (r – correlation coefficient), agreement (ρc – Lin's concordance correlation coefficient) and inter-rater reliability (R^2 – coefficient of determination and ρ – intra-class correlation coefficient mean) of the estimates with and without the use of SADs were calculated as previously described by Dolinski et al. (2017). Lin's concordance correlation coefficient statistics (u, υ, C_b, r, and ρc) were estimated by the epi.ccc function of the epiR package (Stevenson et al., 2020). The built-in boot. sample R function was used for the

TABLE 1 Applied treatments to Rio Verde (GO) (experiment A) and another in Chapadão do Sul (MS) (experiment B).

Treatments[a]	FRAC[b]	Chemical group[c]	Active ingredient (a.i)	a.i. dose (g/L or g/kg)	Commercial product dose (% L or kg/ha)
T1	–	–	–	–	–
T2 (2)	11+3	DMI	Epoxiconazole	160	0.38
		QOI	Pyraclostrobin	260	
T3	M5	ISO	Chlorothalonil	720	2.0
T4 (3)	11+7+3	QOI	Azoxystrobin	93.19	1.0
		DMI	Propiconazole	116.39	
		SDHI	Pydiflumetofen	69.89	
T5 (2)	11+7	SDHI	Fluxapiroxad	167	0.35
		QOI	Pyraclostrobin	333	
T6 (2)	11+7+3	SDHI	Fluxapiroxad	88.9	0.60
		QOI	Pyraclostrobin	177.8	
		DMI	Mefentrifluconazole	133.3	
T7 (4)	11+ M3+ 3	QOI	Azoxystrobin	47	2.0
		DT	Mancozeb	597	
		DMI	Tebuconazole	56	
T8 (5)	11+3+M3	QOI	Azoxystrobin	120	0.60
		DMI	Tebuconazole	160	
			+	+	+
		DT	Mancozeb	800	1.50
T9 (4)	11+3+3+M5	QOI	Azoxystrobin	300	0.5
		DMI	Difenoconazole	200	
			+	+	+
		DMI	Tebuconazole	50	1.50
		ISO	Chlorothalonil	450	
T10	11+3+M5	QOI	Azoxystrobin	120	0.5
		DMI	Tebuconazole	240	
			+	+	+
		ISO	Chlorothalonil	720	1.50

[a]Treatments followed by a number had an adjuvant added to the application. [2]Soy methyl ester, 250 mL/ha; [3] Phosphate alkyl ester, 250 mL/ha; [4] Soy methyl ester, 375 mL/ha and [5] Mineral oil, 500 mL/ha.

[b]FRAC: mechanism of action code for the group according to the Fungicide Resistance Action Committee: 3, inhibitors of sterol biosynthesis upon demethylation; 7, inhibitors of mitochondrial respiration at succinate dehydrogenase complex II; 11, inhibitors of mitochondrial respiration at external quinone complex III; M3, multiple acting dithiocarbamates; and, M5, multiple acting chloronitriles.

[c]Codes of chemical groups: DMI (Demethylation inhibitors), Succinate dehydrogenase inhibitors (SDHI), Quinone outside inhibitors (QOI), ISO (isoftalonitrila) and DT (dithiocarbamates).

equivalence test. The ρ was estimated using the icc function of the irr R package (Gamer et al., 2019). The statistical analyses were performed using R software (R Core Team, 2023).

2.2 | Evaluation of the efficacy of foliar fungicides in controlling corn grey leaf spot under field conditions

Two experiments were conducted to evaluate the efficacy of fungicides in the control of GLS using the previously developed SADs for severity evaluations. Experiment A was conducted in the Campos Agricultural Research in Rio Verde, Goiás, in the geographical coordinates 17°47′06.75″ South and 50°59′55.15″ West, at 766m altitude, using commercial hybrid P3858 PWU susceptible to GLS. Experiment B, carried out at Chapadão Foundation, at the geographical coordinates 18°46′21.7″ South and 052°38′55.0″ West, altitude 840m, in Chapadão do Sul, Mato Grosso do Sul, using the commercial hybrid Formula VIP 2, also susceptible to GLS. Both experiments were conducted during the second corn crop season in Brazil. Experiment A was sown on 31 January 2022 and harvested on 30 June 2022 and experiment B began on 9 February 2022 and was completed on 14 July 2022. Both experiments had the plots harvested and the cultural remains destroyed.

