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Digital assessment of Helminthosporium disease in maize cultivation, in a subtropical environment in Brazil

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ABSTRACT. Maize cultivation has major economic and social importance in Brazil. Being able to identify and manage fungal diseases is one of the main aspects related to good cultivation practices. The purpose of this study was to evaluate the efficiency of using the digital assessment of Helminthosporium disease, associated with the use of preventive fungicide in maize hybrids in low-altitude environments in southern Brazil, in a year with the presence of the La Niña phenomenon. The experiments were conducted in the municipalities of Santa Maria - RS and São Vicente do Sul - RS in the 2021/2022 harvest. The experimental design used was randomized blocks, with treatments in an 8 x 2 factorial scheme, consisting of eight maize hybrids, with and without fungicide application, in a subdivided plot, with fungicide application in bands. Low rainfall and lower disease inoculum pressure have contributed to reducing the severity of the Helminthosporium disease and the rate of disease progression in maize cultivation. The methods for measuring severity digitally and via diagrammatic scale were similar. The use of preventive fungicide in maize cultivation at the V8 and pre-flowering stages did not show positive results for grain yield. Grain length, number of rows per ear, and grain weight per ear are characters that have a high magnitude of association with grain yield in maize cultivation with and without fungicide application in years with the presence of the La Niña phenomenon.

Key words: *Zea mays* L.; Diagrammatic scales; preventive fungicide.

INTRODUCTION

The maize (*Zea mays* L.) cultivation comprises several regions of Brazil with different climate, soil, and level of applied technology, factors that are directly related to the emergence of diseases. Thus, over 20 diseases have already been identified in maize. However, due to the frequency and intensity in which they occur, only some are economically important and have a greater impact on the crop (Souza et al., 2018).

As well as pathogen attack, the cultivation of this species is subject to several factors due to its wide geographic distribution and high genetic variability. Changes in the maize planting system are mainly related to the intensification of soybean production in Brazil and meteorological phenomena, such as El Niño and La Niña. These El Niño and La Niña phenomena are characterized, respectively, by the abnormal heating and cooling of the waters of the central and eastern Equatorial Pacific Ocean, which causes a shift in crop sowing periods, resulting in the diversity of production systems (Guedes et al., 2019).

Among the most relevant diseases that limit production potential, foliar diseases stand out: Southern Corn Rust (*Puccinia polysora*), Helminthosporium (*Helminthosporium turcicum*), Grey Leaf Spot (*Cercospora zeae-maydis*), and White Spot (*Pantoea ananatis*) (Cota et al., 2013). The beginning of an epidemic is marked by the appearance of the first lesion on a host plant. However, for this lesion to have appeared, the pathogen must have been produced and survived somewhere and then be transported and deposited on a healthy host, continuing its cycle. The development of infectious diseases is characterized by the survival, dissemination, infection, colonization, and reproduction of the pathogen (Camera et al., 2020).

Helminthosporium disease is caused by the fungus Exserohilum turcicum (Pass.) K. J. Leonard & E. G. Suggs (synonym Helminthosporium turcicum). The perfect form of the pathogen is Setosphaeria turcica (Luttrell) K. J. Leonard & E. G. Suggs (synonym Trichometasphaeria turcica Luttrell) (Cota et al., 2013). Its symptoms are characterized by necrotic lesions measuring 2.5 to 15 cm in length, which may range from gray-green to brown in color. The first lesions of the disease appear on older leaves, and total burning of the leaf blade may occur in cases of more severe infections (Cota et al., 2013).

During Exserohilum turcicum epidemics, the disease incidence in plants can reach 100%, resulting in a significant drop in yield. Sporadic epidemics occur most frequently in the southern and western regions of Brazil, where they cause severe losses of up to 50% of grain production (De Rossi et al., 2015). The pathogen is able to survive in the residues left in the field, such as infected stems and leaves, and is spread by the wind, which transports conidia over long distances (Hooda et al., 2017). The favorable climatic conditions for the occurrence of epidemics are the air temperatures between 20°C and 25°C and high relative humidity, above 90% (Cota et al., 2013).

However, in addition to knowing the etiology and symptoms of a disease, it is essential to quantify the intensity of the symptoms (Trojan & Pria, 2018). Some of the tools for quantifying are diagrammatic scales designed to determine the quantity or intensity of the disease in leaves and fruits. The popularity of computers and digital cameras has facilitated the development of diagrammatic scales, as well as the use of image analysis to assess severity. This last method allows measuring the lesion severity, size, the classification according to size, and the number of lesions per leaf or area, facilitating the correlation between the symptom and the leaf area. Automated diagnosis of disease symptoms will facilitate decision-making for maize producers (Haque et al., 2022).

The evaluation of diagrammatic scales by manually measuring scores, compared to digital tools such as the Pliman package in R, is limited for maize crops. Therefore, the purpose of this study was to evaluate the efficiency of using the digital assessment of Helminthosporium disease, associated with the use of preventive fungicide in maize hybrids in low-altitude environments in southern Brazil, in a year with the presence of the La Niña phenomenon.

MATERIAL AND METHODS

The experiments were conducted in the 2021/2022 harvest in two locations: Santa Maria (SM; 29º43'28"S, 53º43'41"W, altitude 95 m) and São Vicente do Sul (SVS; 29º42'27"S, 54º41'34"W, altitude 129 m), both located in the State of Rio Grande do Sul, Brazil. The characteristic soil of both locations is classified as sandy Ultisol (or *Argissolo Vermelho Distrófico* in the Brazilian Soil Classification System – SiBCS). The climate classification of both locations, according to Köppen, is *Cfa*, characterized as humid subtropical, with no defined dry season (Alvares et al., 2013). Temperature, precipitation, and relative humidity data was obtained from automatic surface meteorological stations of the Instituto Nacional de Meteorologia (INMET), located 500 m close to the experimental area, which were used to monitor the water status of the maize cultivation in field conditions.

