UNIVERSIDADE FEDERAL DO PARANÁ

MARIA GABRIELA DE CARLOS DA ROCHA

DEVELOPMENT AND VALIDATION OF A STANDARD AREA DIAGRAM TO ASSESS CORN GRAY LEAF SPOT SEVERITY AND FOLIAR FUNGICIDE CONTROL EFFICACY

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Dissertação apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanidade, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Mestre em Ciências.

Orientador: Prof. Dr. Henrique da Silva Silveira Duarte

Coorientador: Pesq. Dr. Adriano Augusto de Paiva Custódio

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **MARIA GABRIELA DE CARLOS DA ROCHA** intitulada: **DEVELOPMENT AND VALIDATION OF A STANDARD AREA DIAGRAM TO ASSESS CORN GRAY LEAF SPOT SEVERITY AND FOLIAR FUNGICIDE CONTROL EFFICACY**, sob orientação do Prof. Dr. HENRIQUE DA SILVA SILVEIRA DUARTE, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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“Faça o teu melhor, nas condições que você tem, enquanto não tem condições melhores para fazer melhor ainda.”

(Mário Sergio Cortella)

RESUMO

A mancha de cercóspora (MC), causada por *Cercospora* spp, tornou-se uma doença foliar muito importante para a cultura do milho de segunda safra, principalmente na América do Sul, podendo causar perdas de até 80%. O estudo teve como objetivo desenvolver e validar uma escala diagramática (ED) para estimar a severidade da MC e aplicar a ED elaborada nesse estudo para testar, em condições de campo, a eficácia de fungicidas registrados no controle da MC. Para isso, o estudo foi realizado em duas etapas: primeiro o desenvolvimento e a validação da ED para a avaliação da severidade da MC; e segundo a aplicação da ED desenvolvida e validada para avaliação da eficácia de nove tratamentos envolvendo fungicidas químicos para o controle da MC em condições de campo. A ED com nove níveis de severidade (0,5; 2; 5; 10; 20; 30; 40; 50 e 60%) melhorou a acurácia e a precisão dos avaliadores. Os parâmetros estatísticos para os 20 avaliadores foram fator de correção do desvio - C_b (sem ED = 0,795, com ED = 0,986); coeficiente de correlação - r (sem ED = 0,899, com ED = 0,951); e coeficiente de correlação concordante de Lin - ρc (sem ED = 0,904, com ED = 0,975). Além disso, as estimativas tiveram maior reprodutibilidade com o uso da ED sendo coeficiente de correlação intraclasse - ρ (sem ED = 0,716, com ED = 0,938) e coeficiente de determinação - R^2 (sem ED = 0,727, com ED = 0,879). Em condições de campo, a ED proposta permitiu avaliar a severidade da MC com até 43% da área foliar afetada pela doença. A ED proposta foi uma ferramenta muito útil para melhorar as estimativas de severidade da MC em folhas de milho. Para avaliar o controle da MC no milho, foram realizados dois experimentos com pulverização de fungicidas químicos, em Rio Verde (GO) (experimento A) e Chapadão do Sul (MS) (experimento B). Ambos em delineamento de blocos casualizados, com 10 tratamentos e quatro repetições. Para a avaliação da severidade, foi utilizada a ED elaborada neste estudo. A partir dos dados de severidade, foram obtidas as curvas de progresso da doença, a área abaixo da curva de progresso da doença (AACPD) e a produtividade. Com base nos valores de AACPD triagem de nove tratamentos para a MC permitiu estabelecer quatro grupos de médias de eficácia de controle da doença. O fungicida Fluxapiroxade + Piraclostrobina + Mefentrifluconazole apresentou a maior média de controle, com 72,75% de eficácia e maior valor de manutenção de produtividade de 43,50%. Os outros tratamentos tiveram eficácia de controle e produtividade intermediárias. A ED proposta é uma ferramenta útil para melhorar as avaliações visuais da severidade da MC nas folhas de milho e os fungicidas testados podem ser usados para o manejo integrado desta doença.

Palavras-chave: Doença foliar; Controle químico; Epidemiologia; Fitopatometria.

ABSTRACT

Gray leaf spot (GLS), caused by *Cercospora* spp, has become a very important foliar disease for second-crop corn production, 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 registered fungicides for GLS control in field trials. For this purpose, the study was carried out in two steps: the development and validation of a SADs for GLS severity evaluation; and the screening in field conditions for GLS control of nine treatments using the proposed SADs developed in this study. The SADs with nine levels of severity (0.5; 2; 5; 10; 20; 30; 40; 50 and 60%) improved accuracy and precision. The statistical parameters for the 20 raters were: bias coefficient factor - C_b (no SADs = 0.795, with SADs = 0.986); correlation coefficient - r (no SADs = 0.899, with SADs = 0.951); and Lin's concordance correlation coefficient - ρc (no SADs = 0.904, with SADs = 0.975). In addition, estimates were more reliable: intra-class correlation coefficient - ρ (no SADs = 0.716, with SADs = 0.938) and inter-rater coefficient of determination - R^2 (no SADs = 0.727, with SAD = 0.879). In field conditions, the proposed SAD allowed evaluating GLS severity with up to 43 % of the leaf area affected by the disease. The SADs proposed was a very useful tool for improving visual assessments of GLS severity on corn old leaves. 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 screening of nine treatments for GLS allowed the establishment of four major groups of efficacy to the disease. The fungicide fluxapyroxad + pyraclostrobin + mefentrifluconazole had the highest value, giving 72.75% 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 the new fungicides that were tested can be used for integrated disease management.

Keywords: Foliar disease; Chemical control; Epidemiology; Phytopathometry.

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SUMMARY

1 INTRODUCTION

Corn (*Zea mays* L.) is one of the most cultivated cereals worldwide, important for human and animal nutrition and for industry (COSER, 2010). In 2021, global production of this grain was 1.403 billion tons, making it the second most produced crop in the world, only behind sugar cane (FAO, 2021). Brazil was responsible for the production of 113.1 million tons of corn in 2021, occupying third place in the ranking of producing countries, behind the leaders United States and China (CONAB, 2022).

The productive 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 second seasons, epidemics of cercospora spot (CS or GLS – gray leaf spot) have become frequent, with a more widespread distribution, limiting the sustainable production of corn (CUSTÓDIO et al., 2020). This foliar disease can cause losses of up to 80% (CASELA, 2006; COTA et al., 2018; CUSTÓDIO et al. 2020). Gray leaf spot was first identified in the United States in 1924 (MUNKVOLD & WHITE, 2016).