The experimental design used was a randomized block design with 10 treatments composed of registered fungicides and four replicates. The fungicide treatments were single molecules, double mixtures, and triple mixtures (Table 1), without or with multi-site fungicides as an important anti-resistance strategy. Treatment 1 was the negative control, without fungicide application, and treatment 2 was the positive control, consisting of the fungicide used by Brazilian growers for many years. Additionally, treatment 3 was a positive control consisting of a multisite fungicide. For both experiments, fungicides were applied three times, the first at V8, the second at VT (pre-tassel), and the third 14days after VT. The plots for severity estimation and spray consisted of four 6m rows, with 6plants/m, and the plots harvested for yield evaluations consisted of two 4m central rows. No phytotoxicity symptoms due to the application of the tested products were observed.

To assess the severity of GLS in corn (10 plants/replicate), the seven leaves (from ear leaf −3 to +3) were observed and the severity was estimated in the leaf with greater severity with the aid of the SADs previously developed. Thus, at each evaluation, the leaf evaluated varied depending on which leaf had greater severity, following Custódio et al. (2019) procedure.

In experiment A, emergence occurred on February 4, 2022, and in experiment B on February 14, 2022. The first severity assessment was done 46days after emergence (DAE) on experiment A and 34 DAE on experiment B with no symptoms observed. Three other severity assessments (V8+15, V8+30, V8+45days) were performed until the harvest on experiment A, and four (V8+15, V8+30, V8+45, V8+60days) on experiment B. The harvest occurred at 120 DAE on experiment A and 150 DAE on experiment B.

From this severity data over time, disease progress curves, the area under the disease progress curve (AUDPC) (Shaner & Finney, 1977), control efficacy and yield maintenance were obtained. The control efficacy of fungicides was calculated using the formula $\%E=[(D-T)/D]\times 100$, where D is the AUDPC in the control treatment and T is the AUDPC in the fungicide treatments. Also, the yield data (kg/ha) were corrected for grain moisture, adjusted to 13% on a wet basis, and related to the number of plants harvested in each experimental plot. The yield maintenance was calculated using the formula $\%G=[(Y-T)/Y]\times 100$, where Y is the yield in the control treatment and T is the yield in the fungicide treatments (Abbott, 1925).

For the disease severity and yield, analysis of variance (ANOVA) was performed for each repetition of experiments A and B. Since the mean square of the residue showed a ratio of less than 7:1 (Banzatto & Kronka, 2013) between the repetitions of the experiments, a joint analysis was carried out with the repetitions of the experiments. In these cases, the transformations obtained were 1/2 for final severity and AUDPC. Subsequently, ANOVA was performed, and the means were compared using the Scott–Knott test at 5% probability. Statistical analyses were performed using the R environment (R Core Team, 2023).

3 | RESULTS

3.1 | Development and validation of SADs for grey leaf spot severity assessment in leaves

The proposed SADs allows for evaluating GLS severity in adult corn plants affected by the disease. Nine illustrations, covering the minimum (0.5%) and the maximum (60%) of grey leaf spot severity comprised the SADs (Figure 1). Based on estimated and actual severity, assessments made by the raters were closer to the actual values using the SADs (Figure 2), as shown by the lines. The absolute error of the estimates reduced significantly when the raters used the SADs (Figure 3).

The 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 2).