Sowing of the experiments took place on September 15, 2021 in SM and October 29, 2021 in SVS. The experimental design adopted was complete randomized blocks with band application of the fungicide, with five replications in experiment I in SM and four replications in experiment II in SVS. The main plot was composed of hybrids, with each plot consisting of five sowing rows, with a row spacing of 0.50 m and a length of 10 m, with a final population of 70,000 plants ha^{-1}.

The treatments consisted of two treatment factors: With and without the use of fungicide, and eight maize hybrids. The main plot was formed by the maize hybrid factor, and the subplot was composed of two levels of the fungicidal factor (with and without) in band. The main portion was composed of hybrids Agroceres 8690, Agroeste 1757, Brevant 2401, Brevant 2418, Dekalb 240, Forseed 670, Pioneer 3016, and Syngenta FEROZ.

The experimental units were fertilized based on the interpretation of the soil analysis carried out in the experimental areas prior to the installation of the experiments and considering the recommendations for maize cultivation from the fertilization and liming manual, using an expected yield of 12 t ha^{-1} (CQFS-RS/SC, 2016). Phosphorus and potassium fertilization was carried out concomitantly with the sowing operation in the planting furrow, using a seeder with furrower. Nitrogen fertilization was carried out through the application of urea fertilizer (46-00-00) at a 320 kg ha^{-1} dose, in which 50% of the total dose was applied when the crop reached the V4 stage, and the remainder 50% was applied when the culture reached the V6 stage.

The fungicide was applied preventively, starting when the maize crop was at the V8 stage, and the second application occurred when the maize crop reached the VT stage (pre-flowering). The fungicide used in the experiment belongs to the strobilurin and triazole chemical groups, with active ingredients of Trifloxystrobin 375 g L^{-1} and Ciproconazole 160 g L^{-1} in the form of Concentrated Suspension (CS). The dose used was 0.2 L p.c. ha^{-1}. For fungicidal treatment applications, a pressurized electric sprayer equipped with Teejet Extended Range 110.02 nozzles, spaced at 0.5 m, was used, providing 200 L ha^{-1} of spray volume. The other crop practices were adopted as per technical indications for maize cultivation in Rio Grande do Sul State (Da Rosa et al., 2017).

To evaluate the yield components, eight plants were used per plot, located in the usable area of the plots. The following were evaluated: Plant height (PH in cm), ear length (EL in cm), number of rows per ear (NR), number of grains per row (NGR), ear diameter (ED in cm), corncob diameter (CD in cm), grain length (GL in mm), grain weight per ear (GWE in g), the humidity of the plot, and the mass of 1,000 grains (MTG in g). EL was measured with the aid of a 50-cm millimeter ruler, and the NR was counted individually, in each ear. ED and CD were measured with an analog caliper, by measuring at the midpoint of the EL, and the GL was calculated from the difference between the diameters.

The collected ears were threshed manually, and, after threshing, the CD was measured. The threshed grains were weighed on a digital scale to obtain the GWE of each ear evaluated. At the end of the individual evaluations of the ears from each plot, a homogenized sample was taken with the grains from the evaluated ears, then 100 grains were counted with two repetitions. Subsequently, by using the mean of these counts and multiplying by 10, the MTG was obtained.

From this same sample, the plot humidity was verified with a portable moisture analyzer, performing two repetitions and using the average of these checks. Humidity was corrected to 13%. Finally, grain yield (YIE) per hectare of each plot was estimated with the weight of the eight ears.

To assess severity, the diagrammatic scale developed by Lazaroto et al. (2012) was used to evaluate the severity of common helminthosporium disease in maize, caused by *Exserohilum turcicum*. Three severity assessments were carried out: One at stage V8, before the first fungicide application, to measure the severity of diseases existing in the area; another at the VT stage (pre-flowering), which coincides with the second fungicide application; and a third 15 to 20 days after the second application, at stage R3. At stage R3, severity was measured digitally by using a smartphone to capture images of maize leaves, with two plants collected per subplot to create images and analyze them subsequently. For visual assessments, all leaves were considered, estimating the percentage of area damaged by the disease on the leaf using the diagrammatic scale. As for the digital evaluation, only the leaves above the ear were measured, using a destructive method, with the help of a smartphone to capture images. During the studies, initial symptoms of Southern Corn Rust (*Puccinia polysora*) and Grey Leaf Spot (*Cercospora zeae-maydis*) were observed, however at low intensity, which made it impossible to measure these diseases. Therefore, we were only able to measure the helminthosporium disease.

Data was initially analyzed regarding adherence of residues, normal distribution, and homogeneity of treatment variances, using the Shapiro Wilk ($p \leq 0.05$) and Bartlett ($p \leq 0.05$) tests, which identified the compliance with statistical assumptions. Subsequently, the data was subjected to analysis of variance (ANOVA) to determine possible treatment and interaction effects. When a significant effect was verified by the F test ($p \leq 0.05$), the appropriate complementary analyzes were carried out, applying the Scott-Knott test, at 5% significance ($p \leq 0.05$). The analyzes were carried out using the R software (R Development Core Team, 2020).

To measure lesions in digital images, the Pliman package of the R software (Olivoto, 2022) was used, which is a suite for carrying out various analyzes on plant images, especially related to leaf analysis, including: Measuring leaf area, recounting objects in an image, calculating the symptomatic area of the disease, extracting RGB values for each object in an image, and calculating object measurements. And to apply the path analysis study methodology, the path_coeff() function from the metan package was used (Olivoto & Lúcio, 2020).

The correlation multicollinearity test was used, according to the criteria by Montgomery, Peca and Vining (2013), using eigenvalues of the correlation matrix and the condition number (CN). The CN was estimated by dividing the largest eigenvalue by the smallest eigenvalue. If the condition is less than 100, multicollinearity is considered weak; if it is between 100 and 1,000, it is considered moderate to strong; and if it is above 1,000, it is considered severe, according to the criteria by Montgomery, Peca and Vining (2013). The multicollinearity diagnosis is as large as the variance of the "inflated" coefficient, which is evaluated using the variance inflation factor (VIF) test. Values above 10 imply multicollinearity. After analysis, 10 variables were selected for trail analysis: PH, EL, GL, CD, MTG, NR, digital severity (DSEV), severity in R2-R3 (SEVR2R3), and severity in pre-flowering (SEVVT). These variables were analyzed in two experiments, one with the use of fungicides and the other without the use of fungicides.

