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Macrospora leaf spot development conditions and resistance/tolerance of Brazilian commercially grown maize genotypes

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ABSTRACT. Macrospora leaf spot (MLS), caused by the fungus *Stenocarpella macrospora*, is one of the most important diseases affecting maize in Brazil. However, there are no MLS-resistant cultivars commercially available. Therefore, this study aimed to investigate the lesion expansion rate of MLS in four maize genotypes, leaf wetness duration (0-, 6-, 12-, 18-, 24-, 30-, 36-, 42-, and 54-hour post-inoculation), disease development severity in three maize genotypes, and resistance/tolerance levels to MLS in 141 maize genotypes commercially grown in Brazil. The estimates were performed using logistic models adjusted to the parameters analyzed, except for resistance/tolerance levels, which were analyzed using proposed severity and resistance scales. The experiment was carried out at the Laboratory of Plant Phytopathology of the Epagri/Cepaf, Santa Catarina State, Brazil, from 2016 to 2020. Disease resistance was significantly different among genotypes and fungal isolates. However, none of the genotypes showed resistance or high tolerance levels to MLS. Leaf wetness duration influenced maximum disease severity, and lesion expansion rate differed significantly among the genotypes tested. All information generated in this study is essential for breeding programs of maize for MLS resistance.

Keywords: corn; leaf wetness duration; lesion expansion rate; severity; *Stenocarpella macrospora*.

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Introduction

Maize (*Zea mays* L.) fields in Brazil occupied more than 21.2 million hectares during the 2021/2022 harvest season, making it the second most important annual crop in the country, with a production estimated to reach 115.6 million metric tons and a value of production of over U$ 30 billion, an increase in production of over 32% when compared to its previous harvest season (Companhia Nacional de Abastecimento [CONAB], 2022). Besides its direct impact on the country's economy, maize is also the basis of human/animal food and feed, serving as well as raw material for many industrial processes, such as ethanol and drug production (Shah, Prasad, & Kumar, 2016; Zhang et al., 2021). Therefore, the search for better agricultural practices, cultivars, and phytosanitary products to help increase yields and decrease disease incidence/severity in maize fields are not only necessary for Brazilian agriculture but worldwide.

Among the most problematic diseases for maize production in Brazil, macrospora leaf spot (MLS) can be highlighted (Silva, Fonseca, Yamada, & Pontes, 2020). Also known as Diplodia Leaf Streak, the disease is caused by a necrotrophic fungus, *Stenocarpella macrospora* (Earle) Sutton (syn. *Diplodia macrospora* Earle in Bull.), and is more common for maize grown under warm and humid conditions of tropical and subtropical regions (Wordell Filho, Casa, & Nesi, 2016; Mário, Gozuen, & Juliatti, 2017). The ideal conditions for conidia germination are relative humidity over 50% and temperatures between 25 and 32°C (Lorenzetti et al., 2019).

Despite difficult measurements (Mueller et al., 2020), yield losses due to MLS infection were estimated at 273.8 million bushels between 2016 to 2019 in the USA and Ontario (Canada). Nevertheless, as this disease can also cause stalk rot and ear rot in maize cultivars (Mário et al., 2017), financial losses in the USA have been estimated at up to US$1.68 billion per harvest season when qualitative parameters, such as incidence of mycotoxins (aflatoxins), are analyzed (Mitchell, Bowers, Hurburgh, & Wu, 2016).

The most common MLS symptoms on maize leaves are small brown spots of water-soaked appearance with a chlorotic halo, which tends to become irregular or elliptical and develop concentric rings, with a reddish or yellow halo as the disease progresses (Siqueira, Machado, Barrocas, & Almeida, 2014; Anderson, Bradley, & Wise, 2021). The main source of primary MLS inoculum is the presence of large amounts of crop residues from previous harvest seasons in areas of no-tillage, which is widely adopted in most of Brazil (Anderson, Bradley, & Wise, 2021). Still, Brazilian farmers have faced the lack of registered fungicides for MLS control (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2022), and no resistant cultivars are commercially available. In this sense, high-quality healthy seeds, crop rotation, biocontrol agents, efficient fungicides, and less susceptible cultivars are crucial to managing and controlling the disease (Munkvold, Munkvold, & White, 2016).

In Brazil, maize hybrids and varieties have been generally classified for their resistance/ tolerance to stalk rot and ear rot and, in some situations, concerning the incidence of burned grains and mycotoxin production. Reports on genetic resistance/tolerance of hybrids to foliar diseases are few and imprecise, especially on the genus *Stenocarpella* (Wordell Filho et al., 2016). In this context, one should bear in mind that disease-resistance selection requires precise phenotypic testing.

Given the above background, the present study aimed to investigate the leaf wetness duration for MLS development in three maize cultivars, MLS lesion expansion rate in four maize cultivars, and genotype resistance/tolerance to MLS in 141 maize cultivars. To our knowledge, the information generated in this study is currently scarce in the literature and may help private/governmental companies/agencies, researchers, and farmers to breed new cultivars with resistance or higher tolerance level to the disease.