Inter-rater reliability of assessments by 20 raters was significantly improved. Without the SADs, the intra-class correlation coefficient mean (ρ) was 0.753, while using the SADs, this value was 0.917. In turn, the mean of the inter-rater coefficient of determination (R^2) of the pairwise comparisons were 0.727 and 0.879 without and with SADs, respectively (Table 3).

3.2 | Efficacy evaluation of foliar fungicides in controlling corn grey spot under field conditions

The proposed SADs were used to evaluate the screening of nine foliar fungicides for the control of GLS on leaves of plants in the vegetative and reproductive stages. The experimental fungicide

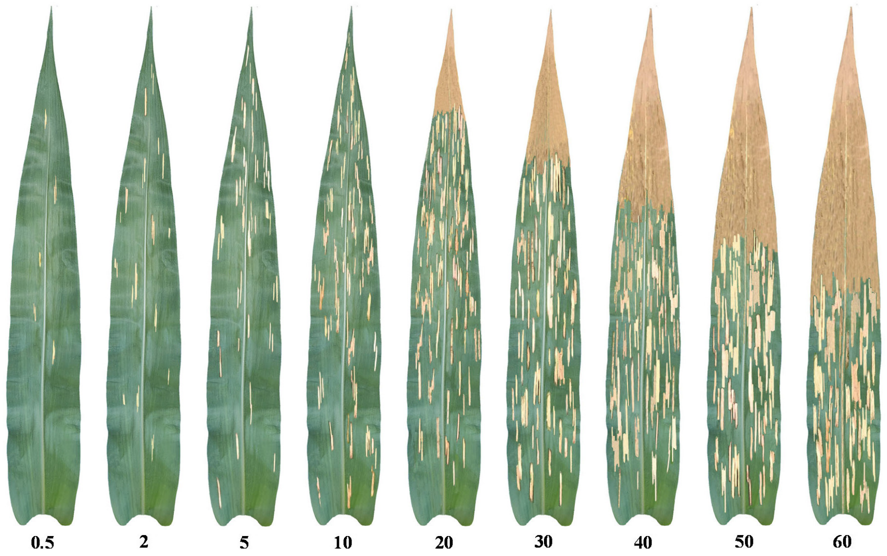

FIGURE 1 Standard area diagram set (SADs) to assess grey leaf spot (GLS) (*Cercospora* spp.) severity on corn leaves. Numbers represent the percentage (%) levels of diseased leaf area (typical lesions and necrosis). Green areas were considered healthy areas.

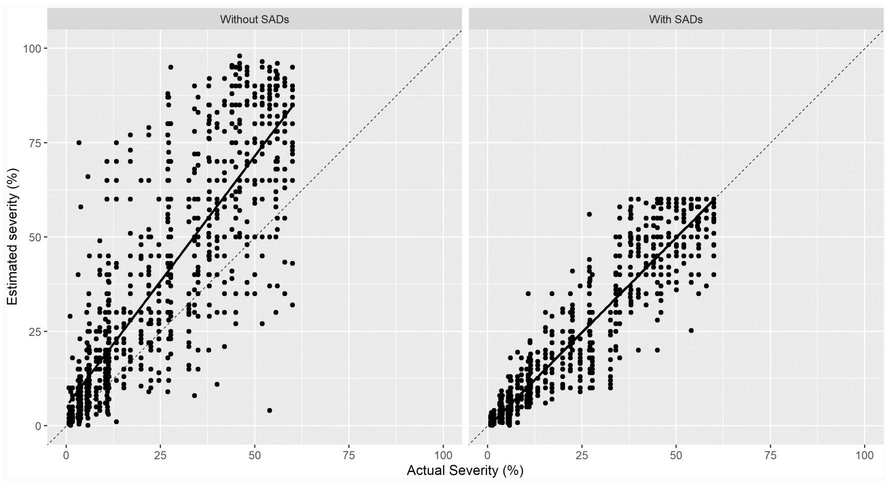

FIGURE 2 Relationship between actual and estimated severity of grey leaf spot on corn (*Cercospora* spp.) without and with the use of a standard area diagram set (SADs) for 50 diseased leaves by 20 raters.

treatments were composed of single molecules, double mixtures, and triple mixtures of active ingredients, without or with the association of multisite fungicides. In each plant, seven leaves were observed between the third leaf below the ear (FE_{-3}), the ear leaf itself (FE) and the third leaf above the ear (FE_{+3}).

