

Reaction of corn lines to bacterial leaf streak







Abstract – The objective of this work was to select experimental corn lines genetically resistant to the bacterial leaf streak caused by *Xanthomonas vasicola* pv. *vasculorum*, in two growing seasons. The treatments were carried out in an experimental randomized block design in the 2019/2020 and 2020/2021 growing seasons, under a covered environment, with open ventilation, with four replicates. Seven lines resistant to and seven susceptible to foliar diseases were evaluated, in addition to two commercial hybrids used as checks. At the corn flowering stage, an artificial inoculation containing 3.78×10^{10} CFU mL⁻¹ of bacterial suspension was performed, and disease incidence and severity were assessed, based on a diagrammatic scale, every seven days. From these evaluations, the areas under the disease progress curve for each line were calculated, and individual and joint analyses of variance were performed. There is genetic variability among the corn genotypes for reaction to bacterial leaf streak, with a significant effect for the areas under the disease progress curve for incidence (AUDPCI) and severity (AUDPCS) between genotypes and between growing seasons, and for the genotype x season interaction. Lines LV1 and L14 are possible source genotypes for genetic resistance to the bacterial leaf streak.

Index terms: *Xanthomonas vasicola* pv. *vasculorum*, *Zea mays*, AUDPC, genetic resistance.

Reação de linhagens de milho à estria bacteriana

Resumo – O objetivo deste trabalho foi selecionar linhagens experimentais de milho geneticamente resistentes à estria bacteriana causada por *Xanthomonas vasicola* pv. *vasculorum*, em duas safras. Os tratamentos foram realizados em um delineamento experimental em blocos ao acaso, nas safras 2019/2020 e 2020/2021, em ambiente coberto e com ventilação aberta, com quatro repetições. Sete linhagens resistentes e sete suscetíveis a doenças foliares foram avaliadas, além de dois híbridos comerciais, utilizados como controles. No estágio de florescimento do milho, foi realizada a inoculação artificial com $3,78 \times 10^{10}$ UFC mL⁻¹ de suspensão bacteriana, e a incidência e a severidade da doença foram avaliadas, com base em escala diagramática, a cada sete dias. A partir destas avaliações, as áreas abaixo da curva de progresso da doença foram calculadas para cada linhagem, e as análises de variância individuais e conjuntas foram realizadas. Há variabilidade genética entre os genótipos de milho quanto à reação à estria bacteriana, com efeito significativo para as áreas sob as curvas de progresso da doença quanto à incidência (AUDPCI) e à severidade (AUDPCS) entre genótipos e entre safras, e quanto à interação genótipo x safra. As linhagens LV1 e L14 são possíveis genótipos fontes de resistência genética à estria bacteriana.

Termos para indexação: *Xanthomonas vasicola* pv. *vasculorum*, *Zea mays*, AUDPC, resistência genética.

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Introduction

Several foliar diseases can affect corn (*Zea mays* L.) during its cycle, directly influencing the yield and final quality of grains. In recent years, a new foliar disease, called bacterial leaf streak, whose etiological agent is the bacterium *Xanthomonas vasicola* pv. *vasculorum* (Cobb, 1894), has been observed in the main corn producing countries, including Brazil (Leite et al., 2019; Tebaldi et al., 2022).

Bacterial leaf streak manifests mainly in areas subjected to irrigation or areas with regular rainfall (Ortiz-Castro et al., 2020), presenting its initial symptoms through small punctures, due to cell extravasation. These spots progress to elongated lesions, surrounded by a yellowish halo limited by the veins. The disease can progress to such a point that it affects a large leaf area, compromising the crop productivity (Leite et al., 2019).

In general, the control of bacterial diseases is, difficult due to the rapid spread of the pathogen, the severity of losses, and the limitations of chemical control (Sundin et al., 2016). The main management measures include the use of windbreaks, equipment disinfection, diseased plants eradication, and the use of copper-based fungicides (Peitl et al., 2017), which are inefficient for the majority of crops affected by phytopathogenic bacteria (Longhi et al., 2022).