Material and methods

The study was conducted at the Laboratory of Plant Phytopathology of the Epagri/Cepaf, Santa Catarina State, Brazil, from 2016 to 2020. In the experiment, maize cultivars were evaluated to establish leaf wetness duration for macrospora leaf spot (MLS) incidence/severity, resistant/tolerant genotypes, and MLS lesion expansion rate.

Leaf wetness duration

For leaf wetness duration determination, we used two single maize hybrids ("P32R48" and "DKB240") and one open-pollinated variety ("SCS155 Catarina"). Maize genotypes were chosen based on their differences in necrotic leaf area measurement by Bermudéz-Cardona, Wordell Filho, and Rodrigues (2015) and Hawerroth et al. (2019). To do so, plants were grown in plastic vases containing about 500 g of sterilized substrate Tecnomax (Composition: peat, *Pinus* bark, expanded vermiculite, dolomitic limestone, and agricultural plaster; pH 5.8; EC 0.7 ± 0.3 mS cm^{-1}, and dry density 101 kg m^{-3}).

The plants were inoculated with a pre-identified monosporic isolate of *S. macrospora* (Dm54). It was obtained from symptomatic leaves of the maize hybrid "P32R48H" collected in the municipality of Abelardo Luz, Santa Catarina (26°33'53" S, 52°19'42" W, 760-m altitude). The inoculum was produced aseptically by transferring the conidia to 9-cm culture plates with oatmeal agar (oat 140 g and agar 15 g in 1 L of distilled water), which were incubated for 30 days at 22ºC with a 12-hour photoperiod (20 W fluorescent lamps emitting 260 to 280 uE m^{-2} s^{-1}). The conidial suspension was prepared and quantified according to Tuite (1969), filtered through two layers of cheesecloth to eliminate mycelial fragments, and added with 100 μL L^{-1} of surfactant (polyoxyethylene-20-sorbitan monolaurate, Tween 20) to facilitate inoculum dispersion on the leaf surface. Viable conidia were determined by transferring the culture plates, 12 hours before inoculation, to 10 mL distilled water (DW), and distributing them into four culture plates containing water agar. After 12-hour incubation at 22ºC, we checked the germination of 100 conidia per plate using a stereomicroscope. A conidium was considered viable if the germ tube was longer than the largest diameter of the conidium. Discrepancies in conidia germination were corrected by knowing inoculum viability.

The plants were inoculated at the phenological stage V1 (Nleya, Chungu, & Kleinjan, 2016), spraying a conidial suspension (viable conidia 5×10^4 mL^{-1}, 2.3 mL $plant^{-1}$) using a DeVilbiss atomizer (model SGA 570, SER 1281; DeVilbiss Co., Somerset, PA) at an air pressure of 55 kPa. Then the plants were transferred to a moist chamber and kept for 0, 6, 12, 18, 24, 30, 36, 42, and 54 hours after inoculation (HAI) at 24 ± 1ºC, 90% relative humidity, and a 12-hour photoperiod. The leaf surface of plants was allowed to dry before returning them to the growth chamber at a constant temperature of 24 ± 0.1ºC until evaluation. All tests were performed in a completely randomized design (CRD), with seven replicates, and repeated three times. Each replicate consisted of a vase containing four maize plants.

Disease evaluations were performed seven days after inoculation, using the severity scale of James (1974). Gompertz model reparametrized by Zeviani, Silva, Carneiro, and Muniz (2013) was adjusted to the necrotic leaf area (disease severity) as a function of leaf wetness duration to estimate the time in which 99% of the maximum severity would be observed, as follows:

$$Y = b_1 e^{log(0.99)e^{b_2\left(1-\frac{T}{b_3}\right)}}$$

where in: Y is the observed severity, T is the leaf wetness duration, b1 is the maximum asymptote (maximum severity), b2 is a parameter without direct interpretation, and b3 is the leaf wetness duration in which a maximum 99% severity is observed. Then, the logistic model reparametrized by Zeviani et al. (2013) was also adjusted, as follows:

$$Y = \frac{b_1}{1 + \left(\frac{1-0.99}{0.99}\right) e^{-b_2(T-b_3)}}$$

where in: Y is the observed severity, T is the leaf wetness duration, b1 is the maximum asymptote (maximum severity), b2 is a parameter without direct interpretation, and b3 is the leaf wetness duration in which a maximum 99% severity is observed.

The model to be used was chosen based on the smallest residual standard error (RSE), the smallest measures of linearity fit [parameter-effect curvature measure (PE) and intrinsic curvature measure (IN)], and the lowest Akaike information criterion (AIC) value.