RESULTS AND DISCUSSION

The accumulated precipitation during the experiments was 388.4 mm in experiment I and 403.8 mm in experiment II (Figure 1). According to Borém et al. (2017), the maize water requirement to reach the maximum production of hybrids is in the range of 400 to 600 mm per production cycle. The low rainfall recorded during the maize cultivation cycle led to a reduction in expected crop yield and disease incidence.

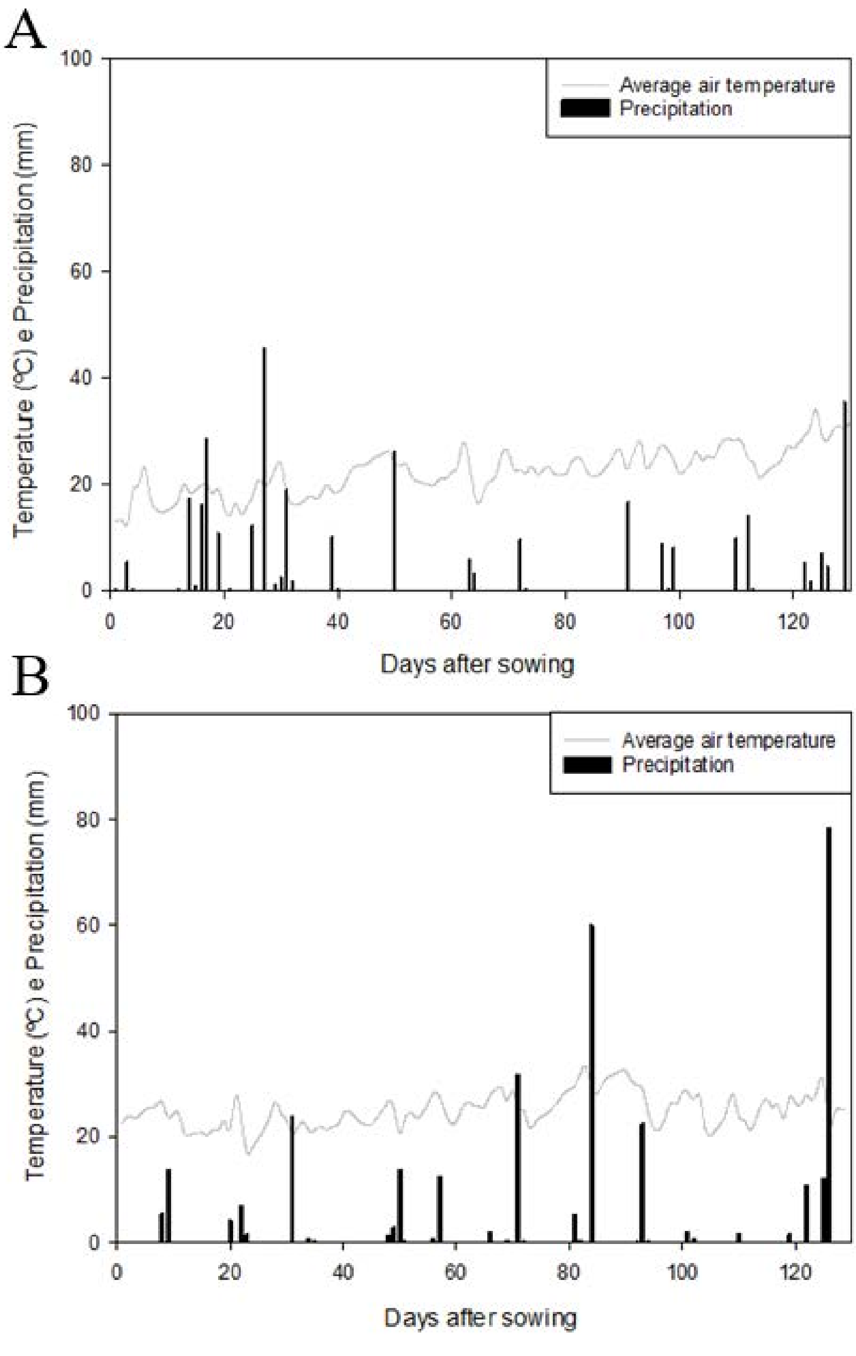
Figure 1. Rainfall volumes and daily air temperature means during experiment I (A) and experiment II (B) in the 2021/22 harvest. According to data obtained from the meteorological station of the INMET, installed on the Campus of the Federal University of Santa Maria and Federal Institute Farroupilha, Campus São Vicente do Sul. Santa Maria, RS, 2022.

The mean air temperature during the experiments was 22.64ºC in experiment I and 24.93ºC in experiment II, while the mean relative humidity was 69.54% in experiment I and 64.52% in experiment II (Figure 2). These characteristics of mild temperatures, combined with relative air humidity above 60%, which occurred during the experiment cycle, may be the main reason for the occurrence of helminthosporium in the experiments (Hooda et al., 2017).

Figure 2. Relative air humidity recorded in the evaluation period of the first harvest, experiment 1 (A) and experiment 2 (B) 2021/22 harvest.

The occurrence of *Exserohilum turcicum* is mainly in places with temperatures of 18 to 27°C and medium to high relative humidity during crop development (Camera et al., 2020). In regions with moderate nighttime temperatures and intermittent periods of cloudy weather, lesions develop significantly due to low light, which favors the development of the pathogen (Camera & Deuner, 2017).