The disease severity values over time in both experiments resulted in the disease progress curve. The highest values of GLS severity were observed in the control treatment with values of 43.1 and 17.0% in experiments A and B, respectively. However, the lowest severity values were observed in the treatment with a triple mixture of fungicides, Fluxapiroxad + Pyraclostrobin + Mefentrifluconazole (T6), which presented severity values equal to 25.7% and 0.47%, for experiment A and B, respectively (Figure 4). Other foliar diseases such as white spot and rusts occurred at a total severity of less than 3%.

Based on the joint analysis of the two trials, the analysis of variance showed a significant difference between the treatments with the fungicides tested ($p < .05$) for the control of grey leaf spot. To the Scott–Knott cluster analysis, the final severity and AUDPC values formed four groups of means, and the productivity values formed three groups of means. The control treatment (T1) had the highest final severity, with a value of 30.05%. On the other hand, the lowest severity values were observed in three treatments with double or triple mixtures of fungicides, being azoxystrobin + propiconazole + pydiflumetofen (T4), fluxapiroxad + pyraclostrobin (T5) and fluxapiroxad + pyraclostrobin + mefentrifluconazole (T6) which presented 14.44%, 13.76% and 13.09%, respectively (Table 4). The highest severity values were observed in fungicide treatments Azoxystrobin, Difenoconazole + Tebuconazole, Chlorothalonil (T9) and azoxystrobin + mancozeb + tebuconazole (T7), which presented 19.54 and 18.54%, respectively (Table 4). respectively (Table 4).

All AUDPC and yield values differed from the control treatment (T1) (Table 4). For AUDPC, four different statistical groups were formed while for yield only three. The fungicide fluxapiroxad + pyraclostrobin + mefentrifluconazole (T6) showed greater efficacy in controlling the disease, resulting in 7501.69 kg/ha of yield and 43.5% yield maintenance, the highest values observed among the other treatments. When comparing the treatment widely used by

