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INCIDENCE OF CHARCOAL ROT (*Macrophomina phaseolina*) IN MAIZE HYBRIDS IN DIFFERENT ENVIRONMENTS DURING OFF-SEASON

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ABSTRACT – Maize cultivation in Brazil can occur during the summer or off-season. Diseases are significant contributors to production losses. Charcoal rot (*Macrophomina phaseolina*) is the primary disease affecting off-season maize crops. This study aimed to assess the incidence of charcoal rot in commercially available maize hybrids under different soil water availability conditions during the off-season. The experiments were conducted at the Embrapa Research Facility, located at Invernadinha Farm. The experimental design was a randomized block design with three replicates. The first experimental crop was sown on February 21, 2022, in a red-yellow Latosol soil environment (Environment 1). The second was sown on March 3, 2022, in a Concretionary Petric Plinthosol soil environment (Environment 2). Ten hybrids were used in each experiment. After the plants reached physiological maturity, plant height was measured, and ten stalk samples were collected from each plot. Afterwards, the microsclerotia of *M. phaseolina* were identified visually in the laboratory. Data are expressed as percentages and subjected to a joint analysis of variance. These results indicate that soil conditions of higher water stress, in conjunction with elevated temperatures, significantly enhanced the development of charcoal rot and directly affected plant productivity. A joint analysis of the environments revealed that the AS1820, AS1822, and P3707 hybrids had the lowest average incidence of charcoal rot and the highest yields.

Keywords: corn disease, latosol, plinthosol, gray stalk rot, *Zea mays*.

INCIDÊNCIA DE PODRIDÃO DO CARVÃO *(Macrophomina phaseolina)* EM HÍBRIDOS DE MILHO EM DIFERENTES AMBIENTES DURANTE A SAFRA

RESUMO - O cultivo de milho no Brasil é realizado em duas épocas, no verão (primeira safra) e no inverno (safrinha ou segunda safra). As doenças contribuem, significativamente, para as perdas na produção deste cereal e a podridão de macrophomina no colmo é uma das principais doenças que afetam os cultivos de milho na safrinha. O objetivo deste estudo foi avaliar a incidência da podridão de macrophomina em híbridos comerciais de milho sob diferentes condições de disponibilidade de água no solo durante a safrinha. Os experimentos foram conduzidos no área experimental da Embrapa, localizado na Fazenda Invernadinha. Foi utilizado o delineamento experimental de blocos casualizados com três repetições. O primeiro experimento foi semeado em 21 de fevereiro de 2022, em ambiente de Latossolo Vermelho-Amarelo (Ambiente 1) e o segundo foi semeado em 03 de março de 2022, em ambiente de Plintossolo Pérrico Concrecionário (Ambiente 2). Em cada experimento foram utilizados dez híbridos. Após a fase de maturação fisiológica, a altura das plantas foi medida e dez amostras de caule foram coletadas de cada parcela. A presença de microescleródios de *M. phaseolina* foi identificada visualmente em laboratório. Os dados foram expressos em porcentagem e submetidos à análise de variância conjunta. Os resultados indicaram que sob condições de maior estresse hídrico no solo, associado a temperaturas elevadas, houve aumento significativo no desenvolvimento da podridão de macrophomina no colmo e impactaram diretamente a produtividade das plantas. Os híbridos AS1820, AS1822 e P3707 demonstraram a menor incidência média da doença e as maiores produtividades.

Keywords: doença do milho, latossolo, plintossolo, podridão cinzenta do colmo, *Zea mays*.

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Maize is one of the most economically valuable, high-yield crops worldwide. The Food and Agriculture Organization of the United States (FAO, 2022) reported that global maize production increased by 73.36% from 2010 to 2020. The top maize-producing countries are the United States, China, and Brazil (United States Department of Agriculture, 2022a). Brazil is the third largest producer and exporter of maize, accounting for 17.98% of the total exports during the 2021/2022 crop season (United States Department of Agriculture, 2022b).