The progression of helminthosporium on maize leaves in both experiments is observed in Figure 3. The first signs of the disease were observed 50 days after sowing the maize. The symptoms



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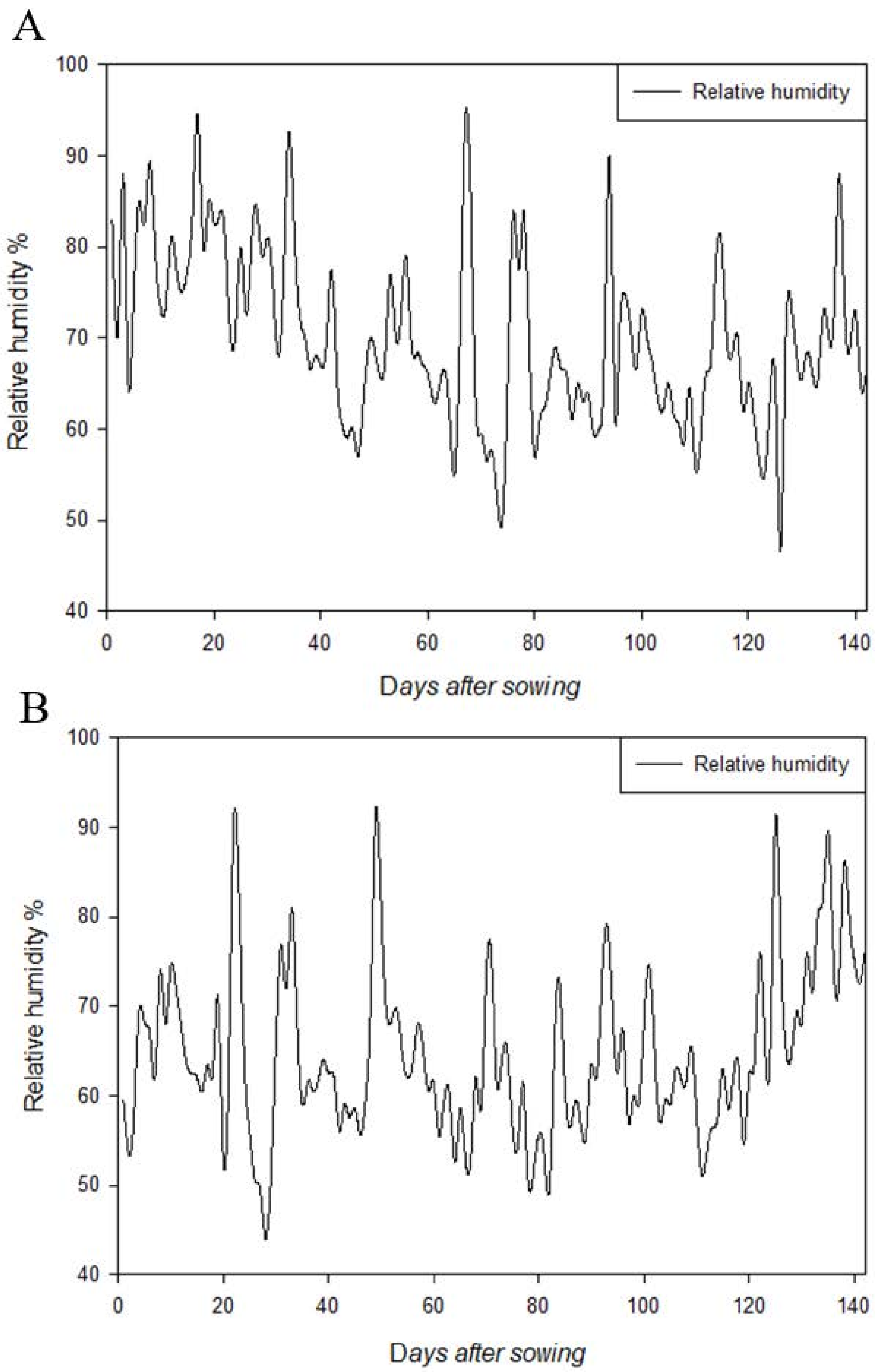

Figure 2. Relative air humidity recorded in the evaluation period of the first harvest, experiment 1 (A) and experiment 2 (B) 2021/22 harvest.

observed were straw-colored, necrotic lesions, with uneven distribution on the leaves, measuring between 5 and 8 cm in length and 2 to 3 cm in width, as well as those observed by Kotze et al. (2018). The onset of the disease will depend on weather conditions and the susceptibility of the hybrids (Hooda et al., 2017). However, the most susceptible hybrids tend to be infected and show symptoms earlier than hybrids with some degree of resistance to the disease, ranging from the V10 (Carpane et al., 2020), VT, and R1 (Mallowa et al., 2015) stages.

Figure 3. Progress curve of helminthosporium disease in maize hybrids, (A) with fungicide application and (B) without fungicide, experiment I, (C) with fungicide application and (D) without fungicide, experiment II.

According to the catalogs of hybrids in the study, we found that the genetic resistance of maize hybrids to helminthosporium leaf spot is as follows: Agroeste 1757 and Forseed 670 hybrids have no information; the Agroceres 8690 hybrid is tolerant to the disease; and hybrids Brevant 2401, Brevant 2418, and Syngenta Feroz are moderately tolerant. The Dekalb 240 hybrid is moderately susceptible, and the Pioneer 3016 hybrid is susceptible to the helminthosporium disease. It is noteworthy that the best way to control the disease is through the genetic resistance of hybrids (Hooda et al., 2017).