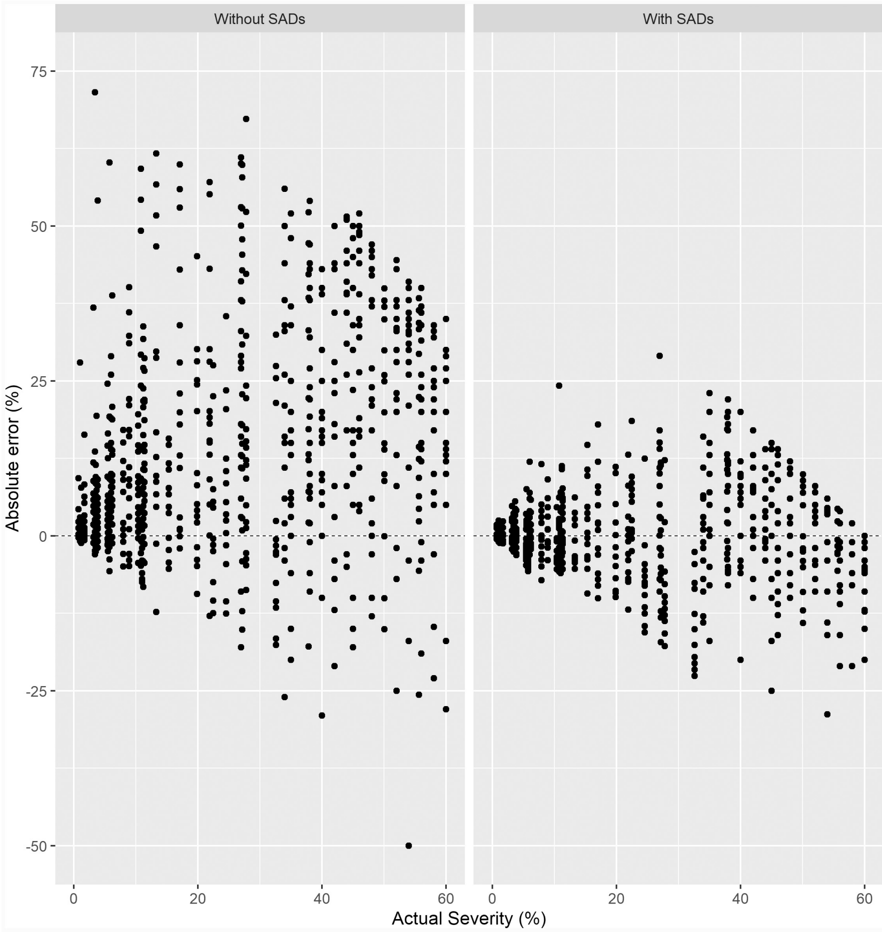

FIGURE 3 Absolute error (estimated severity minus actual severity) of the estimates without standard area diagram set (SADs) and with SADs for the 50 diseased leaves by 20 raters.

TABLE 2 Effect of using a standard area diagram set (SADs) as an assessment aid on the bias, accuracy, precision and agreement of severity assessments of corn grey leaf spot (*Cercospora* spp.), on 50 leaves as estimated by 20 raters.

	Means[a]			
Variables	Without SADs	With SADs	Difference between means[b]	95% CIs of the difference[c]
Scale (υ)[d]	1.474 (0.293)	1.056 (0.089)	−0.418 (0.068)	−0.549 to −0.285
Location (u)[e]	0.533 (0.362)	−0.018 (0.137)	−0.551 (0.084)	−0.715 to −0.384
Coefficient of bias (C_b)[f]	0.795 (0.136)	0.986 (0.010)	0.192 (0.030)	0.132–0.250
Correlation coefficient (r)[g]	0.899 (0.039)	0.951 (0.013)	0.052 (0.009)	0.034–0.069
LCCC (ρ_c)[h]	0.716 (0.134)	0.938 (0.016)	0.222 (0.029)	0.164–0.279

[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 the 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 bias correction factor (C_b) measures how far the best-fit line deviates from 45° 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.

producers epoxiconazole + pyraclostrobin (T2), the treatment with the best control efficacy (T6) showed a greater control efficacy by 17.40%.

Next, the foliar fungicides azoxystrobin + propiconazole + pydiflumetofen (T4) and fluxapyroxad + pyraclostrobin (T5) had the second and third highest control efficacy of 60.55% and 66.55%, respectively. These treatments presented yields of 6553.26 and 6971.68 kg/ha, respectively (Table 4). The other treatments had lower control efficacy than the other fungicides and presented an average of 45.6%.

4 | DISCUSSION

The fungus *Cercospora* spp. causes GLS, an endemic foliar corn disease that has become a reemergent problem for growers around the world such as in many places of Paraná, Goiás and Mato Grosso do Sul, Brazil (Custódio et al., 2020). SADs to identify and quantify the disease target under field conditions are essential to allow different types of studies on GLS, such as screening for foliar fungicide efficacy to the control in early (vegetative) and late (reproductive) stages of the corn plants. In our studies, the SADs developed improved accuracy, precision and reliability of grey leaf spot severity assessments. As a result, it constitutes a useful tool for estimating disease severity in different areas of knowledge such as epidemiological and fungicide efficacy studies, evaluation of disease management strategies and selection of pathogen-resistant genotypes for disease control.