Resistance of genotypes

MLS resistance was evaluated for 141 maize genotypes commercially grown in Brazil. Table 1 displays the classification of each genotype. Inocula used were the pre-identified monosporic isolates of *S. macrospora* (Dm54 and Dm58) obtained from maize leaves of the variety "SCS155 Catarina", which were collected in the municipalities of Abelardo Luz and Chapecó (27°05'47" S, 52°37'06" W, Elevation 674m), Santa Catarina State, Brazil. Inoculated plants (phenological stage V1) were kept in a moist chamber (98% relative humidity, 12-hour photoperiod, 24 ± 0.5°C) for 30 HAI. Thereafter, the plants were transferred to a growth chamber (24 ± 0.2°C) and kept until evaluation. All tests were performed in a completely randomized design (CRD), with four replicates, and repeated twice. Each replicate consisted of a vase containing four plants. The disease was evaluated seven days after inoculation, measuring the proportion of leaf area necrosed by *S. macrospora* and using the severity scale proposed by James (1974), as well as the resistance scale of Olatinwo, Cardwell, Deadman, and Julian (1999), wherein: highly resistant (no symptom - VR), resistant (0.1 to 4% infected leaf area - R), moderately resistant (5 to 10% infected leaf area - MR), high/intermediate (11 to 20% infected leaf area - HI), intermediate (21 to 30% infected leaf area - I), low/intermediate (31 to 40% infected leaf area - LI), moderately susceptible (41 to 50% infected leaf area - MS), susceptible (51 to 60% infected leaf area - S), and highly susceptible (>60% infected leaf area - HS).

Table 1. Classification of each maize genotype used during the experiments.

Classification of the genotype	Commercial name
Single-cross hybrid	P30F36; P3989; P30F53H; P30R50; P30S31; P32R48; P30S40; Fórmula; P32R22H; P30F35; P30B39; P30R32; BG7046; P3161H; BG7318H; DKB240; P30K75; DOW2A106; AGROMEN30A06; 526012; AG9040; DKB330; DOW2B707; PRE22S11; GNZ2500; AS1555; GNZ0729; AS1551; ASP1039; AG8015; AS1572; DKB234; BX945; AGROMEN 30A05; AS1565; AS1577; AS1579; AS1545; AG8021; P3646; SHS7070; Status; GNZ9501; P1630H; CD316; AGROMEN30A03; SHS7090; CD351; AS1560; CD386; SHS7080; SHS7311; P30F90; CD393; DKB177; AS1550; AS1575; 2A550HX; 30A68; 359003; 2B587HX; AS1535; P30K64; HS20653; AG8021YG; 30A77HX; P2530.
Double-cross hybrid	PRE32D10; SHS4080; RG01; SHS4050; DKB747; SHS4070; 318010; DKB979; DKB615; AG2020; RG02A; AG6020; PRE22D11; SHS4060; AGROMEN2012; AG6040; AS32; BM207.
Triple-cross hybrid	BG7065; BG7050; P30B30; BG7060; BG6070H; RG03; GNZ2005; SHS5050; AG8011; AG5011; DOW2B655; 20A55; AGROMEN20A06; DKB566; AG6018; XH121; SHS5070; 30P34; AS3466; AS3430; BG7049H; SHS5080; SHS5090; CD397; SG6418; 2B688HX; 2B433HX; BM128; 20A78HX.
Open-pollinated variety	SCS154 Fortuna; SCS155 Catarina.
No information	P2323; AO1052; XH131; SMX1007; XH117; ASV173; SMX1004; SYN3507; AS48; SMX1002; XH101; DG3; XH104; SMX1001; SMX1008; SMX1003; BF8029; SMX1005; BM739; SMX1011; HS6206; SMX1006; SMX1010; BM7205.

Data on necrotic leaf area as a function of the genotype were subjected to analysis of variance to test homogeneity and normality assumptions. In the case of non-normal and/or inhomogeneous distribution, the data were transformed by the Box-Cox parameter. When needed, the transformation was indicated in the header of each table.

Lesion expansion rate of *S. macrospora*

Lesion expansion rate of *S. macrospora* was evaluated in four maize genotypes: three single hybrids ("P1630", "DKB240", and "30K75Y") and one open-pollinated variety ("SCS155 Catarina"). These genotypes were chosen based on their resistance level to the disease and the size of the cultivated area in southern Brazil. The inoculum used was the pre-identified monosporic isolate Dm54. The methods used for plant growth, inoculum preparation, and inoculation were the same as those described for the "leaf wetness duration" and "resistance of genotypes" experiments. Inoculated plants (phenological stage V1) were kept in a moist chamber (98% relative humidity, 12-hour photoperiod, 24 ± 0.5°C) for 30 HAI. Thereafter, plants were transferred to a growth chamber (24 ± 0.2°C) and kept until evaluation. The length and width of 20 lesions, randomly selected per repetition, were measured every two days, with the help of a digital caliper (Worker Mark). Subsequently, the necrosed area (mm^2) of each lesion was calculated. All tests were performed in a completely randomized design (CRD), with four replicates, and repeated twice. Each replicate consisted of a vase containing four plants.

The logistic model was adjusted to the necrotic leaf area data as follows:

$$Y = \frac{b_1}{1 + \left(\frac{1}{b_2} - 1\right) e^{-b_3 \times x}}$$

where in: Y is the lesion area, x is the time after inoculation, b1 estimates the maximum area (lesion) of the lesion, b2 estimates the initial inoculum and b3 estimates the lesion expansion rate.