Maize is cultivated in Brazil during two distinct seasons: the first crop season (summer crop season) and the second crop season (off-season). Summer harvest is widely practiced across most states during the rainy season, which begins in August in the southern region and extends to mid-October or November in the midwestern, southeastern, and northern regions. Off-season crops refer to cultivating drought-resistant maize seeded between January and March following the harvest of early soybeans in the summer (Companhia Nacional de Abastecimento, 2019).

Weather conditions crucial for maize cultivation in Brazil are favorable for the emergence of diseases that harm the yield and quality of the grain (Silva et al., 2020). These diseases have been estimated to cause annual losses of 10–15% in maize crops (Mueller et al., 2016).

Charcoal rot of maize is a prevalent disease in off-season maize crops and is caused by the fungus Macrophomina phaseolina (Tassi) Goid. The disease is most likely to occur in hot and dry weather conditions, commonly found in the low-altitude Brazilian Cerrado region, where off-season maize is grown (Almeida et al., 2014). M. phaseolina is a native soil inhabitant and can infect numerous agriculturally important species. In the absence of hosts, the fungus can persist in the soil by producing resistance structures known as microsclerotia (Costa et al., 2020).

Microsclerotia are resistance structures that form when hyphae are compacted. These structures enable the fungus to persist in soil and crop residues for extended periods and serve as the main source of primary inoculum for the fungus (Ishikawa et al., 2018). In addition, because M. phaseolina fungus is polyphagous, the inoculum produced in one crop during the growing season can spread to infect a subsequent crop, as seen in the off-season maize crop grown after soybean (Costa et al., 2020).

Monitoring conducted by Embrapa in Tocantins (TO) revealed the presence of M. phaseolina in 88% of samples collected from the state's off-season maize production regions (Costa et al., 2020). In addition, the authors detected fungi in all areas where the samples were obtained and observed decreases in yield ranging from 27% to 47%, which varied depending on the location and hybrids used.

Management of Charcoal rot is complex because of the broad host range of the fungus, which limits the use and efficiency of control methods such as crop rotation and foliar fungicide applications, compounded by the fungus's location in the soil or inside the root and stalks tissues (Nascimento et al., 2016).

Genetic resistance is considered one of the main strategies for managing the charcoal rot of maize. However, information regarding the resistance levels of commercial hybrids is limited. This lack of information prevents farmers from

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choosing and planting more resistant hybrids in areas where the disease is endemic and has a high incidence. To address this, Embrapa has been continuously conducting trials to evaluate commercial maize hybrid resistances to charcoal rot to guide farmers in choosing the most suitable cultivars for cultivation in the low-altitude Cerrado regions of Brazil.

We aimed to evaluate the incidence of charcoal rot of maize in commercial hybrids grown in different soil water availability environments.

Material and Methods

The experiments were conducted at the Embrapa experimental station at the Invernadinha Farm, Paraíso do Tocantins, Brazil. The coordinates of the location are 10°11’16.383”S, 48°40’55.484”W. The first experiment was planted on February 21, 2022, in Red-yellow Latosol soil (Environment 1). The second was planted on March 3, 2022, in Concretionary Petric Plinthosol soil (Environment 2). The region’s climate is classified according to the Köppen system as Aw, tropical Cerrado, with dry winters.

The experimental design was a randomized block design with ten treatments (maize hybrids) and three replicates in both experiments. The list of evaluated hybrids is shown in Table 1. The FS715 hybrid was used as the susceptibility standard for charcoal rot of maize. The plots consisted of four 5.0-m rows, with 0.5-m spacing between them and an average plant density of 60,000 plants per hectare. Two central rows from each plot were used for evaluation and harvest. Sowing was performed using the no-tillage system, with 400 kg per hectare of 15-15-15 for base fertilization and 187 kg per hectare of 32-00-00 for topdressing in the V4 stage (four fully expanded leaves). Other crop practices, such as pest, disease, and weed management, were performed following the standard practices of the farm to obtain high yields.