TABLE 3 Inter-rater reliability of assessments of grey leaf spot by 20 raters on 50 leaves of corn without and with the use of a standard area diagram set (SADs).

Statistics	Without SADs	With SADs
Intra-class correlation coefficient (ρ)	0.753 (CI 0.661–0.833)	0.917 (CI 0.881–0. 946)
Mean inter-rater coefficient of determination (R^2)[a]	0.727 (0.418–0.921)	0.879 (0.722–0.963)
	Mean difference[b] = 0.152 (0.008), 95% CIs 0.137–0.167	

Note: Inter-rater reliability is measured by the intra-class correlation coefficient (ρ) and coefficient of determination (R^2).

[a]Mean coefficient of determination estimated from pairwise comparisons of assessments by all visual raters.

[b]Mean of the difference between each rating, with standard errors in parentheses (bootstrap calculated value), confidence intervals (CIs) were based on 10,000 bootstrap samples. If the CIs embrace zero, the difference is not significant ($\alpha = 0.05$).

SADs have been widely used as a tool to improve visual severity estimation of several foliar diseases in different crops (Del Ponte et al., 2017). For foliar corn diseases, the SADs have also improved visual estimates for eyespot (Camochena et al., 2008), white spot (Capucho et al., 2010; Malagi et al., 2011; Sachs et al., 2011), northern leaf blight (Lazaroto et al., 2012), diplodia leaf streak (Lorenzetti et al., 2019) and bacterial leaf streak (Braga et al., 2020) in field for adult plants. However, to our knowledge, no SADs had been developed to evaluate the severity of GLS in corn leaves.

In this context, the number of diagrams used in the SADs proposed for GLS is considered sufficient to obtain accurate, precise, and reliable severity estimates, as suggested by Del Ponte et al. (2017). Besides the development and validation of the SADs, it is important to standardize a methodology for severity assessment of foliar diseases in field experiments. This is because multiple experiments have been carried out in Brazil since 2016 through a cooperative network (https://www.fitossanidadetropical.org.br/informacoes-tecnicas/publicacoes) to know the control efficacy of foliar fungicides in different locations. Therefore, as well as SADs, a standardized methodology is also important to allow comparisons of the results from multiple experiments and even to make possible future meta-analytical studies of the historical series of this database.

The control effectiveness of the fungicides was evident by the lower AUDPC values and yield maintenance provided by all treatments in relation to the control without foliar fungicide. In Brazil, previous studies by Juliatti et al. (2004) and Pinto et al. (2004) tested fungicides from the QoI (quinone outside inhibitors) and DMI (sterol demethylation inhibitors) groups, demonstrating efficacy for