Statistical analyses

All analyses were performed with the statistical software R (RRID: SCR_001905) (R Core Team, 2022), using the packages 'nlme' (RRID: SCR_015655) (Pinheiro, Bates, Debroy, Sarkar, & R Core Team, 2017) and ExpDes.pt (Ferreira, Cavalcanti, & Nogueira, 2013).

Results

The genotypes tested showed significant differences concerning the incidence and severity of MLS as a function of the leaf wetness duration, inocula used, and lesion expansion rate (Tables 2, 3, 4, and 5). None of the genotypes were resistant or showed high tolerance to *S. macrospora*.

Leaf wetness duration

The adjustment of the Gompertz model to the MLS severity progress was performed to obtain a better fit for the results (Table 2 and Figure 1). The maize genotypes differed for maximum severity when exposed to different leaf wetness times (Table 2).

Table 2. Results of the non-linear regression analysis when the Logistic (Log) and Gompertz (Gomp) models were adjusted to the macrospora leaf spot (MLS) severity data in maize leaves as a function of the leaf wetness duration. CI (95% confidence interval), AIC (Akaike information criterion), RSE (residual standard error of the model), PE (parameter-effect curvature measure), IN (intrinsic curvature measure), b_1 (maximum severity), and b_3 (leaf wetness duration in which a maximum 99% severity is reached).

Estimated Parameters	DKB240		SCS155 Catarina		P32R48	
	Log	Gomp	Log	Gomp	Log	Gomp
b_1	78.6	79.1	47.6	48.4	45.3	46.5
(95% CI)		77.9-80.2		47.3-49.5		45.1-48.0
b_3	21.3	25.1	35.8	43.1	17.9	27.5
(95% CI)		23.2-27.2		39.6-46.7		22.4-33.5
AIC	870.67	860.59	780.94	771.74	903.25	886.63
RSE	5.33	5.14	3.87	3.74	5.99	5.64
R^2	0.96	0.97	0.96	0.96	0.89	0.91
PE	0.56	0.24	0.71	0.35	1.63	0.51
IN	0.08	0.06	0.09	0.1	0.09	0.11

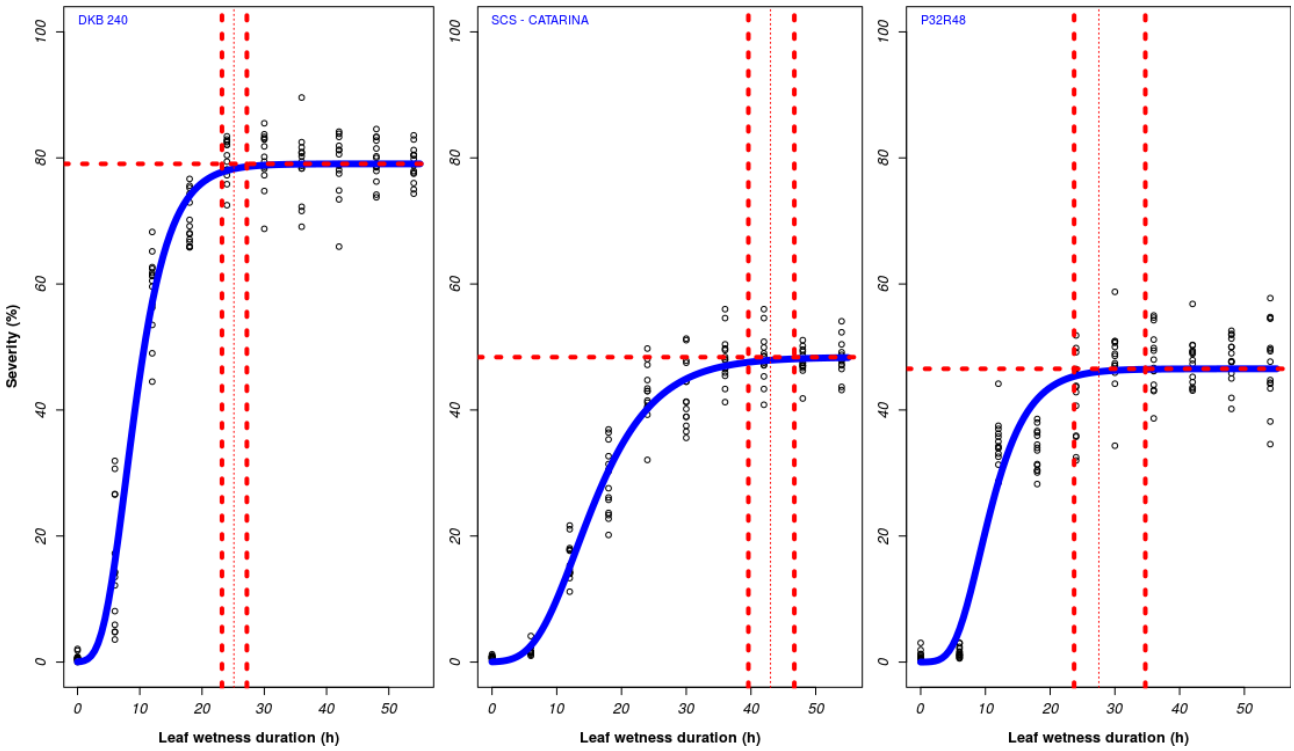

Figure 1. Progress curves of macrospora leaf spot (MLS) severity in maize leaves as a function of the leaf wetness duration. Blue lines refer to the adjusted model, and vertical lines represent the confidence interval for the estimation of b_3 (leaf wetness duration in which a maximum of 99% severity is reached).