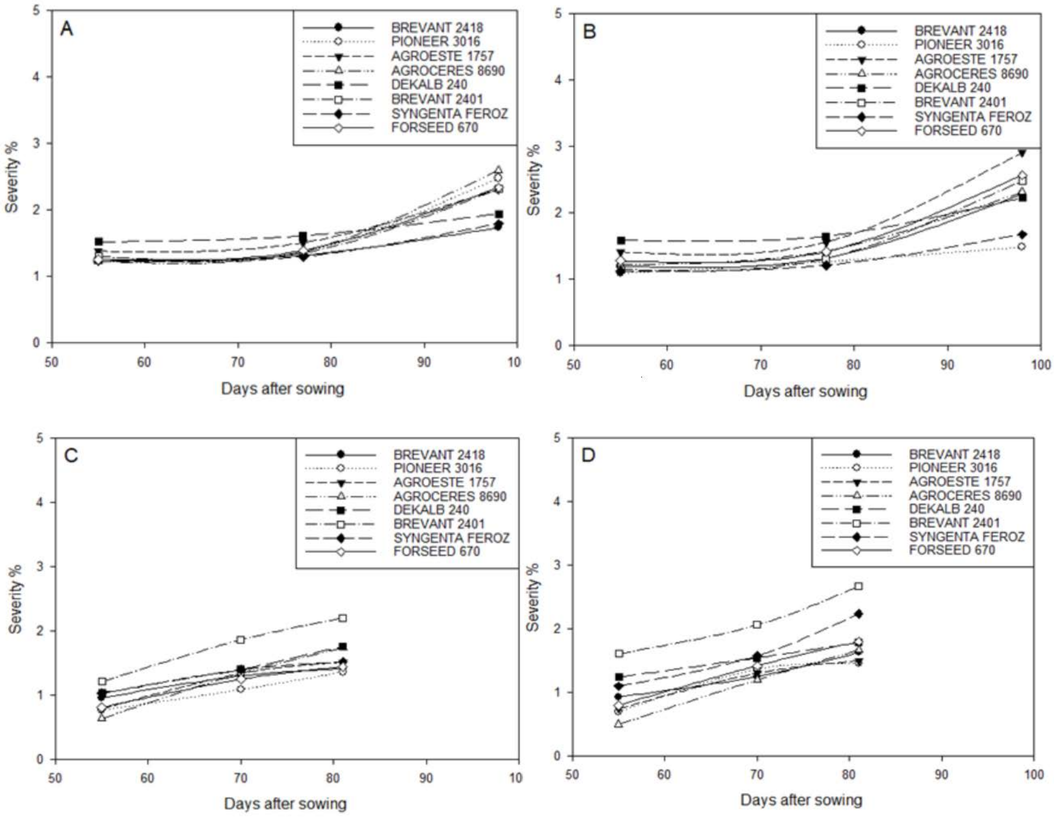

Figure 3. Progress curve of helminthosporium disease in maize hybrids, (A) with fungicide application and (B) without fungicide, experiment I, (C) with fungicide application and (D) without fungicide, experiment II.

There was no statistical difference for the fungicidal factor, with and without fungicide application. However, the application of fungicides resulted in a reduction in the incidence of the helminthosporium disease in the average of the two experiments carried out, despite the different meteorological conditions and hybrids used. When analyzing Figure 3 (B), we observe that the hybrid Agroeste 1757 showed greater severity of helminthosporium at 98 days after sowing, with 2.9% severity in experiment I, demonstrating greater sensitivity to the presence of the pathogen.

We can also observe the difference in the behavior trend of the disease progress curves. In experiment I, 50 days after sowing, meteorological conditions were more conducive to the disease development, as the previous period had more frequent and voluminous rainfall, consequently presenting higher relative humidity, a longer period of shading, and lower temperatures when compared to experiment II, which had a slightly drier meteorological condition. Therefore, the initial severity was slightly higher in experiment I. Mesquini et al. (2020) observed high rainfall at 80 and 100 days after sowing the crop, observing that, at these times, the epidemics were more explosive, with a 20ºC mean air temperature and relative humidity between 70% and 98%.

From the first evaluation onwards, the behavior of the meteorological variables was inverted between the two experiments. In experiment II, precipitation was more regular and voluminous, while in experiment I it was milder. Observing the disease progress curves, we saw that in experiment II, the severity of the disease had an increasing trend until the last evaluation, at 98 days. While in experiment I, the progress curve tends to increase only from 78 to 98 days, remaining stable from 50 to 78 days after sowing.

In general, during the experiments, a low level of helminthosporium severity was observed in the different hybrids and at different moments of crop development. This is largely due to environmental conditions, such as low relative humidity, high temperatures, and mild rainfall, during the average period when the experiments were conducted.

There was no significant interaction between Hybrid × Fungicide in experiments I and II for severity estimates using the diagrammatic scale and digital image analysis (Tables 1 and 2). However, on average, there was a decrease in severity with the use of fungicide in both study environments. In general, fungicide applications reduce the disease severity, especially when applied at the beginning of the disease, which can vary between maize phenological stages, especially in hybrids with susceptibility. Therefore, preventive management is essential (Carpane et al., 2020).

The more likely the conditions are for the development of the disease, the earlier the applications should be, since the disease severity varies due to numerous factors, such as the type of soil, type of cultivation, humidity, and temperature (Dalavai & Kalappanavar, 2017). The work by Blandino et al. (2012) showed that applications in V12 to V15 were more effective in reducing the disease when at high pressure, whereas when at low pressure, applications were effective even when made in VT to R3.

Table 2 presents the severity scores estimated by digital image evaluation, using the R software. It is worth mentioning that object segmentation is a fundamental step for image analysis, and the background of the images must contain elements with low contrast in relation to the foreground, such as plants, leaves, and soil. Other sources of noise include brightness gradients and overexposure (Olivoto, 2022). We can observe that the estimated values are similar to those in Table 1 carried out with the aid of a diagrammatic scale. Trojan and Pria (2018) found that the use of diagrammatic scales provided greater accuracy in estimated severity values in maize, even for evaluators with some experience. The value divergences in the two tables are directly related to the

Table 1. Severity of the helminthosporium disease, evaluated with the aid of a diagrammatic scale, for eight maize hybrids, when subjected to fungicide application (CF) and without fungicide application (SF).