In Brazil, gray leaf spot is mainly 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 ascomycete 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). The first report of the disease in the country dates back to 1934, but the first epidemic records occurred in the southwest of Goiás in the first season of 1999/2000 and in the second season of 2000 (REIS et al., 2004). The high severity of this disease, at epidemic levels, occurs in the main Brazilian corn producing regions (JULIATTI et al., 2004; BRITO et al., 2008), mainly in monoculture areas in direct planting system in straw (REIS et al., 2004).

Normally, the first symptoms are observed two to three weeks before the phenological tassel emergence stage (VT), 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 gray in color and in highly susceptible hybrids they present extensive necrotic areas (LATTERELL & ROSSI, 1983; CUSTÓDIO et al., 2019). 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 et al 2005; WARD et al., 1999).

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 gray leaf spot in second-crop corn is necessary in South America. In Brazil, it is one of the main control measures used to protect the yield potential of hybrids in the vegetative and reproductive phenological stages of corn (CUSTÓDIO et al., 2022; 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 gray leaf spot, whether synthetic, phytochemical, microbiological or plant resistance inducer products.

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 (AMORIM & BERGAMIN FILHO, 2011). For gray leaf spot, 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. 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 (NUTTER et al., 1991; BERGAMIN FILHO & AMORIM, 1996; LOPES et al., 2014). However, SADs for the foliar assessment of corn gray leaf spot has not yet been developed and validated. 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 measures.

The present work aimed to develop and validate a SADs to assist in estimating the severity of gray leaf spot on corn under field conditions. Furthermore, the proposed SADs was used to evaluate the control efficacy of registered foliar fungicides and obtain important information for integrated disease control.

2 LIERATURE REVIEW

2.1 CORN CROP IN BRAZIL

Corn has been an important cereal for humans since the Paleolithic era, and in the Americas since the Holocene (CORTELETTI, 2015). Its importance in the world market today demonstrates its important role as one of the main commodities.

Cultivated in all regions of the country, corn is present in more than two million agricultural establishments (MIRANDA, 2018). According to Hugo (2016), corn is the second most important crop in agricultural production in Brazil, surpassed only by soybeans, which leads grain production in the country.

Since the 2017/18 season, Brazil has been the third largest corn producer in the world, behind the USA and China, being among the two largest exporters with approximately 18% of international trade in this period (SOUZA et al., 2023). Production in the national territory occurs in the first crop season, with sowing concentrated in spring and summer, and in the second crop season, with sowing during summer and autumn. The second crop, an important initiative that started in the southern region of the country, fits into a context of crop succession, generally cultivated after soybean sowing and harvesting, promoting an alternative crop. For producers, this crop came as a new option for harvesting in autumn-winter; however, only a small portion of national production adopted it immediately, a fact that named this crop as "safrinha" and "2nd crop" or "winter crop" (MATTOS & SILVEIRA, 2018).

According to Helfand & Rezende (1998), the expansion and establishment of corn in the second crop season were linked to the greater adoption of direct planting of soybeans since there was a need for a successor crop to contribute to soil coverage after harvest of the legume. Furthermore, the development of earlier soybean cultivars and more efficient inputs contributed to the dynamism of the 2nd crop of corn in Brazil, allowing an increase in total grain yield and the intensification of land use (SANCHES et al., 2019).

The establishment of the second-crop corn in Brazil had numerous effects. This greater availability of grain promoted a change in the relationship between internal supply and demand throughout the year. What once reached the market only in the first half of the year, was now also offered in the second half of the year, a fact that enabled Brazil's growing participation in world exports of corn (SOUZA et al., 2023). As a reflection of Brazil's growing role in global trading of this cereal, the sector attracted greater participation from growers, which increased the volume of trading on the Brazilian stock exchange, B3 (Brasil, Bolsa, Balcão). B3 records show that corn futures contracts went from 364 thousand in 2010 to 3.4 million in 2021, which represents an annual growth of 22% (Brasil, Bolsa e Balcão, 2022).

Thus, there was significant growth in the second crop, especially in the Central-West. Corn production in this region went from an annual average of 3.2 million tons in the 1980s to 38.7 million in the 2010s, with around 92% of this last number referring to the second crop. Mato Grosso stands out in this context – with production concentrated in the second crop, it accounted for 27% of national production in the 2010s; consequently, the share of corn production in the Central-West in relation to the national total increased from 14% to 47% between 1980 and 2010 (SOUZA et al., 2023).

For the 2022/23 season, a record total production in the country's history is expected. Between the first, second and third crops, it is estimated that 128 million tons of this grain will be harvested. A slight drop in the summer harvest was observed when compared to the previous season, but with an increase in both area and productivity in relation to the second crop (CONAB, 2022). This performance, in addition to showing the importance of the corn agro-industrial chain in Brazilian agriculture, places the country among the biggest players in the production and export of the commodity on the world market.

Currently, the state of Mato Grosso stands out as the largest producer of Brazilian corn, followed by Paraná and Mato Grosso do Sul. Mato Grosso do Sul is expected to surpass Goiás in the current crop, followed by Minas Gerais. Mato Grosso's production is even higher than that of each other region of the country. In Paraná, the 22/23 season was completed, with more than 10% reduction in yield, mainly due to pests and drought (CONAB, 2021).

Among the factors that can contribute to the reduction in the yield of agricultural crops, it is common to list abiotic causes, such as water stress, nutritional deficiency and adverse weather conditions, and biotic causes, such as insects and diseases.

In corn cultivation, there are several foliar diseases that occur during the growth and development cycle. The most common foliar diseases are Southern corn rust (SCR) (*Puccinia polysora* Underw. 1897), Northern corn leaf blight (NCLB) (*Exserohilum turcicum* (Pass.) K.J. Leonard & Suggs 1974), Southern corn leaf blight (SCLB) (*Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker 1959) cercospora leaf blight or gray leaf spot (*Cercospora* spp.) and white spot disease of corn (*Pantoea ananatis* corrig. (Serrano 1928) Mergaert et al. 1993 or *Phaeospharia maydis* (Henn.) Rane, Payak & Renfro 1967) (COTA et al., 2013) (CASELA et al., 2006). The importance of each of these diseases varies depending on the crop and the region, so it is not possible to say that any of them is of greater importance in relation to the others (CASELA et al., 2006).