The experiment was conducted using a natural fungal inoculum in the soil. At the end of the cycle, after the physiological maturity of the plants and before harvest, the height of the plants was measured, and ten stalk fragments were randomly collected from the two central rows of each plot. Fragments were collected between the two nodes at the base of the stalks and taken to the laboratory for analysis of the presence of *M. phaseolina* microsclerotia. In the laboratory, the stalks fragments were opened longitudinally, and microsclerotia in the stalks tissues was visually identified using a stereoscopic magnifying glass (Figure 1). The number of stalks fragments exhibiting microsclerotia formation was counted relative to the total number of fragments collected. The data were expressed as a percentage of infected stalks, reflecting the incidence of charcoal rot.

All ears of the two central rows of each plot were harvested, threshed, and their weight and moisture content were measured. The grain weight was adjusted to a moisture content of 13%, and the yield was expressed in bags ha^{-1}.

Data on the percentage of infected stalks were transformed to $\sqrt{x+1}$.

The transformed data, and data on productivity, plant height, and thousand-grain weight, were then subjected to joint variance analysis in two different growing environments. Finally, the means were compared using the

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Figure 1. Sample of maize stalks with charcoal rot symptoms (A); Presence of *Macrophomina phaseolina* microsclerotia in the vascular tissues of the stalk (B and C) (Source: Rodrigo Véras da Costa, Embrapa Milho e Sorgo).

Scott-Knott test at a 5% significance level when necessary using the statistical software Sisvar, version 5.6.

Precipitation and temperature data were collected from a local weather station in the experimental area, including the maximum, average, and minimum values. Using the Penman-Monteith method, the collected data were then used to calculate Potential Evapotranspiration (ET0). Subsequently, water balances (Figure 2) were determined for each experiment based on the methodology established by Thornthwaite and Mather (1955) and Camargo (1962) using temperature, precipitation, and ET0 data. The available water capacity (AWC) adopted for the Latosol experiment was 72 mm, and the Petric Plinthosol experiment was 27.55 mm (Bouwer & Rice, 1984).

Results and Discussion

The first environment was defined by the sowing of maize hybrids during the optimal period

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Table 1. List of maize hybrids evaluated in experiments conducted in the experimental area of Invernadinha Farm, Paraíso do Tocantins, TO, off-season 2022.

TREAT	Hybrids	Technology	Company
1	20A38	VIP3	Sempre Sementes
2	AG7098	TRECEPTA	Sementes Agroceres
3	AS1820	PRO3	Agroeste
4	AS1822	PRO3	Agroeste
5	FS715	PWU	Forseed Sementes
6	MG580	PW	Morgan Sementes
7	NS45	VIP3	Nidera Sementes
8	P3707	VYH	Pioneer Sementes
9	P3898	Conventional	Pioneer Sementes
10	SHS7940	PRO3	Santa Helena Sementes

Source: Seed companies, 2022 crop season.

for cultivating off-season maize crops (February) in a Latosol soil type, characterized by a high clay content and, thus, a high capacity for water retention. The second environment was defined by sowing maize hybrid in March, the period considered the riskiest for cultivating off-season maize crops in the region. This environment featured a Plinthosol soil type, abundant in gravel and with a lower water retention capacity. Therefore, the first environment has higher water availability based on the soil type and sowing time. In contrast, the second environment is associated with a higher water deficit (Figure 2).

According to the joint analysis of variance, a significant interaction was found between the "environmental" and "hybrid" factors for charcoal rot incidence, plant height, and grain yield (Table 2). Although the highest occurrence of charcoal rot was observed in Environment 2, which had the highest water stress (Figure 3), there was a difference in the ranking of the hybrids across the two environments, which accounted for the interaction between the factors for that particular variable. In contrast, plant height, grain weight, and grain yield were higher in the environment with greater soil water availability (Environment 1) (Fig. 3).