Hybrids	Severity R2-R3			
	Experiment I		Experiment II	
	SF	CF	SF	CF
Dekalb 240	1.18 ns	1.34	0.90 ns	0.69
Brevant 2401	2.01	1.49	1.36	1.52
Pioneer 3016	1.27	1.08	0.57	1.21
Agroeste 1757	0.85	0.79	1.85	1.37
Forseed 670	1.64	1.15	1.60	1.68
Agroceres 8690	0.35	0.87	1.37	0.78
Syngenta Feroz	1.93	0.82	0.75	1.29
Brevant 2418	1.28	0.45	1.63	0.69
Average	1.31 ns	1.00	1.25 ns	1.15
CV 1 - F (%)	74.71		95.27	
CV 2 - H (%)	19.37		49.58	
CV 3 - F*H (%)	75.64		80.27	

ns = not significant. Coefficient of variation (CV%), Fungicide (F), Hybrid (H).

Table 2. Severity of the helminthosporium disease, digital evaluation with Pliman package, for eight maize hybrids, when subjected to fungicide application (CF) and without fungicide application (SF).

Hybrids	Severity R2-R3			
	Experiment I		Experiment II	
	SF	CF	SF	CF
Dekalb 240	1.07 ns	0.63	1.96 ns	1.52
Brevant 2401	2.05	1.36	2.98	1.91
Pioneer 3016	1.97	0.60	1.69	1.62
Agroeste 1757	2.38	1.65	0.86	0.75
Forseed 670	2.20	1.74	2.34	1.79
Agroceres 8690	1.78	1.73	0.22	1.14
Syngenta Feroz	0.93	0.75	3.18	1.35
Brevant 2418	1.71	0.65	1.88	0.29
Average	1.76 ns	1.14	1.89 ns	1.30
CV 1 - F (%)	78.53		115.80	
CV 2 - H (%)	86.96		109.35	
CV 3 - F*H (%)	104.57		114.61	

ns = not significant. Coefficient of variation (CV%), Fungicide (F), Hybrid (H).

low severity found in the experiments and the presence of other diseases, which were not able to be visually estimated due to their low incidence and severity.

YIE remained below expectations in the relevant studies. We can observe the YIE of the 8 maize hybrids for experiments I and II (Table 3). There was no statistical difference for the fungicidal factor, only statistical difference for the hybrid factor. We observed the formation of 3 groups in experiment I. The first group was formed by the Pioneer 3016 hybrid, which differed statistically

Table 3. Grain yield of commercial maize hybrids, cultivated in the municipalities of Santa Maria - RS and São Vicente do Sul - RS, subjected to fungicide application (CF) and without application (SF), in the 2021/22 harvest.

Hybrids	Grain productivity (kg ha^{-1})			
	Experiment I		Experiment II	
	SF	CF	SF	CF
Pioneer 3016	9,200 a	10,475 a	10,330 a	8,566 a
Agroeste 1757	8,328 b	8,130 b	9,912 a	9,414 a
Agroceres 8690	7,868 b	7,495 b	8,037 b	7,080 b
Brevant 2418	7,498 b	7,461 b	6,903 c	7,835 a
Dekalb 240	6,653 b	7,149 b	8,169 b	6,860 b
Syngenta Feroz	5,828 b	6,454 b	6,459 c	6,100 b
Brevant 2401	5,874 b	6,413 b	4,890 c	4,792 b
Forseed 670	2,695 c	3,837 c	146 d	342 c
Average	6,743 ns	7,177	6,856 ns	6,374
CV 1 - F (%)	26.29		25.10	
CV 2 - H (%)	10.59		17.98	
CV 3 - F*H (%)	17.22		14.34	

ns = not significant; *means followed by the same lowercase letter in the column do not differ according to the Scott-Knott test, at 0.05 significance (P>0.05).

from the other hybrids with and without fungicide application using the Scott-Knott test, at 5% significance. This hybrid achieved a YIE of 10,475 kg ha^{-1} with the use of fungicide and 9,200 kg ha^{-1} without the use of fungicide. Group 2 was composed of hybrids Agroeste 1757, Agroceres 8690, Brevant 2418, Dekalb 240, Syngenta FEROZ, and Brevant 2401, with a YIE of 8,130, 7,495, 7,461, 7,149, 6,454, and 6,413 kg ha^{-1} with the use of fungicide, respectively, and a YIE of 8,328, 7,868, 7,498, 6,653, 5,828 and 5,874 kg ha^{-1} without the use of fungicide, respectively. Group 3 was formed by the hybrid Forseed 670, with a YIE of 3,837 kg ha^{-1} with the use of fungicide and 2,695 kg ha^{-1} without the use of fungicide.

When observing the YIE of maize hybrids in experiment II (Table 3), we verified the formation of three groups with fungicide application and four groups without fungicide application. We observed that with the fungicide application hybrids Agroeste 1757, Pioneer 3016, and Brevant 2418 differed statistically from the other hybrids, according to the Scott-Knott test, at a significance of 5%, reaching a YIE of 9,414, 8,566, and 7,835 kg ha^{-1}, respectively. Group 2, formed by hybrids Agroceres 8690, Dekalb 240, Syngenta FEROZ, and Brevant 2401, achieved a YIE of 7,080, 6,860, 6,100, and 4,792 kg ha^{-1}, respectively. Group 3 was formed by the hybrid Forseed 670, with a 342 kg ha^{-1} YIE.

Without fungicide application, hybrids Pioneer 3016 and Agroeste 1757 formed group 1, which differed statistically from the other hybrids using the Scott-Knott test, at 5% significance, reaching 10,330 and 9,912 kg ha^{-1} YIE, respectively. Group 2 was formed by hybrids Dekalb 240 and Agroceres 8690, with 8,169 and 8,037 kg ha^{-1} YIE. Group 3 was formed by hybrids Brevant 2418, Syngenta FEROZ, and Brevant 2401, with 6,903, 6,459, and 4,890 kg ha^{-1} YIE, respectively. Group 4 was formed by hybrid Forseed 670, with 146 kg ha^{-1} YIE.