Among the diseases, gray leaf spot is one of the main foliar diseases of corn crops in Brazil, both due to the potential damage caused and its wide distribution, being found in all producing regions (BRITO et al., 2008).

In Brazil, gray leaf spot was reported for the first time in 1953 (CHUPP, 1953) and, for a long period, it was considered a secondary disease, due to its sporadic occurrence and low severity. At the end of the 1990s, epidemics were reported in the southwest of Goiás in municipalities such as Rio Verde and Jataí (GO), starting a series of epidemic outbreaks throughout the central-southern region of Brazil. These alerted growers and research institutions (BRUNELLI, 2004) and was responsible for a greater adoption of foliar fungicides in commercial corn crops.

2.2 GRAY LEAF SPOT OF CORN

Gray leaf spot of corn in Brazil is caused by the etiological agents *Cercospora zeina* Crous & U. Braun 2006, *Cercospora zeae-maydis* Tehon & E.Y. Daniels 1925, with *C. zeina* predominant over other species in the regions with the highest corn production in the country (NEVES et al., 2015).

Data showed that the incidence of this disease has increased in several parts of the world, resulting in corn production damage of up to 25% in endemic regions of the USA and 65% in susceptible hybrids in South Africa (DONAHUE et al., 1991, WARD et al., 1999). This reduction occurs due to the attack of the pathogen on the leaves, thus reducing the photosynthetic area. Indirectly, the weakening of the plant also occurs, which favors stem rot, leading to lodging.

Cercospora zeae-maydis and many other species of the genus *Cercospora* spp. produce a phytotoxin, cercosporin, which acts as a photosensitizing agent in leaf cells, causing death in the presence of light. This toxin results in the production of reactive forms of oxygen that damages cell membranes and causes loss of electrons (AGRIOS, 1997).

In periods of high humidity, the fungus present in crop residues sporulates, producing conidia that are disseminated by the wind, infecting corn plants, with the lower leaves being the primary sites of infection. Under these favorable environmental conditions of high relative humidity and in the presence of dew, the lesions resulting from the initial infection produce spores that will be transported by wind or rain splashes to the upper leaves. A favorable condition for infection and lesion development is the formation of a microclimate, which forms 1-2 mm above the leaf blade. By remaining saturated with humidity for a long period of time, it does not require continuous periods of high relative humidity for the infection to occur, since the pathogen can remain latent until favorable environmental conditions return (WARD et al., 1999, KOSHIKUMO, 2007).

The latent period for gray leaf spot varies from 14 days for more susceptible hybrids to 21 days for moderately resistant hybrids. The rate of development of gray leaf spot is determined by three factors that interact in time and space: the initial amount of inoculum or disease; the reproduction rate of the pathogen in the same culture cycle and the proportion of healthy tissue to be infected. In this way, the survival of the pathogen in crop residues on the soil surface contributes to an increase in the severity of the disease in the same area (CASELA & FERREIRA, 2003).

The characteristic symptom of the disease is the linear-rectangular appearance of the lesions, which are generally delimited by leaf veins (LATTERELL & ROSSI,1983). These spots are brownish to olive green in color, and, under conditions of high relative humidity, they become covered with spores, giving them a grayish color. Due to this characteristic, it is known as gray leaf spot (CHUPP, 1953).

The onset of symptoms normally occurs during the flowering phase with the colonization of the leaf blade in the lower third of the plant, causing extensive necrotic areas (LIPPS, 1987; WARD et al., 1999). According to Casela and Ferreira (2003), the severity of gray leaf spot increases in conditions of high relative humidity (90%) and daytime temperatures between 22 and 32 °C, as well as cold nights and dew formation. The fact that the pathogen colonizes a large part of the leaf tissue reduces the photosynthetic area, leading to early senescence and, consequently, reduced productivity (BRITO et al., 2007). In an experiment carried out in the state of São Paulo, during the off-season from 2004 to 2008, Fantin et al. (2008) evaluated the influence of gray leaf spot severity on corn yield in more than 44 simple and triple hybrids and demonstrated that, even under conditions of low disease severity, there is a significant reduction in crop yield.

To conduct studies on the epidemiology of this pathosystem, control efficacy, damage assessment, and disease progress curve modeling, it is necessary to use efficient tools to quantify the disease in the field (LOPES et al., 2014).

2.3 QUANTIFICATION OF PLANT DISEASES

The quantification of plant diseases is a fundamental part of the correct interpretation of epidemiological and control studies. The most used measures are incidence and severity. The decision on which type of measure to use will depend on the characteristics of the pathosystem, the objectives and the time available for the assessment (NUNES & ALVES, 2012; AMORIM & BERGAMIN FILHO, 2011).

To quantify the severity of diseases, standard area diagram sets (SADs) are used as tools to help evaluators estimate the percentage of disease in the plant with precision and accuracy (CAMPBELL & MADDEN, 1990). These SADs contain diagrams with increasing levels of disease severity that are used for the evaluator to guide their assessments, allowing to estimate intermediate severity values from those contained in the scale (LOPES et al., 2014).

After obtaining plant material (leaves, fruits, whole plants, etc.) with symptoms of the disease to which the SAD is going to be developed, there are different methodologies for its preparation. The traditional method, which most diagrammatic scales followed, considers the following steps: I) determine the real severity of each plant sample; II) determine the severity values that the SAD will cover, corresponding to the maximum intensity of the disease and the pattern of injuries observed in the field and III) determine the intermediate levels of the SAD (DUARTE et al., 2014). This last topic can follow two different methodologies: the traditional one, which spaces these severity levels by logarithmic values, or the modern methodology currently adopted by researchers, in which the intervals between the diagrams must be linearly distributed (NUTTER & ESKER, 2006; BOCK et al., 2009).