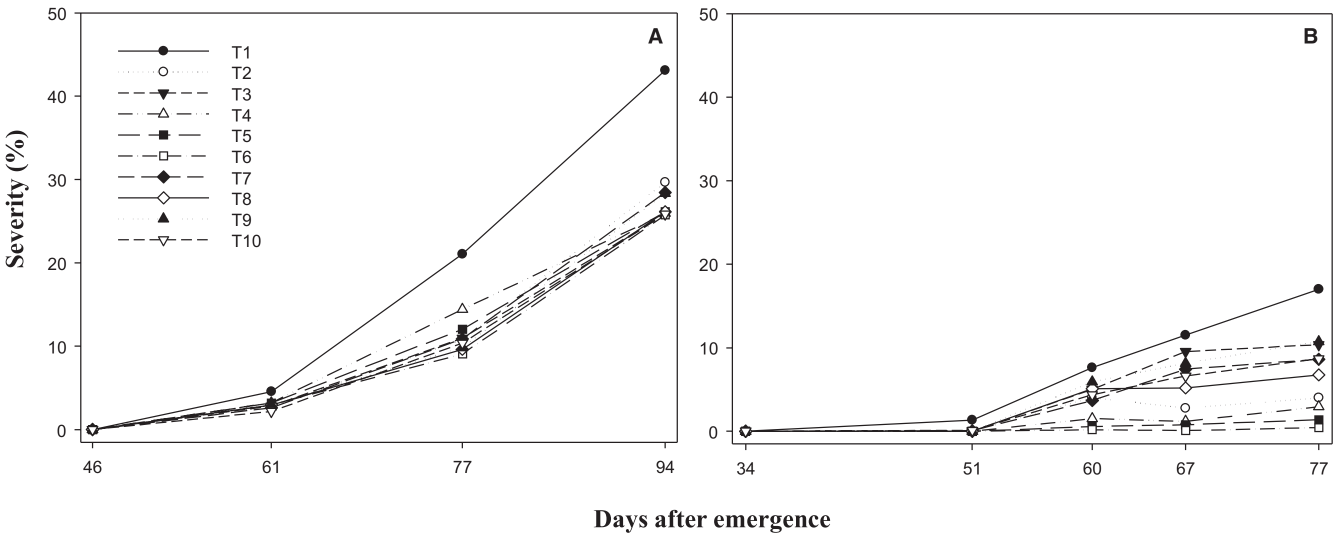

FIGURE 4 Disease progress curves of different fungicide treatments against grey leaf spot during the plant cycle. Experiment A (Rio Verde, GO) and experiment B (Chapadão do Sul, MS). Treatments: control (T1), epoxiconazole + pyraclostrobin (T2); chlorothalonil (T3); azoxistrobin + propiconazol + pydiflumetofen (T4); fluxapiroxad + pyraclostrobin (T5); fluxapiroxad + pyraclostrobin + mefentrifluconazole (T6); azoxystrobin + mancozeb + tebuconazole (T7); azoxystrobin + tebuconazole + mancozeb (T8), azoxystrobin + difenoconazole + tebuconazole + chlorothalonil (T9); azoxystrobin + tebuconazole + chlorothalonil (T10).

Treatments[1]	Final severity[2]		AUDPC[2]		%E	Yield		%M
T1	30.05	a[3]	522.77	a[3]	0.00	5219.40	c[3]	0.00
T2	16.83	c	274.62	b	55.35	6822.47	b	30.50
T3	18.13	b	309.02	b	38.45	6320.74	b	21.00
T4	14.44	d	272.65	c	60.55	6553.26	b	25.50
T5	13.76	d	244.21	c	66.55	6971.68	b	33.50
T6	13.09	d	209.37	d	72.75	7501.69	a	43.50
T7	18.54	b	299.33	b	44.35	6758.06	b	29.50
T8	16.44	c	272.70	b	50.10	6669.75	b	27.50
T9	19.54	b	310.33	b	38.70	6693.47	b	28.50
T10	17.28	c	280.57	b	46.80	6969.01	b	33.50
CV%	8.42		9.58			7.24		

[1] Treatments: control (T1), epoxiconazole + pyraclostrobin (T2); chlorothalonil (T3); azoxystrobin + propiconazole + pydiflumetofen (T4); fluxapyroxad + pyraclostrobin (T5); fluxapyroxad + pyraclostrobin + mefentrifluconazole (T6); azoxystrobin + mancozeb + tebuconazole (T7); azoxystrobin + tebuconazole + mancozeb (T8), azoxystrobin + difenoconazole + tebuconazole + chlorothalonil (T9); azoxystrobin + tebuconazole + chlorothalonil (T10).

[2] Data were transformed into $(x)^{1/2}$ for analysis but are presented in the original scale.

[3] Means followed by the same letters in the column do not differ by Scott–Knott test at 5% probability.

TABLE 4 Final severity, area under the disease progress curve (AUDPC), control efficacy (%*E*), yield (kg/ha) and yield maintenance (%*M*).

grey leaf spot control in corn. The difference in control between fluxapyroxad + pyraclostrobin + mefentrifluconazole (T6) and the other treatments can be explained because it is a triple mixture of active ingredients (carboxamide + strobilurin + triazole) (Custódio et al., 2020), combining the curative action of DMIs and preventive of QoIs and SDHIs (succinate dehydrogenase inhibitor) (FRAC, 2018).