Plant genetic resistance comes as a possibility of control, and it is more efficient and less costly to the farmer, as it allows of the adoption of other techniques (Sousa et al., 2020). For diseases caused by species of the *Xanthomonas* genus, the genetic resistance is the main strategy for reducing losses (Bernal et al., 2020), since some species are insensitive to copper and, under high disease pressure, these molecules lose effectiveness (Liao et al., 2019).

To obtain hybrids that are resistant to a plant pathogen, it is necessary to evaluate different genotypes from different origins, which may provide phenotypes of interest. The existence of genetic variability is important for the study of these genotypes, so that they can be included in a breeding program (Faleiro et al., 2008).

The objective of this work was to select experimental corn lines which are resistant to bacterial leaf streak, for two seasons.

Materials and Methods

The experiments were carried out in the municipality of Guarapuava, in the state of Paraná (PR), Brazil, in the Department of Agronomy of Universidade Estadual do Centro-Oeste (Unicentro), in the Cedeteg Campus (25°23'00"S, 51°29'38.50"W, at 1,024 m altitude), in the 2019/2020 and 2020/2021 growing seasons. The climate of the region is Cfb, mesothermal humid subtropical, according to the Köppen-Geiger's classification.

The bacteria isolate *Xanthomonas vasicola* pv. *vasculorum* (Xvv) was obtained from corn plants in the region of Guarapuava. For taxonomic confirmation of the isolate, the material was sent to a specialized laboratory and identified by a genetic sequencing of the 16S region. The isolate is stored in the phytopathological collection of the phytopathology laboratory of Unicentro, where subcultures are carried out periodically.

The corn lines used to conduct the experiment came from the corn breeding program of Unicentro, taking into account the productive potential and some level of resistance to foliar diseases (Table 1). Based on the history of disease assessments in previous seasons, fourteen lines with different levels of susceptibility or resistance to foliar diseases were selected. In addition to these, the B2401PWU and BG7318YH commercial hybrids were used as standard checks of resistance and susceptibility to bacterial leaf streak (BLS), respectively, based on field observations by experienced researchers.

An area covered with 150-micra transparent plastic film, with open windows and ample ventilation was used during two growing seasons for the experiment, and the genotypes were sown on November 11, 2019 and November 13, 2020. An experimental randomized block design was carried out with 16 genotypes (Table 1), which composed the treatments, and four replicates. Each plot consisted of three pots containing one plant each, totaling three plants per plot per replicate. The plants were grown in 18 L polypropylene pots, containing a mixture of soil (50%), sand (25%) and bio-stabilized pine bark-based substrate (25%). The soil used is classified as a Brown Oxisol (Santos et al., 2018), with a very clayey texture. At sowing, the substrate was fertilized with the 08-30-20 NPK formulation.

Seed of the evaluated lines were subjected to treatment with imidacloprid + thiodicarb, to protect them against initial pests. Three seed were sown per pot, with one plant remaining after thinning 20 days after sowing. The plants were irrigated by drip, and cultural treatments related to nitrogen fertilization and insect management were carried out in accordance with the technical recommendations for the crop (Pauletti & Motta, 2019).

A bacterial suspension (Xvv) in 0.85% saline solution (NaCl) was prepared using young colonies (48 hours). The suspension was adjusted to 0.6 absorbance in a spectrophotometer (OD_{580 nm}, BEL Photonics, model UV-M51), with 3.78x10¹⁰ CFU mL⁻¹, according to Lautenchleger et al. (2023).

Plant irrigation was performed two hours before the inoculation to fully open the stomata. Plant inoculation was carried out at full flowering, by spraying the bacterial suspension with a manual sprayer on the adaxial surface of all plant leaves, until they drained (Lautenchleger et al., 2023).

In the 2019/2020 growing season, the inoculation was carried out in the thirteenth week, and the evaluation period lasted for six weeks. In the 2020/2021 growing season, in the 12th week, the plants were subjected to inoculation and evaluated until the twentieth week. As the experimental area is a space with open ventilation, climate data from the external environment, coming from the local meteorological station, were considered. In the 2019/2020 growing season, the average temperature at the experimental site was 18.8°C. and the relative humidity (RH) was 67%. In the 2020/2021 growing season, the averages were higher: 20.5°C and 80%. From the inoculation time until the end of the evaluations, the averages were 21°C and 73% RH, and 20.5°C and 78% RH for the 2019 and 2020 seasons, respectively (Figure 1).