The traditional method elaborates severity levels based on the Webber-Fechner law, which proposes that the visual acuity of the human eye is proportional to the logarithm of the stimulus intensity (HORSFALL & BARRATT, 1945). Thus, this law states that the human eye is more accurate in quantifying extreme values of severity than intermediate values, and, therefore, logarithmic values are used in SADs levels. However, with the advancement of knowledge, and the need for closer severity intervals to evaluate diseases in the field, the modern method has been used more in recent years in SAD published in scientific journals (DUARTE et al., 2014.; DEL PONTE et al., 2017; VIDAL et al., 2019; FRANCESCHI et al., 2020; BRAGA et al., 2020;)

In order for the prepared SAD to be used and recommended as a standard method for quantifying the severity of a disease, it is necessary to validate it. This process requires a wide sampling of symptomatic leaves, so that the severity levels represented in the proposed diagram are covered. This set of leaves with symptoms of the disease has the severity estimated by evaluators without, and later with the use of SAD, so that the performance of estimated values can be compared with and without the aid of SAD to obtain results on improvement in the accuracy, precision, and reproducibility of estimates. After this process, it is possible to know whether the scale produces satisfactory results and guarantees a significant improvement in the severity estimate. If not validated, the SAD must be created and validated again (KRANZ, 1988; BERGAMIN FILHO, 2010; DUARTE et al., 2014).

Among the parameters evaluated in the validation of a SAD, accuracy refers to the proximity of the estimated values of a given set of samples in relation to the real value, previously determined (VALE et al., 2003). Thus, in an accurate method, the estimated values are very close to the real values, so that overestimates and underestimates do not prevail. Precision refers to repeatability, that is, a repetitive method is one that presents a low variation associated with estimating the quantity of the disease (BOCK et al., 2010). Reproducibility refers to the variability of estimated values between different evaluators combined in pairs. Therefore, a reproducible method is one in which the values estimated by different evaluators are close to each other (YADAV et al., 2013). In disease assessment, both accuracy and precision are important to correctly evaluate management strategies, quantify and model the progress of disease in time and space, predict future quantities of disease, and elucidate the relationship between injury and harm (NUTTER et al., 1991; MADDEN & NUTTER, 1995).

The progress of gray leaf spot generally results from the occurrence of two distinct processes; increase in the number of lesions per unit of leaf area through new infections and the increase in the size of existing lesions through expansion or coalescence of lesions (KOSHIKUMO, 2007). The use of a standard area diagram to assess severity of this disease can be useful to conduct field experiments for epidemiological studies.

2.4 GRAY LEAF SPOT CONTROL

The yield potential of corn is influenced by the occurrence of epidemics of multiple foliar diseases, especially in susceptible hybrids (MUELLER et al., 2013; MUNKVOLD; WHITE, 2016; WISE et al., 2016). Gray leaf spot epidemics have become typical, mainly due to the increase in second-crop season. Currently, this endemic foliar disease, of multiple occurrence and more widespread distribution, limits the sustainable production of corn (REIS et al., 2004; FANTIN & DUARTE, 2009; CUSTÓDIO et al., 2019).

An effective method of managing gray leaf spot involves the integration of a series of practices, such as the use of more resistant hybrids available on the market, rotation with non-host crops such as soybeans and wheat, managing straw from previous harvests with practices to accelerate the decomposition of crop residues, and application of foliar fungicides (CASELA et al., 2006; REIS et al., 2004; VIEIRA et al., 2011, COTA et al., 2018).

Genetic resistance is the most effective and used control measure not only for gray leaf spot, but for most corn diseases (MUELLER et al., 2013; WISE et al., 2016). However, after susceptible hybrids of corn are already sowed and conditions favorable to infection by *Cercospora* spp. occur, application of a foliar fungicide is the main way to control the disease. The main classes of fungicides that have been used to control gray leaf spot are demethylation inhibitors (DMI) and quinone outside inhibitors (QoI) (NEVES & BRADLEY, 2019; BRADLEY & AMES, 2010; PINTO et al., 2004; WARD et al., 1999; AGROFIT, 2023). However, recently new options for fungicide molecules and classes have been introduced in Brazil (CUSTÓDIO et al., 2020).

The frequency of fungicide use in commercial corn crops has increased in recent years also due to high grain prices on the international market (COSTA et al., 2012, WISE et al., 2016, CUSTÓDIO et al., 2019). The use of these products in corn cultivation is recommended in potential situations of high disease severity, which result from a combination of factors such as: use of susceptible genotypes, climatic conditions favorable to the development of diseases, direct planting without crop rotation and continuous sowing of corn in the area (COSTA et al., 2021).

Foliar fungicides will contribute to maintain corn yield by acting on crop productivity components, such as the number of plants per area, number of ears per plant, number of rows per ear, number of grains per row and thousand grain weight. Thus, the productive potential of corn is defined until vegetative tassel (VT), a stage at which the yield component number of grains per row has already been established. From that moment on, the production potential will only be preserved through grain filling. Therefore, fungicide applications in the pre-tassel and post-tassel phases will interfere with the last component of yield and will act to preserve the productive potential of the crop through protection against damage caused by foliar diseases. In that way, the application of fungicides prevents crop losses due to the protection provided during the grain filling period. Considering that the leaves above the ear contribute, on average, for more than 90% of the production of corn, the presence of disease symptoms on this leaf, in susceptible hybrids, associated with climatic conditions favorable to the development of diseases, indicate the need for the application of fungicides (COTA et al., 2021).

In Paraná and several states in Brazil, the fungicides registered for the control of corn foliar diseases are, for the most part, site specific, belonging to the chemical groups of triazoles, strobilurins, and more recently carboxamides. These active ingredients are formulated alone or in double and triple mixtures, associated or not with multisite fungicides (AGROFIT, 2023). In several studies, there were reports of the efficacy of fungicides from the triazole plus strobilurin groups in corn production, with an increasing reduction in the severity of the disease (JULIATTI et al., 2004; BRITO et al., 2007; COSTA, 2007; CUSTÓDIO et al., 2020, COTA et al., 2021, BRAGA, 2022). However, the constant use of the same active ingredient or chemical group can select resistant isolates and make future disease control in crops difficult (FRAC, 2018). Therefore, studies to compare the efficacy of different fungicides for controlling leaf spots and corn rust are important.

3 MATERIAL AND METHODS

The study was conducted in two stages. Initially, a SADs was proposed to assess the severity of GLS in corn. Then, the control efficacy of nine treatments over GLS was evaluated using the SADs previously developed. The development of the SAD was conducted at the epidemiology lab of Universidade Federal do Paraná (UFPR), Curitiba, PR, and Instituto de Desenvolvimento Rural do Paraná - IAPAR-EMATER (IDR-Paraná), Londrina, PR. The field study was conducted at the experimental station Campos Pesquisa Agrícola, in Rio Verde, GO, and Fundação Chapadão in Chapadão do Sul, MS.