The treatments azoxystrobin + propiconazole + pydiflumetofen (T4), fluxapyroxad + pyraclostrobin (T5) and fluxapyroxad + pyraclostrobin + mefentrifluconazole (T6) stood out in terms of control efficacy, and all these mixtures have an active ingredient from the carboxamide group. Silva et al. (2018) reported excellent performance of products combined with carboxamides in controlling diseases in corn in the field, including GLS, in addition to providing greater leaf area maintenance and greater yield. Recently, new fungicide formulations containing this second generation of carboxamides have been labelled and introduced for the management of corn foliar diseases in Brazil because they are considered medium risk for the emergence of fungicide resistance (Custódio et al., 2019, 2020).

The classes of fungicides that have been most used to control grey leaf spot are DMIs QoIs (AGROFIT, 2023). The continuous use of the same active ingredient or the same chemical group favours the development and predominance of pathogen populations resistant to fungicides. According to the FRAC (2018), DMIs represent a medium risk of selection of resistant isolates, and QoIs represent a high risk of selection of resistant isolates of fungal pathogens (Neves & Bradley, 2019). SDHIs fungicides prevent fungal activity by inhibiting the activity of the enzyme succinate dehydrogenase (SDH), a component of complex II of the mitochondrial electron transport chain (Kuhn, 1984). They have a medium risk of causing pathogen resistance to the fungicide, being a better alternative to managing this disease, in accordance with FRAC recommendations.

In the present work, the fungicide fluxapyroxad + pyraclostrobin + mefentrifluconazole (T6) was granted registration in Brazil in 2022. Mefentrifluconazole is the first isopropanol triazole fungicide developed and it has very good efficacy in controlling several pathogenic agents of the Ascomycota phylum in laboratory conditions, such as *Fusarium* spp. (Liu et al., 2022), *Alternaria alternata*, *Cercospora beticola* and *Zymoseptoria tritici* (Ishii et al., 2021). In field experiments with corn, mefentrifluconazole had good efficacy in controlling *Fusarium verticillioides* (He et al., 2023). To date in Brazil, there are few studies available on the control of GLS with triple mixtures of site-specific fungicides associated or not with fungicides with multisite activity in corn. Furthermore, difficult-to-control epidemics of corn stunt caused by mollicutes and viruses and fungal stalk rots complex (Costa et al., 2023) have increased the adoption of hybrid tolerant to these problems in recent years by Brazilian corn growers to the detriment of genetic resistance to leaf spots and rusts that can be controlled by highly effective foliar fungicides (Custódio et al., 2019). Therefore, the results of this study have important contributions to improve the integrated management of second-crop corn diseases, in particular to field evaluations aimed at controlling GLS by new foliar fungicides.

In conclusion, the SADs improved raters' ability to estimate grey leaf spot severity accurately, precisely, and reliably on corn leaves. All foliar fungicides controlled GLS, with emphasis on Fluxapyroxad + Pyraclostrobin + Mefentrifluconazole, which provided the highest yield maintenance.

AUTHOR CONTRIBUTIONS

M. G. C. Rocha: Conceptualization, Software, Formal analysis, Resources, Data Curation, Writing – Original Draft. A. A. P. Custódio: Conceptualization, Methodology, Resources, Writing – Review

& Editing, Supervision, Project administration. L. H. Fantin: Methodology, Resources, Investigation, Supervision. K. B. Oliveira: Methodology, Resources, Investigation, Supervision. H. D. Campos: Methodology, Investigation. Marcelo Giovanetti Canteri: Supervision. H. S. S. Duarte: Conceptualization, Writing – Review & Editing, Supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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DATA AVAILABILITY STATEMENT

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

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