From the inoculation date on, the genotypes' reaction to the pathogen were assessed every seven days. In each plot, symptomatic leaves were counted to calculate the incidence, and severity scores were assigned with basis on the diagrammatic scale proposed by Braga et

Table 1. Lines and hybrids of corn (*Zea mays*) selected for genetic resistance to bacterial leaf streak (*Xanthomonas vasicola* pv. *vasculorum*), in the 2019/2020 and 2020/2021 growing seasons, in the municipality of Guarapuava, in the state of Paraná, Brazil.

Genetic material	Origin	Generation
B2401PWU	Corteva	Hybrid ⁽¹⁾
BG7318YH	Corteva	Hybrid ⁽²⁾
LV1	AG8080	S ₈
LV4	DKB350	S ₈
LV5	AG6018	S ₈
L160	POP30 (P30P37 x 8460)	S ₇
L18	POP30 (P30P37 x 8460)	S ₇
L54.1	POP102 (P30B39)	S ₅
L4.1	POP103 (AS1572)	S ₅
L52.3	POP103 (AS1572)	S ₅
LV2	CD303	S ₈
LV6	P30F33	S ₈
L14	POP29 (Penta x P30F53)	S ₇
L20	POP31 (Penta)	S ₇
L71	POP31 (Penta)	S ₇
L73.1	POP103 (AS1572)	S ₄

⁽¹⁾Resistant check. ⁽²⁾Susceptible check.

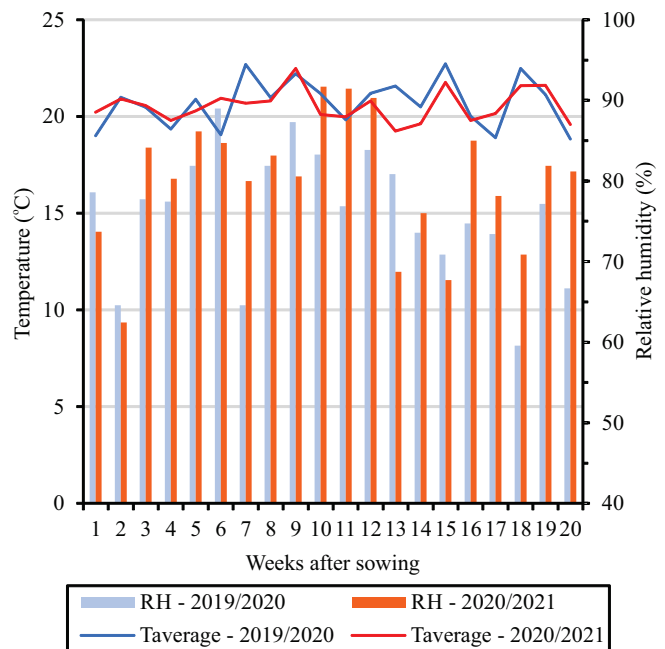


Figure 1. Weekly average temperature and relative humidity (RH) data from the meteorological station, in the municipality of Guarapuava, in the state of Paraná (PR), Brazil, during the 2019/2020 and 2020/2021 corn (*Zea mays*) growing seasons.

al. (2020). The incidence and severity values obtained were submitted to the calculation of the area under the disease progress curve (AUDPC) according to Shaner & Finney (1977).

Individual and joint analyses of variance were performed for the two growing seasons. Hybrids and lines were evaluated together, due to the unavailability of other genotypes, especially lines that showed resistance to the disease. Then, the significant variables were submitted to comparison of the means by the Scott-Knott's test, at 5% probability, using the Sisvar program, version 4.0 (Ferreira, 2019).

Results and Discussion

There was a significant effect for the areas under the progress curve of incidence (AUDPCI) and severity (AUDPCS) among genotypes, between seasons, and for the genotypes x seasons interaction. For the developments of the interaction, there was a significant difference for AUDPCI (Table 2), in both seasons, and for only AUDPCS (Table 3) in the 2020/2021 season.