The maximum severity (b_1) was observed for the hybrid "DKB240", which ranged between 77.9 and 80.2%. This genotype differed significantly from the variety "SCS155 Catarina" and the hybrid "P32R48", which did not differ from each other and had severity rates between 45.1 and 49.5%. However, "SCS155 Catarina" required between 39.6 and 46.7 hours of leaf wetness to reach 99% of the maximum severity (b_3).

In the present study, MLS severity was significantly lower on leaves of the genotype "SCS155 Catarina" when compared to the hybrids "P32R48" and "DKB240".

Resistance of genotypes

The evaluated maize genotypes showed different behaviors for both *S. macrospora* isolates used at the seedling stage (Tables 3 and 4). For the monosporic isolate Dm54, ten distinct groups of cultivars were observed according to the percentage of necrotic leaf area. For the group with the lowest severity, the necrotic area ranged from 17.37 to 24.92%, whereas for the group with the highest severity it ranged from 86.68 to 90.22% (Table 3).

Based on the resistance scale of Olatinwo et al. (1999), none of the studied genotypes were classified as moderately resistant, resistant, or highly resistant. Moreover, none of them were fully resistant to the disease, with 57.44% being highly susceptible, 16.31% susceptible, 9.92% moderately susceptible, 10.63% low/intermediate, 4.25% intermediate, and 1.41% high/intermediate. The hybrids "AG8021YG", "SMX1006", "30A77HX", "SMX1010", "BM207", and "P2530" had an intermediate behavior, while "20A78HX" and "BM7205" had a high/intermediate behavior.

For the isolate Dm58 (Table 4), the necrotic leaf area of hybrids with the lowest percentage of severity ranged between 15.31 to 20.03%, while for those with the highest severity it varied between 77.24 to 82.37%. None of the genotypes studied showed complete resistance to the disease, with 63.82% of the genotypes being highly susceptible, 12.05% susceptible, 14.89% moderately susceptible, 2.83% low/intermediate, 4.25% intermediate, and 2.12% high/intermediate according to the resistance scale of Olatinwo et al. (1999).

The hybrids "SMX1002", "AS1551", "AS1550", "P30B39", "AS3430", and "DKB177" had an intermediate behavior, while "SMX1003", "AS3466", and "SMX1001" showed a high/intermediate behavior, thus presenting a partial resistance to the disease. The hybrids "P30R50" and "P30F36" showed similar behavior for both isolates tested and were considered highly susceptible (HS) to MLS.

Table 3. Percentage of necrotic leaf area (severity) caused by *S. macrospora* using as inoculum the monosporic isolate Dm54. Before analysis, data were transformed $y=y^{1.6}$ according to the Box-Cox transformation parameter. Averages are presented in the original scale.