3.1 DEVELOPMENT AND VALIDATION OF A SADS FOR GRAY LEAF SPOT SEVERITY ASSESSMENT IN ADULT LEAVES

To develop the SADs, corn leaves with typical GLS symptoms, infected by natural inoculum, were collected and photographed 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” software. Necrotic areas were considered diseased areas and green tissue and midvein as healthy areas. Based on the highest and lowest severity level observed in these processed leaves, the SADs illustration was elaborated from a standard corn leaf with the aid of CorelDRAW Graphical Suite (2022) and Photo Impression (6.0 version) 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 method of ED development.

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, with 30 seconds per leaf, the raters had 30 seconds 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 gray leaf spot severity with the aid of the SADs, also having 30 seconds per leaf.

The accuracy, precision, and inter-rater reliability of the estimates with and without the SADs were calculated as previously described (DOLINSKI et al., 2017). The statistical analyses were performed using R software (R Core Team 2023). The LCCC statistics were estimated by the epi.ccc function of the epiR package (STEVENSON et al., 2020). The builtin boot. sample R function was used for the equivalence test. The ρ was estimated using the icc function of the irr R package (GAMER et al., 2019).

3.2 EVALUATION OF THE EFFICACY OF REGISTERED FOLIAR FUNGICIDES IN CONTROLLING CORN GRAY 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 Agricultural Research Field in Rio Verde, Goiás, in the geographical coordinates 17º 47' 06.75" S and 50º 59' 55.15" W, at 766m altitude, using hybrid P3858 PWU. Experiment B, carried out at Fundação Chapadão, at the geographical coordinates 18º 46' 21.7" S and 052º 38' 55.0" W, altitude 840m, in Chapadão do Sul, Mato Grosso do Sul, used the hybrid Formula VIP 2. Both experiments were conducted during the second corn crop season in Brazil. Experiment A was installed on 01/31/2022 and harvested on 06/30/2022 and experiment B began on 02/09/2022 and was finished on 07/14/2022. 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 4 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. Additionally, treatment 3 was a positive control showing multisite activity. Treatment 1 was the negative control, without fungicide application, and treatment 2 was the positive control, consisting of the standard product used by growers for many years. For both experiments, fungicides were applied three times, the first at V8, the second before at VT (pre-tassel), and the third 14 days after VT. The plots for severity estimation and spray consisted of four 6m rows, with 6 plants/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.

TABLE 1 – APPLIED TREATMENTS TO EXPERIMENTS A AND B

Treatments	**FRAC[1]**	**Active Ingredient (a.i)**	**a.i. dose (g/l or kg/kg)**	**Commercial product dose (% L or Kg)/ha**
T1	-	-	-	-
T2	11+3	Epoxiconazole Pyraclostrobin[2]	160 260	0.38

T3	M5	Chlorothalonil	720	2.0
T4	11+7+3	Azoxystrobin Propiconazole Pydiflumetofen[3]	93.19 116.39 69.89	1.0
T5	11+7	Fluxapiroxad Pyraclostrobin[2]	167 333	0.35
T6	11+7+3	Fluxapiroxad Pyraclostrobin Mefentrifluconazole[2]	88.9 177.8 133.3	0.60
T7	11+ M3+ 3	Azoxystrobin Mancozeb Tebuconazole[4]	47 597 56	2.0
T8	11+3+M3	Azoxystrobin Tebuconazole + Mancozeb[5]	120 160 + 800	0.60 + 1.50
T9	11+3+3+M5	Azoxistrobin Difenoconazole + Tebuconazole Chlorothalonil[4]	300 200 + 50 450	0.5 + 1.50
T10	11+3+M5	Azoxystrobin Tebuconazole + Chlorothalonil	120 240 + 720	0.5 + 1.50

SOURCE: The author (2023).

LEGEND:

[1] 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.
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

[5] Mineral oil, 500 ml/ha

To assess the severity of GLS in corn, 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.

In experiment A, emergence occurred on July 2, 2022, and in experiment B on July 2, 14, 2022. The first severity assessment was done at the V8 corn stage, 46 days 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+45 days) were performed until the harvest on experiment A, and four (V8+15, V8+30, V8+45, V8+60 days) 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, area under the disease progress curve (AUDPC) (SHANER & FINNEY, 1977), control efficacy, and yield maintence were obtained. The control efficacy of fungicides was calculated using the formula %E = [(D-T)/D]x100, where D is the AUDPC in the control treatment and T is the AUDPC in the fungicide treatments. For the disease severity data, 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 with a 5% probability of error. Statistical analyses were performed using the R environment (R Core Team 2022).

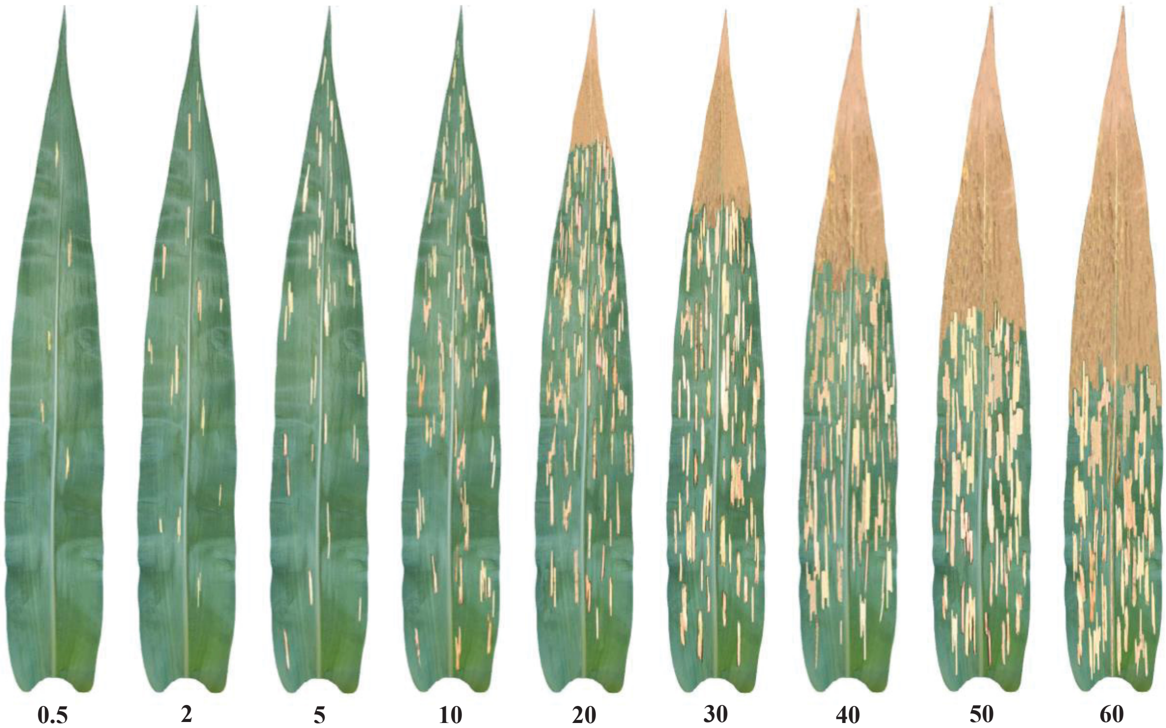
Also, the yield data (kg ha^{-1}) were corrected for grain moisture, adjusted to 13% on a wet basis, and related to the number of plants harvested in each experimental plot. The yield maintance was calculated using the formula %G = [(Y-T)/Y]x100, where Y is the yield in the control treatment and T is the yield in the fungicide treatments.