Table 2. Breakdown of the genotype x season interaction, based on the means of the area under the incidence progress curve of bacterial leaf streak caused by *Xanthomonas vasicola* pv. *vasculorum* in corn (*Zea mays*), in the municipality of Guarapuava, in the state of Paraná, Brazil⁽¹⁾.

Genotype	Season	
	2019/2020	2020/2021
B2401PWU	0.00 Aa	2377.26 Ba
BG7318YH	1029.00 Ab	3220.99 Ba
LV1	147.00 Aa	2920.28 Ba
LV4	401.62 Aa	3704.52 Bb
LV5	141.75 Aa	3003.58 Ba
L160	663.25 Aa	3602.80 Bb
L18	1404.37 Ab	3811.22 Bb
L54.1	1411.37 Ab	3986.74 Bb
L4.1	113.75 Aa	4118.43 Bb
L52.3	130.37 Aa	3579.44 Bb
LV2	1250.37 Ab	3561.79 Bb
LV6	289.62 Aa	3540.46 Bb
L14	336.00 Aa	3117.85 Ba
L20	1544.37 Ab	3912.80 Bb
L71	925.75 Ab	3950.82 Bb
L73.1	512.75 Aa	4238.54 Bb

⁽¹⁾Means followed by equal letters, lowercases in the columns and uppercases in the rows, belong to the same group, by Scott-Knott's test, at 5% probability.

When considering season within each genotype, AUDPCI was significant for both growing seasons. For AUDPCS, there was significance for the LV4, LV5, L160, L18, L54.1, L4.1, L20, L71, and L73.1 lines.

In the 2020/2021 growing season, the averages for AUDPCI (Table 2) and AUDPCS (Table 3) of bacterial leaf streak in corn were significantly higher than in the 2019/2020 season. Considering that the experiment was under the influence of external climatic variables, variations of temperature and humidity over the years may have influenced the expression of the disease (Figure 1).

All genotypes showed higher AUDPCI values in the second growing season than in the first one (Table 2). The AUDPCS was higher in the second season only for some lines (Table 3), showing that the expression of the disease is influenced by environmental factors, such as air temperature and relative humidity, as well as for other foliar diseases in corn (Hooda et al., 2017; Ortiz-Bustos et al., 2019).

When analyzing each growing season separately, the genotypes also showed differences for the incidence

Table 3. Breakdown of the genotype x season interaction, based on the means of the area under the severity progress curve of bacterial leaf streak, caused by *Xanthomonas vasicola* pv. *vasculorum* in corn (*Zea mays*), in the municipality of Guarapuava, in the state of Paraná, Brazil⁽¹⁾.

Genotype	Season	
	2019/2020	2020/2021
B2401PWU	0.00 Aa	31.00 Aa
BG7318YH	16.72 Aa	35.66 Aa
LV1	0.72 Aa	33.19 Aa
LV4	4.16 Aa	48.66 Ba
LV5	0.71 Aa	64.63 Bb
L160	6.52 Aa	64.60 Ba
L18	20.99 Aa	97.44 Bb
L54.1	30.63 Aa	93.15 Bb
L4.1	0.92 Aa	142.45 Bc
L52.3	0.89 Aa	39.34 Aa
LV2	14.62 Aa	51.87 Aa
LV6	2.32 Aa	36.49 Aa
L14	3.49 Aa	42.95 Aa
L20	15.04 Aa	280.06 Bd
L71	8.83 Aa	61.14 Ba
L73.1	2.87 Aa	91.31 Bb

⁽¹⁾Means followed by equal letters, lowercases in the columns and uppercases in the rows, belong to the same group, by Scott-Knott's test, at 5% probability.

and severity of the disease. The B2401PWU hybrid, considered a resistant check, showed low values for both AUDPCI and AUDPCS, within the genotypes. The BG7318YH hybrid, used as susceptible check, showed high incidence values of area under the progress curve, in the 2019/2020 season, but showed no statistical difference in comparison with the resistant control in the 2020/2021 season. The L4.1 and L20 lines showed higher means for AUDPCS and were even more susceptible to the pathogen than the susceptible check itself in the 2020/2021 season.