Hybrid	Severity	Hybrid	Severity	Hybrid	Severity	Hybrid	Severity	Hybrid	Severity
P30F36	90,22 [a]	RG03	76,60 [c]	20A55	68,33 [e]	SCS155 Catarina	57,74 [f]	CD356	42,65 [h]
P3989	88,16 [a]	GNZ2005	76,31 [c]	AGROMEN 20A0	67,79 [e]	AGROMEN30A03	57,59 [f]	30A68	42,01 [h]
P30F53H	87,80 [a]	SHS5050	76,19 [c]	XH131	67,65 [e]	SHS7090	56,96 [f]	2B688HX	40,76 [h]
P30R50	86,68 [a]	526012	75,31 [d]	DKB615	67,14 [e]	PRE22D11	56,84 [f]	2B433HX	40,26 [h]
P30S31	85,35 [b]	AG8011	75,29 [d]	SCS154 Fortuna	66,82 [e]	BG7049H	56,14 [f]	359003	40,03 [h]
P32R48	85,18 [b]	AG9040	75,27 [d]	AG2020	66,77 [e]	SHS5080	55,12 [f]	AS32	39,67 [h]
P30S40	84,67 [b]	SHS4050	74,98 [d]	SMX1007	66,30 [e]	SHS5090	54,63 [g]	2B587HX	39,60 [h]
Fórmula	84,57 [b]	DKB330	74,79 [d]	DKB566	65,74 [e]	SMX1002	54,41 [g]	SMX1003	39,44 [h]
BG7065	84,30 [b]	DOW2B707	74,33 [d]	AS1545	65,72 [e]	SHS4060	54,39 [g]	BF8029	38,92 [h]
P32R22H	83,99 [b]	PRE22S11	73,98 [d]	AG8021	65,52 [e]	AGROMEN2012	53,77 [g]	AS1535	38,46 [h]
P30F35	83,34 [b]	GNZ2500	73,87 [d]	AG6018	65,17 [e]	CD351	53,33 [g]	P30K64	37,74 [h]
BG7050	82,72 [b]	AS1555	73,52 [d]	XH121	65,11 [e]	AS1560	53,21 [g]	SMX1005	37,02 [h]
P30B39	82,45 [b]	GNZ0729	73,34 [d]	P3646	65,06 [e]	CD397	52,84 [g]	BM739	36,46 [h]
P30R32	81,92 [b]	AS1551	72,99 [d]	SHS7070	64,90 [e]	AG6040	52,12 [g]	SMX1011	34,90 [h]
BG7046	81,74 [b]	DKB747	72,74 [d]	RG02A	64,80 [e]	CD386	52,08 [g]	HS20653	33,46 [i]
P3161H	81,39 [c]	ASP1039	72,55 [d]	AG6020	64,71 [e]	XH101	51,97 [g]	HS6206	33,31 [i]
PRE32D10	81,11 [c]	AG8015	72,42 [d]	XH117	64,30 [e]	SG6418	51,21 [g]	BM128	32,75 [i]
P2323	80,11 [c]	AS1572	72,16 [d]	Status	64,08 [e]	SHS7080	50,96 [g]	AG8021YG	29,18 [i]
AO1052	79,18 [c]	SHS4070	71,87 [d]	SHS5070	63,96 [e]	SHS7311	50,93 [g]	SMX1006	27,86 [i]
SHS4080	78,67 [c]	DKB234	71,41 [d]	ASV173	63,79 [e]	P30F90	50,79 [g]	30A77HX	27,11 [i]
BG7318H	78,04 [c]	BX945	71,35 [d]	GNZ9501	63,48 [e]	CD393	50,70 [g]	SMX1010	27,07 [i]
DKB240	77,91 [c]	318010	71,29 [d]	P1630H	61,99 [e]	DKB177	50,18 [g]	BM207	24,92 [j]
P30K75	77,86 [c]	AG5011	71,15 [d]	CD316	60,95 [f]	AS1550	50,14 [g]	P2530	24,01 [j]
DOW2A106	77,69 [c]	DOW2B655	70,84 [d]	30P34	59,93 [f]	AS1575	48,69 [g]	20A78HX	18,12 [j]
RG01	77,65 [c]	AGROMEN 30A05	70,60 [d]	AS3466	59,71 [f]	DG3	48,00 [g]	BM7205	17,37 [j]
P30B30	76,90 [c]	DKB979	70,54 [d]	SMX1004	59,53 [f]	XH104	47,98 [g]		
AGROMEN 30A06	76,86 [c]	AS1565	69,94 [d]	SYN3507	59,49 [f]	SMX1001	45,30 [h]		
BG7060	76,78 [c]	AS1577	69,63 [d]	AS3430	59,38 [f]	SMX1008	44,68 [h]		
BG6070H	76,75 [c]	AS1579	69,53 [d]	AS48	59,10 [f]	2A550HX	44,52 [h]		

Means followed by equal letters do not differ from each other according to the Scott-Knott test.

Table 4. Percentage of necrotic leaf area (severity) caused by *S. macrospora* using as inoculum the monosporic isolate Dm58. Before analysis, data were transformed $y = y^{1.5}$ according to the Box-Cox transformation parameter. Averages are presented in the original scale.