4 RESULTS

4.1 DEVELOPMENT AND VALIDATION OF A SADs FOR GRAY LEAF SPOT SEVERITY ASSESSMENT IN ADULT LEAVES

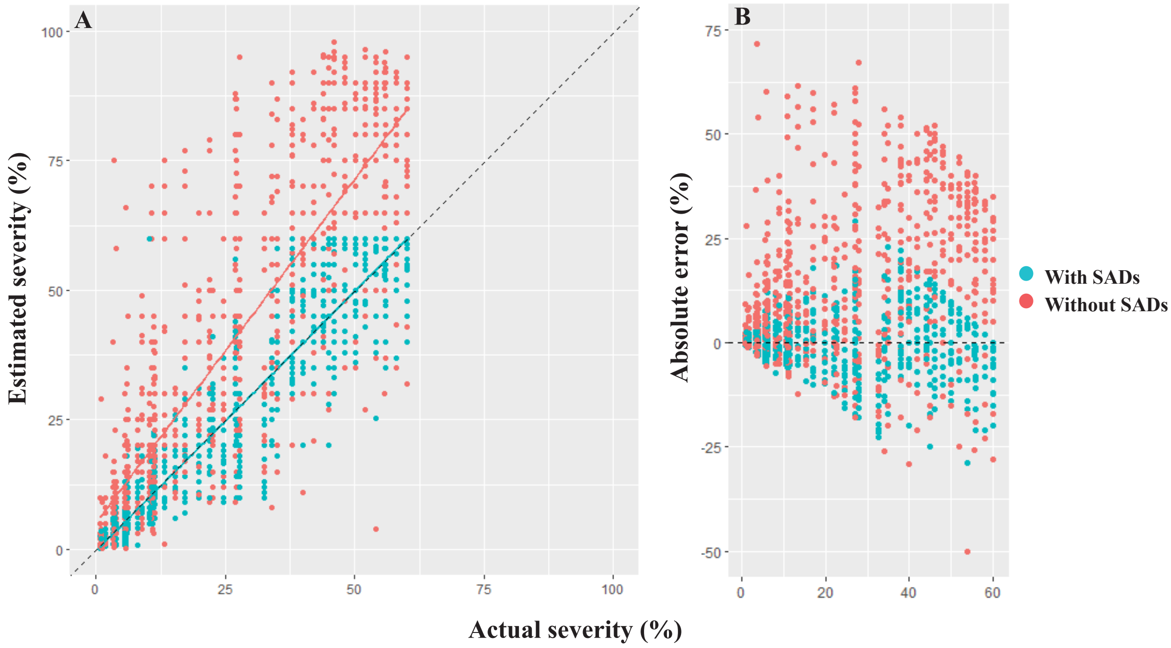
The proposed standard area diagram allows for evaluating GLS severity in adult corn plants affected by the disease. Nine illustrations, covering the minimum (0.5%) and the maximum (60%) of gray leaf spot severity comprised the SADs (Figure 1). Based on estimated and actual severity, assessments made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

FIGURE 1 – STANDARD AREA DIAGRAM SET TO ASSESS GRAY LEAF SPOT (GLS) (*Cercospora* spp.) SEVERITY. NUMBERS REPRESENT THE PERCENTAGE (%) LEVELS OF DISEASED LEAF AREA (TYPICAL LESIONS AND NECROSIS). GREEN AREAS WERE CONSIDERED HEALTHY AREAS.



SOURCE: The author (2023).

FIGURE 2 - RELATIONSHIP BETWEEN ACTUAL AND ESTIMATED SEVERITY (A) OF GRAY LEAF SPOT ON CORN (*Cercospora* spp.) WITHOUT (RED SPOTS) AND WITH (BLUE SPOTS) THE USE OF A STANDARD AREA DIAGRAM SET (SADS) FOR 50 DISEASED LEAVES BY 20 RATERS. ABSOLUTE ERROR (B) (ESTIMATED SEVERITY MINUS ACTUAL SEVERITY) OF THE ESTIMATES WITHOUT SADS AND WITH SADS FOR THE 50 DISEASED LEAVES.



SOURCE: The author (2023).

The statistical parameters (υ, 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. The statistical parameters values were: scale bias - υ (no SADs = 1.474, with SADs = 1.056) (confidence intervals - CI = -0.549 - -0.285), bias coefficient factor - C_b (no SADs = 0.795, with SADs = 0.986) (CI = 0.132 - 0.250); correlation coefficient - r (no SADs = 0.899, with SADs = 0.951) (CI = 0.034 - 0.069); and Lin's concordance correlation coefficient - ρc (no SADs = 0.716, with SADs = 0.938) (CI = 0.164 - 0.279). As the CI did not embrace zero, the difference was significant ($\alpha = 0.05$).

The statistical parameter location bias (u) was not significantly different (no SADs = 0.533, with SADs = -0.018) (CI = -0.715- 0.384). The means of the parameter for both assessments, without and with SADs were close to its ideal condition (u =0) (Table 2).