Genotypes resistant to a pathogen may have some form of limitation to their multiplication, or even delay their development (Råberg, 2014). When evaluating a foliar disease, it is expected that lines that have resistance genes will show no or little progress of the disease over time. However, as most disease resistance inheritance in corn has a polygenic origin, it is natural that there are different levels of incidence and severity between genotypes and between seasons (Ferreira et al., 2024). Even so, it is important that stable genotypes (those showing similar responses between environments and seasons) be identified (Bocianowski et al., 2020).

Preliminary studies indicate that corn resistance to BLS is quantitative (Qiu et al., 2020). Historically, this is the main type of resistance implemented in commercial hybrids, and it is preferable in relation to the qualitative resistance because, as it is governed by a large number of genes, it is generally effective against several races of the same pathogen, thus becoming more durable over time (Miedaner & Juroszek, 2021). The fact that the checks showed discrepant values between seasons suggests that the performances can vary, even in genotypes considered stable, due to climatic variables (Figure 1) and the nature of resistance. This highlights the complexity of managing bacterial diseases in corn and the need to comprehensively assess the susceptibility to BLS in commercial hybrids. It is worth pointing out that, at the time this experiment was carried out, there was little information on the susceptibility of commercial hybrids, and that this work was a pioneer in studies regarding genetic resistance to the disease.

Combinations of genetic control with other cultural practices, such as crop rotation, and the use of chemicals can help manage the disease (Duin et al., 2022). Bacterial leaf streak was quantified as a function

of commercial corn hybrids, associated with chemical control and identified resistant and susceptible genotypes by Pietrobon et al. (2021). However, in susceptible material, the adoption of chemical control allowed of 55% reduction in AUDPC in relation to the control.

The L18, L54.1, LV2, L20, and L71 lines undergone high incidence of disease in both growing seasons, which could be a negative aspect. Even with a significant difference for incidence between the growing seasons, the LV1, LV5, and L14 lines stood out positively, showing lower incidence values in both seasons (Table 2).

In the evaluation period of the 2019/2020 growing season, in which incidence and severity values were lower, a peak of the average temperature was recorded (22.5°C), together with the minimum average RH, throughout the entire period (60%) (Figure 1). In the 2020/2021 growing season, the weekly average RH, in the evaluation period, was not less than 69%, and the average maximum temperature was 21.8°C. These data indicate that higher RH favored the development of the disease, corroborating the results found by Hartman et al. (2020), who identified an increase in the severity of BLS, in conditions of 100% relative humidity. Any irrigation system in corn cultivations, combined with high relative humidity, favors the occurrence of the disease, as this combination allows of longer stomatal opening time, the main gateway to XvV (Korus et al., 2017).

When comparing the progression of incidence and severity, especially in the 2020/2021 growing season, it can be observed that the incidence was high in practically all genotypes (Table 2). However, the same performance was not observed for severity (Table 3). This indicates that the pathogen was able to infect plants, but conditions were not favorable for its colonization, suggesting that these lines have some type of defense mechanism to the bacteria (Amaral et al., 2019). These results corroborate preliminary studies which report that resistance to BLS is quantitative in nature and that genetic control can be used in the disease management, together with other techniques, especially chemical control.

Therefore, the lines that showed the lowest disease progress curves are indicated to make part of a breeding program, in which they can be crossed with other promising genotypes with high productive potential,

and their combining capabilities can be estimated (Lima & Borém, 2018).

Conclusions

1. There is genetic variability among the evaluated corn (*Zea mays*) genotypes regarding the reaction to corn bacterial leaf streak (*Xanthomonas vasicola* pv. *vasculorum*).

2. A significant effect for incidence and severity of bacterial leaf streak occurs among genotypes and among seasons, as well as in the genotype x season interaction.

3. Lines LV1 and L14 show the lowest values of area under the incidence and severity progress curve, and are presented as possible genotypes as source of corn resistance to bacterial leaf streak.

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