Hybrid	Severity	Hybrid	Severity	Hybrid	Severity	Hybrid	Severity	Hybrid	Severity
P30R50	82,37 [a]	BG7050	74,08 [b]	P3161H	68,53 [c]	SMX1011	60,56 [d]	AS32	46,38 [f]
P32R22H	82,04 [a]	30A68	74,01 [b]	DOW2B707	68,45 [c]	GNZ0729	60,49 [d]	AG6018	45,81 [f]
P30F36	81,74 [a]	BG7060	73,99 [b]	P30S31	68,30 [c]	DKB566	60,29 [d]	AS1575	45,53 [g]
BG7046	81,70 [a]	P3989	73,68 [b]	SHS5050	67,98 [c]	2A550HX	59,37 [d]	AS48	45,17 [g]
AG8015	81,15 [a]	CD356	73,46 [b]	2B587HX	67,71 [c]	AG9040	57,54 [e]	DKB979	44,25 [g]
30A77HX	79,88 [a]	XH121	73,42 [b]	DKB234	67,58 [c]	HS20653	57,16 [e]	AS1560	43,57 [g]
BG7318H	78,42 [a]	CD351	73,40 [b]	AS1565	67,51 [c]	SMX1007	57,16 [e]	DKB330	43,54 [g]
Fórmula	78,40 [a]	AGROMEN 30A6	73,24 [b]	AG6040	66,80 [c]	P30K75	57,07 [e]	BM7205	43,52 [g]
BM739	78,36 [a]	P3646	73,20 [b]	XH101	66,77 [c]	DKB615	56,75 [e]	AS1577	42,82 [g]
HS6206	78,30 [a]	P1630H	72,13 [b]	XH117	66,30 [c]	GNZ2005	56,58 [e]	SMX1005	42,43 [g]
P32R48	78,17 [a]	AO1052	72,10 [b]	PRE22S11	66,13 [c]	RG02A	56,29 [e]	ASP1039	41,47 [g]
P30R32	77,66 [a]	P30B30	71,68 [b]	BM207	66,12 [c]	AS1535	56,08 [e]	GNZ9501	41,17 [g]
BG6070H	77,58 [a]	XH104	71,18 [c]	359003	65,92 [d]	RG 01	55,84 [e]	P2530	39,61 [g]
P30F35	77,35 [a]	AGROMEN 30A3	71,17 [c]	XH131	65,74 [d]	AS1579	54,52 [e]	SMX1010	38,31 [h]
P30F90	77,24 [a]	DOW2B65	70,86 [c]	GNZ2500	65,68 [d]	AS1545	53,72 [e]	AS1572	35,04 [h]
2B433HX	76,50 [b]	P2323	70,81 [c]	BM128	65,30 [d]	20A78HX	53,53 [e]	SMX1004	34,40 [h]
BG7049H	76,14 [b]	RG 03	70,74 [c]	SHS7070	65,01 [d]	SMX1006	52,57 [e]	SMX1002	28,57 [i]
AG8021	76,14 [b]	SHS4070	70,49 [c]	SHS7080	64,71 [d]	20A55	51,95 [e]	AS1551	28,53 [i]
PRE32D10	75,95 [b]	SHS5090	70,46 [c]	2B688HX	63,71 [d]	AG2020	51,71 [e]	AS1550	26,91 [i]
SHS5070	75,54 [b]	BX945	70,37 [c]	DKB240	63,67 [d]	SG6418	51,69 [e]	P30B39	26,48 [i]
P30F53H	75,30 [b]	318010	70,23 [c]	CD397	63,64 [d]	BG7065	50,99 [e]	AS3430	23,66 [i]
SHS4080	75,04 [b]	P30P34	70,09 [c]	Status	63,59 [d]	AG8021	50,18 [f]	DKB177	23,33 [i]
526012	74,93 [b]	CD 316	69,78 [c]	SHS7090	63,16 [d]	SHX 7311	48,76 [f]	SMX 1003	20,03 [j]
BF8029	74,78 [b]	AGROMEN 30A5	69,65 [c]	DKB747	62,54 [d]	ASV173	48,66 [f]	AS3466	18,96 [j]
P30K64	74,75 [b]	SHS5080	69,64 [c]	AGROMEN201	61,94 [d]	AS1555	48,66 [f]	SMX1001	15,31 [j]
DG3	74,46 [b]	AGROMEN 20A6	69,53 [c]	CD386	61,60 [d]	SYN3507	48,37 [f]		
SHS4050	74,24 [b]	SHS4060	69,30 [c]	CD393	61,39 [d]	SMX1008	48,35 [f]		
AG8011	74,17 [b]	AG6020	68,95 [c]	AG5011	60,95 [d]	PRE22D11	48,30 [f]		
DOW2A10	74,16 [b]	P30S40	68,64 [c]	SCS 155 Catarina	60,79 [d]	SCS 154 Fortuna	48,09 [f]		

Means followed by equal letters do not differ from each other according to the Scott-Knott test.

Lesion expansion rate of *S. macrospora*

Lesion expansion rate of *S. macrospora* (b_3) differed significantly only between "DKB240" and "P1630H" (Table 5). Under ideal controlled conditions for fungal development, rates ranged from 0.1190 mm^2 day^{-1} for the genotype "SCS155 Catarina" to 0.2562 mm^2 day^{-1} for the susceptible hybrid "P1630H".

Table 5. Logistic model parameter estimates for MLS lesion expansion rate as a function of post-inoculation time. b_1 = maximum severity (lesion area) of the disease, b_2 = initial inoculum, and b_3 = lesion expansion rate. The *p*-value for the difference between the observed estimates of the genotypes concerning the hybrid DKB240.

Hybrid	b_1	*p*-value	b_2	*p*-value	b_3	*p*-value
DKB 240	0.0567	0	0.1787	0	0.1313	0
30K75Y	0.0359	0.0608	0.2528	0.0428	0.1910	0.2913
P1630H	0.0550	0.8755	0.1506	0.2596	0.2562	0.0073
SC 155 Catarina	0.0863	0.0223	0.1335	0.0352	0.1190	0.6306

Concerning maximum disease severity (b1) after inoculation, the hybrids "P1630H" and "30K75Y" did not differ from "DKB240", which was used as a comparison standard because it has the largest sowing area in southern Brazil among all genotypes used. Only the variety "SCS155 Catarina" differed significantly from the standard hybrid for parameter b1. Based on the estimate of the initial inoculum of the disease (b2), the genotypes "30K75Y" and "SCS155 Catarina" differed significantly from the standard hybrid "DKB240".

The hybrid "30K75Y" had an initial inoculum about 42% higher than the standard hybrid (DKB240), while the variety "SCS 155 Catarina" showed an initial inoculum about 25% lower than the standard hybrid.