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 GRAY LEAF SPOT (*Cercospora* spp.), ON 50 LEAVES AS ESTIMATED BY 20 RATERS.

Variables	Means[a]		Difference between means[b]	95% CIs of the difference[c]
	Without SADs	With SADs		

Scale *(υ)*[d]	1.474 (0.293)	1.056 (0.089)	-0.418 (0.068)	-0.549 – -0.285
Location (*u*)[e]	0.533 (0.362)	-0.018 (0.137)	-0.551 (0.084)	-0.715 – -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

SOURCE: The author (2023).

LEGEND: [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] 10000 bootstrap samples were used to obtain the confidence intervals (CIs). If the CIs embrace zero, the difference was not significant (α = 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 correction factor (C_b) measures how far the best-fit line deviates from 45^0 and is a way to measure accuracy.

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

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

Inter-rater reliability of assessments by 20 raters was significantly improved. Without the SADs, the intra-class correlation coefficient mean (ρ) was 0.753 (confidence intervals = 0.661-0.833), while using the SADs, this value was 0.917 (confidence intervals = 0.881-0.946). In turn, the mean of the inter-rater coefficient of determination (R^2) of the pairwise comparisons were 0.727 (minimum = 0.418, maximum = 0.921) and 0.879 (minimum = 0.722, maximum = 0.963) without and with SADs, respectively. he 95% confidence interval (CI) of this mean was 0.137 to 0.167. As the CI did not embrace zero, the difference was significant (α = 0.05) (Table 3).

TABLE 3 - INTER-RATER RELIABILITY OF ASSESSMENTS OF GRAY LEAF SPOT BY 20 RATERS ON 50 LEAVES OF CORN WITHOUT AND WITH THE USE OF A STANDARD AREA DIAGRAM SET (SADS). INTER-RATER RELIABILITY IS MEASURED BY THE INTRA-CLASS CORRELATION COEFFICIENT (ρ) AND COEFFICIENT OF DETERMINATION (R^2).

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	

SOURCE: The author (2023)

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

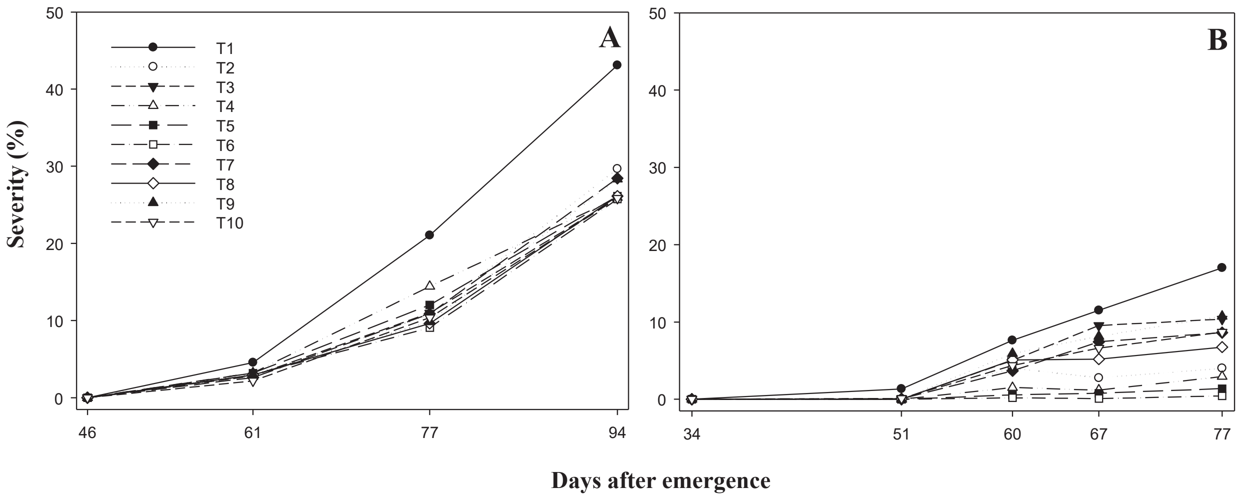
[b] Mean of the difference between each rating, with standard errors in parentheses (bootstrap calculated value), confidence intervals (CIs) were based on 10000 bootstrap samples. If the CIs embrace zero, the difference is not significant (α = 0.05).

4.2 EFFICACY EVALUATION OF REGISTERED FOLIAR FUNGICIDES IN CONTROLLING CORN GRAY SPOT UNDER FIELD CONDITIONS

The proposed standard area diagram set was used to evaluate the screening of nine foliar fungicides registered for the control of GLS on leaves of plants in the vegetative and reproductive stages. The experimental fungicide treatments were composed of single molecules, double mixtures and triple mixtures of active ingredients, without or with the association of multissite fungicides. In each plant, seven leaves were observed between the third leaf below the ear (EF_{-3}), the ear leaf itself (EF) and the third leaf above the ear (EF_{+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 3). Other foliar diseases such as white spot and rusts occurred at a total severity of less than 3%.

FIGURE 3 - DISEASE PROGRESS CURVES OF DIFFERENT FUNGICIDE TREATMENTS AGAINST GLS DURING THE PLANT CYCLE. EXPERIMENT A (RIO VERDE – GO) AND EXPERIMENT B (CHAPADÃO DO SUL – MS).



SOURCE: The author (2023).

LEGEND: 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).

Based on the joint analysis of the two trials, the analysis of variance showed a significant difference between the treatments with the fungicides tested ($P<0.05$) for the control of gray leaf spot. Based on Scott-Knott cluster analysis, the final severity and area under the disease progress curve (AUDPC) values formed four groups of means, and the yield 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 with a single molecule or triple mixture of fungicides chlorothalonil (T3) and azoxystrobin + mancozeb + tebuconazole (T7), which presented 18.13 and 18.54%, respectively (Table 4).

All AUDPC and yield values differed from the control treatment without fungicide (T1) (Table 4). For AUDPC, four groups of means were formed while for yield only three. The fungicide fluxapiroxad + pyraclostrobin + mefentrifluconazole (T6) showed greater efficacy in controlling the disease, resulting in 7,501.69 kg ha^{-1} of yield and 43.5% yield maintenance, the highest values observed among the other treatments. When comparing the treatment widely used by producers epoxiconazole + pyraclostrobin (T2), the treatment with the best control efficacy (T6) showed a greater maintenance of yield by 17.40%. When comparing to the treatment widely used by growers, epoxiconazole + pyraclostrobin (T2), the treatment with the best control efficacy (T6) showed a greater yield maintenance, of 17.40%.