The linear association between the variables, observed in the vertical line (Figure 4), shows that both in treatments with fungicide and in treatments without fungicide, traits PH, EL, GL, CD,

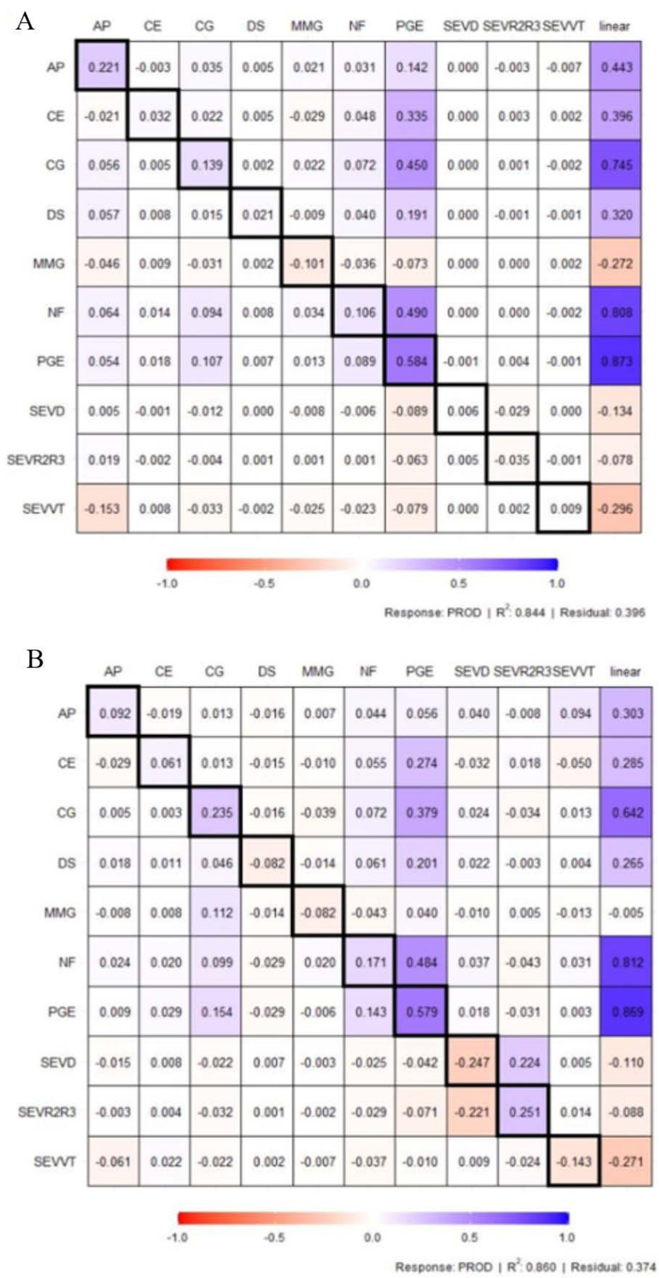

Figure 4. Linear correlation (vertical line), estimation of direct effects (diagonal) and indirect effects (horizontal line) of traits (PH) plant height, ear length (EL), grain length (GL), corncob diameter (CD), mass of one thousand grains (MTG), number of rows (NR), digital severity (DSEV), severity in R2-R3 (SEVR2R3), severity in pre-flowering (SEVVT) on total grain yield, for treatments with use of fungicides (A) and without use of fungicides (B), collected from 144 samples (two experiments) carried out in the municipalities of Santa Maria - RS and São Vicente do Sul - RS.

and NR were positively correlated with YIE, especially NR and GL, presenting values of 0.808 and 0.745, respectively, with fungicide, and 0.812 and 0.642, respectively, without fungicide. On the other hand, MTG, DSEV, SEVR2R3, and SEVVT showed a negative correlation with YIE, with an emphasis on the SEVVT values, -0.296 and -0.271, for treatments with and without fungicide, respectively.

Figure 4. Linear correlation (vertical line), estimation of direct effects (diagonal) and indirect effects (horizontal line) of traits (PH) plant height, ear length (EL), grain length (GL), corncob diameter (CD), mass of one thousand grains (MTG), number of rows (NR), digital severity (DSEV), severity in R2-R3 (SEVR2R3), severity in pre-flowering (SEVVT) on total grain yield, for treatments with use of fungicides (A) and without use of fungicides (B), collected from 144 samples (two experiments) carried out in the municipalities of Santa Maria - RS and São Vicente do Sul - RS.

In the study of cause-and-effect relationships between the variables, we observed that PH, GL, NR, and GWE have a direct positive effect on YIE in treatments with the application of fungicides (A) (0.221, 0.139, 0.106, and 0.584, respectively). A similar result was obtained with the same direction between treatments (B), except for PH, (0.235, 0.171, and 0.579, respectively).

There is a direct, negative effect of DSEV and SEVVT on YIE in treatments without fungicide application (B), with values of -0.247 and -0.143, respectively. This result is not observed in treatments using fungicide (A). There was also a positive direct effect of SEVR2R3 on YIE. This relationship may be associated with the fact that late infections, in which symptoms appear at advanced stages of plants, are not capable of causing such severe damage to YIE when compared to infections that appear at earlier stages, such as VT (Sartori et al., 2017). The more distant the infection occurs from the stigma fertilization, the smaller the reduction in YIE compared to infections prior to fertilization (Formento, 2010).

The results obtained corroborate the conclusions of Bortolini and Gheller (2012) and Wise et al. (2019), who highlight the positive impact of the use of fungicides, through an increase in maize YIE. Therefore, it is evident that the management of fungicides in maize crops has an influence on the relationships between variables, affecting the estimates of Pearson's correlation coefficients and, consequently, the direct and indirect effects between the variables obtained in the cause-and-effect analysis.

Thus, hybrids with high productive potential, associated with adequate phytosanitary management, lead to greater grain yield and economic gains. Therefore, we recommend further studies using new maize hybrids, associated with fungicide product management, especially in years with greater rainfall, which favors the incidence of diseases.