Discussion

In the present study, several genotypes were evaluated to identify cultivars with different levels of resistance/tolerance to MLS. None of the tested cultivars presented a complete resistance to the disease. However, many of them showed promising results to be used in further studies and in maize breeding programs to reduce MLS damaging effects in fields.

To reduce MLS-related problems in maize crops through genetic resistance, information about sources and types of resistance should be gathered. Thus, studies of this type must continue to identify genotypes with different levels of resistance/tolerance. In short, pathogen variability and cultivar genetic resistance/tolerance should be monitored to improve cultivar rotation practices.

Leaf wetness duration

The longest leaf wetness duration required for the variety "SCS155 Catarina" may be associated with its greater tolerance to MLS. As for the fungal isolate DM54 (Table 3), although the open-pollinated cultivar "SCS155 Catarina" showed a susceptible level of resistance, it was higher than the cultivars "P32R48" and "DKB240". Similar results were reported in studies performed by Bermudéz-Cardona et al. (2015) and Hawerroth et al. (2019).

For the tested hybrids, a maximum severity was reached after 23.2 hours of leaf wetness. Therefore, it is an important parameter to differentiate maize genotypes regarding resistance to MLS. By testing the genotypes "SCS155 Catarina" and "P32R48", Bermudéz-Cardona et al. (2015) observed that the former (variety) differed significantly from the latter (hybrid) concerning MLS severity on maize leaves. These authors also found a significant MLS effect on the photosynthetic parameters of the cultivars, with "SCS155 Catarina" showing a disease tolerance 36% higher than "P32R48" after 168 hours post-infection. In brief, leaf wetness duration is a major parameter for maize breeding programs focused on selecting MLS-resistant materials.

Resistance of genotypes

The different behaviors observed among the maize genotypes tested can be associated with their susceptibility to different fungus isolates, polymorphism, and different pathogenicity of *S. macrospora*. It was demonstrated by Piletti et al. (2014) when analyzing 25 maize hybrids inoculated with four different *S. macrospora* isolates. According to Young et al. (1959), isolates can modify their pathogenicity and become more aggressive as a function of their place of origin. There are no reports in the literature about the presence of *S. macrospora* strains; however, it is known the existence of variations in the pathogenicity and aggressiveness between different isolates of the pathogen (Piletti et al., 2014).

During the last harvest seasons, the hybrids "P30F53" and "DKB240" occupied a large maize cultivation area in southern Brazil, raising the risk of an epidemic outbreak of MLS, as both cultivars have a similar behavior towards the disease. Casa et al. (2011) studied MLS incidence in southern Brazil and reported an increase in leaf ears of about 10 times that of white rot and burnt grains in ears, hence decreasing grain quality. On the other hand, Mendes et al. (2018) observed that the presence of burnt maize grains is more related to harvest time, weather conditions, fungicide application, and planted hybrid than to MLS incidence in leaf ears. Nevertheless, it is of great importance, mainly to the food and feed industries, to identify whether MLS is a strong driver for the incidence of burned grains in maize, mainly to help improve the final quality of harvested grains and recommend genotypes with higher resistance to MLS.

Despite the unavailability of commercial hybrids with complete resistance to *Stenocarpella* spp. (Hawerroth et al., 2019), the genetic variability for resistance/tolerance to this fungus among different cultivars suggests the potential for the development of resistant hybrids through specific breeding programs.

Lesion expansion rate of *S. macrospora*

The differences observed in lesion expansion rates can be used to quantify the resistance of maize hybrids and varieties. For Bove, Bavaresco, Caffi, and Rossi (2019), lesion size, spore infection efficiency, and infectious duration are among the most important components of resistance.

Regarding the distinction between susceptible and resistant genotypes, Berger, Bergamim Filho, and Amorin (1997) related more than 40 pathosystems for which lesion expansion rate has already been measured and highlighted the difference of this character between susceptible and resistant genotypes.

Interestingly, the hybrid "DKB240", which is highly cultivated under southern Brazil climatic conditions, besides being highly susceptible to MLS (Tables 3 and 4) and requiring short leaf wetness durations to reach 99% maximum severity (Table 2), showed a lesion expansion rate much lower than the hybrid "P1630H", which is also highly susceptible to the disease, but with 10% less susceptibility than the hybrid DKB240 (Table 3).

Conclusion

In this study, several genotypes were evaluated to identify their levels of resistance/tolerance to macrospora leaf spot (MLS). However, none of them had a complete resistance to the disease. Still, many cultivars showed to be promising for further studies and maize breeding programs aimed at minimizing MLS damaging effects. For instance, 4.25% of the tested genotypes showed intermediate behavior and 1.41% high/intermediate against the fungus isolates DM54 and DM58. Moreover, the hybrids "P30R50" and "P30F36" showed the same level of tolerance to MLS against both fungus isolates. Minimum leaf wetness durations of 22.4 hours are required to trigger MLS infection and development on maize ears. To reduce MLS-related problems in maize crops through genetic resistance, information about sources and types of resistance should be gathered. Thus, studies of this type must continue to identify genotypes with different levels of resistance/tolerance. Lastly, pathogen variability and cultivar genetic resistance/tolerance should be monitored to improve cultivar rotation practices.

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