Next, the foliar fungicides azoxystrobin + propiconazole + pydiflumetofen (T4) and fluxapiroxad + pyraclostrobin (T5) had the second and third highest control efficacy, of 60.55% and 66.55%, respectively. These treatments presented yield of 6,553.26 kg ha^{-1} and 6,971.68 kg ha^{-1}, respectively (table 4). The other treatments had lower control efficacy than the other fungicides and presented an average of 45.6%.

TABLE 4 - FINAL SEVERITY, AREA UNDER THE DISEASE PROGRESS CURVE (AUDPC), CONTROL EFFICACY (%E), YIELD (kg ha^{-1}) AND YIELD MAINTENANCE (%M).

Treatments	Final severity**		AUDPC**		%E	Yield		%M
T1	30.05	a*	522.77	a*	0.00	5,219.40	c*	0.00
T2	16.83	c	274.62	b	55.35	6,822.47	b	30.50
T3	18.13	b	309.02	b	38.45	6,320.74	b	21.00
T4	14.44	d	272.65	c	60.55	6,553.26	b	25.50
T5	13.76	d	244.21	c	66.55	6,971.68	b	33.50
T6	13.09	d	209.37	d	72.75	7,501.69	a	43.50
T7	18.54	b	299.33	b	44.35	6,758.06	b	29.50
T8	16.44	c	272.70	b	50.10	6,669.75	b	27.50

T9	19.54	b	310.33	b	38.70	6,693.47	b	28.50
T10	17.28	c	280.57	b	46.80	6,969.01	b	33.50
CV%	8.42		9.58			7.24		

SOURCE: The author (2023)

LEGEND: Joint analysis of experiments A and B; 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).

* Means followed by the same letters, lowercase in the column do not differ, respectively, by the ANOVA and Scott-Knott tests at 5% probability.

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

5 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). Reliable 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 gray leaf spot severity assessments. As a result, it constitutes a useful tool for estimating disease severity to aid epidemiological studies, evaluation of disease management strategies, such as fungicide efficacy, and selection of pathogen-resistant genotypes for disease control.

Standard area diagram sets have been widely used as a tool to determine the severity 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; VIERA et al. 2014), 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 a 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.

Therefore, we suggest that field trials should be conducted following the methodology standardized in this study: a) the plot (experimental unit) consists of at least 4 lines of 6 meters in length spaced 0.80 to 1.0 m between lines without sowing flaws; b) the chosen hybrid must be susceptible to the target disease and tolerant or resistant to non-target diseases; c) only the plants on the two central lines must be evaluated; d) at least 10 plants must be randomly evaluated per plot; e) on each plant, the severity of the disease must be observed on seven leaves in the vegetative and reproductive stages, among leaf -3, ear leaf and +3, and the severity estimate must be made only on that leaf with the greatest severity of the disease, and the leaf evaluated on the plant may vary over time; f) at least five evaluations should be carried out during the culture cycle from V6 onwards at 15-day intervals; g) severity assessments should start with the negative control treatment (control without fungicide), followed by any positive control treatments, and only then for the other treatments; h) the experimental design in randomized blocks must have at least four replications, and the experimental treatments (foliar fungicide) must be sprayed three times sequentially at intervals of a minimum of 14 and a maximum of 21 days, being: first application: early season, at the eight-leaf vegetative stage (V8), or earlier if severity reaches 1% in the control treatment without fungicide; second application: midseason, in the vegetative stage in pre-tassel of 11 leaves (V11); and, third application: late season, at the reproductive stage in post-tassel blister grain (R2), after complete emission of the tassel and pollination.

The control effectiveness of the fungicides was evident by the lower AUDPC values and yield maintance 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 and DMI groups, demonstrating efficacy for gray leaf spot control in corn. The difference in control between fluxapiroxad + 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 (FRAC, 2018).

The treatments azoxystrobin + propiconazole + pydiflumetofen (T4), fluxapiroxad + pyraclostrobin (T5) and fluxapiroxad + 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 labeled 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, CUSTÓDIO et al., 2020).

The classes of fungicides that have been most used to control gray leaf spot are sterol demethylation inhibitors (DMI) and quinone outside inhibitors (QoI) (AGROFIT, 2023). The continuous use of the same active ingredient or the same chemical group allows for a greater selection of fungal isolates resistant to fungicides, which can spread quickly (BRENT & HOLLOMON, 2007). According to 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). Succinate dehydrogenase inhibitor (SDHI) 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, it contains the active ingredient triazole, the first isopropanol (new subclass of DMIs) developed and efficient in controlling several pathogenic agents of the Ascomycota phylum in laboratory conditions, such as *Fusarium* spp. (LIU et al., 2022), *Alternaria alternata, Cercospora beticola, 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 and stalk rot complex (COSTA et al., 2023) have increased the adoption of hybrids 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 for field evaluations aimed at controlling GLS by new foliar fungicides.

6 CONCLUSION

The SADs improved raters' ability to accurately, precisely, and reliably estimate gray leaf spot severity on corn leaves. All foliar fungicides controlled GLS, with emphasis on Fluxapiroxad + Pyraclostrobin + Mefentrifluconazole, which provided the highest yield gain maintenance. However, co-formulations of fungicides with QoI,DMI and SDHI molecules, associated with fungicides with multisite activity that show low risk of fungal selection, is an important anti-resistance strategy that should be adopted.

7 FINAL CONSIDERATIONS

The standard area diagram set and methodology used in this study can be recommended so that estimates for assessing the severity of foliar disease have greater accuracy, precision, and reproducibility.

The fungicide fluxapyroxad + pyraclostrobin + mefentrifluconazole can be recommended for controlling corn gray leaf spot in second crop corn. This control option can help mitigate the risk of selecting isolates resistant to other site-specific fungicides, especially if associated with fungicides with multisite activity.

Two treatments with triple mixtures and which have fungicides with multisite activity in their composition showed lower efficacy in controlling foliar disease compared to fungicides with only triple mixtures of site-specific fungicides. However, these fungicides also showed superior control performance and are important additional tools for resistance management.

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