CONCLUSIONS

Low rainfall and lower disease inoculum pressure contribute to reducing the severity of the helminthosporium disease and the progress rate of the disease in maize crops in low-altitude environments in southern Brazil. The methods for measuring disease severity, digitally and using a diagrammatic scale, showed similarity in years with the presence of the La Niña phenomenon. The use of preventive fungicide in maize crops at the V8 and pre-flowering stages did not show positive results for grain yield in environments with the presence of the La Niña phenomenon. Grain length, number of rows per ear, and grain weight per ear are traits that have a high magnitude of association with grain yield in maize with and without fungicide application in maize cultivation.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alvares CA, Stape JL, Sentelhas PC. (2013). Koppen's climate classification map for Brazil. *Meteorologische Zeitschrift*. 22: 711–728.

Blandino M, Galeazzi M, Savoia WE. (2012). Timing of azoxystrobin + propiconazole application on maize to control northern corn leaf blight and maximize grain yield. *Field Crops Research*. 139: 20-29.

Bortolini AMM, Gheller JA. (2012). Aplicação de diferentes fungicidas no controle de doenças foliares na cultura do milho em relação à produtividade. *Revista Brasileira de Energias Renováveis*. 1: 109-121.

Borém A, Galvão JCC, Pimentel MA. (2017). Milho: do plantio à colheita. 2th ed. Viçosa, MG: Universidade Federal de Viçosa.

Camera JN, Deuner CC (2017). Limiares térmicos para a germinação de conídios de Exserohilum turcicum. *Revista Ceres*. 64: 138-142.

Camera JN, Koefender J, Schoffel A. (2020). Expansão da lesão da helmintosporiose em diferentes híbridos de milho. *Holos*. 8: 1-12.

Carpane PD, Peper AM, Kohn F. (2020). Management of Northern Corn Leaf Blight using Nativo (Trifloxistrobin + Tebuconazole) Fungicide Applications. *Crop Protection*. 127: 104982.

Comissão de Química e Fertilidade do Solo - RS/SC. (2016). Manual de adubação e calagem para os Estados do Rio Grande do Sul e Santa Catarina. 11th ed. Viçosa, MG: Sociedade Brasileira de Ciência do Solo.

Cota LV, Da Costa RV, Sabato EO. (2013). Histórico e perspectivas das doenças na cultura do milho. Sete Lagoas, MG: Embrapa Milho e Sorgo.

Dalavai PA, Kalappanavar IK. (2017). Investigations of turcicum leaf blight (TLB) and common rust (CR) of maize in northern Karnataka. *Journal of Farm Sciences*. 30: 431-434.

Da Rosa APSA, Emygdio BM, Bispo NB. (2017). Indicações técnicas para o cultivo de milho e de sorgo no Rio Grande do Sul safras 2017/2018 e 2018/2019. Sertão, RS: Instituto Federal Sul-Rio-Grandense.

De Rossi RL, Reis EM, Brustolin R. (2015). Morfologia de conídios e patogenicidade de isolados de Exserohilum turcicum da Argentina e do Brasil em milho. *Summa Phytopathologica*. 41: 58-63.

Formento A, Norma. (2010). Enfermedades foliares reemergentes del cultivo de maíz: royas (Puccinia sorghi y Puccinia polysora), tizón foliar (Exserohilum turcicum) y mancha ocular (Kabatiella zeae). INTA, Argentina.

Guedes HAS, Priebe OS and Manke EB (2019). Tendências em séries temporais de precipitação no Norte do Estado do Rio Grande do Sul, Brasil. *Revista Brasileira de Meteorologia*. 34: 283-291.

Haque MA, Marwaha S, Deb CK. (2022). Deep learning-based approach for identification of diseases of maize crop. *Scientific Reports*. 12: 6334.

Hooda KS, Khokhar MK, Shekhar M. (2017). Turcicum leaf blight—sustainable management of a re-emerging maize disease. *Journal of Plant Diseases and Protection*. 124: 101–113.

Kotze RG, Van der Merwe CF, Crampton BG. (2018). A histological assessment of the infection strategy of Exserohilum turcicum in maize. *Plant Pathology*. 68: 504-512.

Lazaroto A, Dos Santos I, Konflanz VA, (2012). Escala diagramática para avaliação de severidade da helmintosporiose comum em milho. *Ciência Rural*. 42: 2131-2137.

Mallowa SO, Esker PD, Paul PA. (2015). Effect of Maize Hybrid and Foliar Fungicides on Yield Under Low Foliar Disease Severity Conditions. *Disease Control and Pest Management*. 105: 1080-1089.

Mesquini RM, Mattos AP, Rissato BB. (2020). Progresso temporal de doenças da cultura do milho. *Summa Phytopathologica*. 46: 140-144.

Montgomery DC, Peck EA, Vining GG. (2013). Introduction to linear regression analysis. John Wiley & Sons: Wiley series in probability and statistics.

Olivoto T. (2022). Lights, camera, pliman! An R package for plant image analysis. *Methods in Ecology and Evolution*. 13: 789-798.

Olivoto T, Lúcio AD. (2020). Metan: An R package for multi-environment trial analysis. *Methods in Ecology and Evolution.* 11: 783-789.

R Development Core Team. (2020). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Souza SCR, Rey MS, Bernardi C. (2018). Severidade de manchas foliares do milho sob diferentes níveis de adubação nitrogenada. *Applied Research & Agrotechnology.* 11: 61-67.

Sartori M, Nesci A, García J. (2017). Efficacy of epiphytic bacteria to prevent northern leaf blight caused by Exserohilum turcicum in maize. *Revista Argentina de Microbiología.* 49: 75-82.

Trojan DG, Pria MD. (2018). Validação de escala diagramática para quantificação da severidade da antracnose da folha do milho. *Summa Phytopathologica.* 44: 56-64.

Wise KA, Smith D, Freije, A. (2019). Meta-analysis of yield response of foliar fungicide-treated hybrid corn in the United States and Ontario, Canada. *Plos One.* 14: e0217510.