In both environments, the maize hybrids were categorized into three groups based on the incidence of charcoal rot (Table 2). In the first environment, 40% of the maize hybrids had a low disease incidence. However, in the second environment, with higher soil water stress, only 20% of the evaluated hybrids were in the low-incidence group. The hybrids AS1820 and P3707 were the most resistant to charcoal rot in both environments. However, hybrid AS1822 was still able to maintain a high yield in the second environment, although it was not in the group of the most resistant hybrids (Table 2). Although the incidence of charcoal rot was relatively high in AS1822, the visible symptoms on the stalks were mild, which could explain the high yield observed in the second environment.

This study observed increased symptoms associated with charcoal rot in water-stressed

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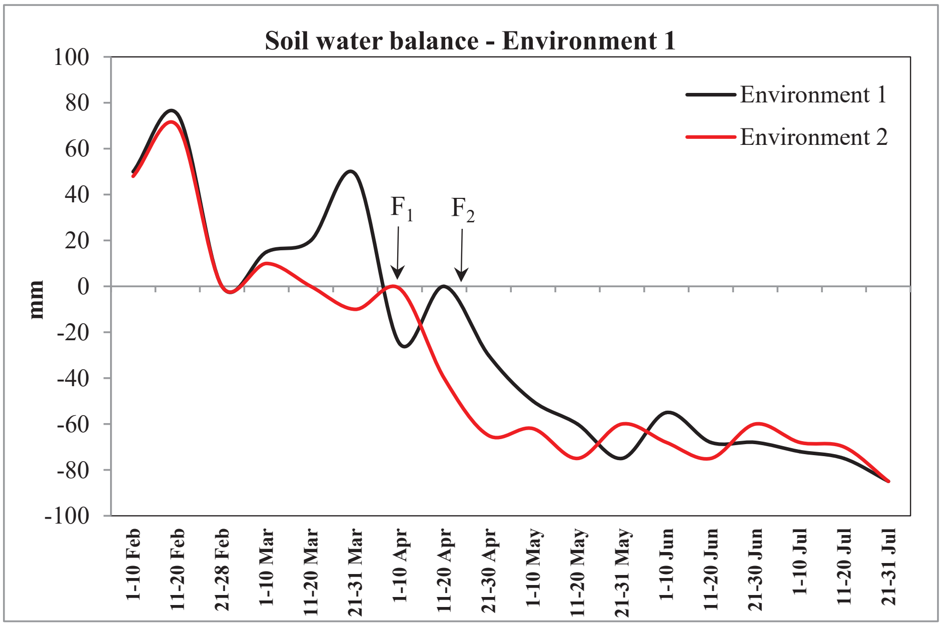

Figure 2. Water balance for the experiments conducted in two environments in the 2022 off-season, in Paraíso do Tocantins, TO. Environment 1: Latosol areas sown on 02/21/2022; Environment 2: Plinthosol area with sowing on 03/03/2022. F1: Average flowering of cultivars in Environment 1; F2: Average flowering of cultivars in Environment 2.

soils. The average incidence of charcoal rot was 38.5% in environments with low water restriction and 62.4% in environments with high water restriction, with a difference of approximately 24% (Figure 3). These results align with prior literature, highlighting the relationship between soil water availability and the incidence of charcoal rot in various critical agronomic crops. According to Luna et al. (2017) and Costa et al. (2019), charcoal rot of maize symptoms is commonly observed in water-stressed areas, such as the outskirts of crops, compacted soils, and slopes, especially in years with high temperatures and low soil moisture. Ashraf et al. (2017) found that an increase in temperature resulted in increased mycelial growth in 24 isolates of *M. phaseolina*, which were collected from maize plants. However, Lodha and Mawar (2020) reported that low soil moisture levels had a more significant impact on the development of the disease caused by *M. phaseolina* than high temperatures. Jordaan et al. (2019) studied the effects of irrigation on the severity of the disease caused by *M. phaseolina* in soybean crops. The authors found that irrigation did not prevent plant infection but could significantly reduce the severity of the disease in infected plants.

A study on the impact of moisture levels on the survival of *M. phaseolina* in two types of soil, sandy loam and clay loam (Dhingra & Sinclair, 1975), found that in both soil types, the population of *M. phaseolina* declined significantly as soil moisture and incubation time increased. The authors stated that a decline

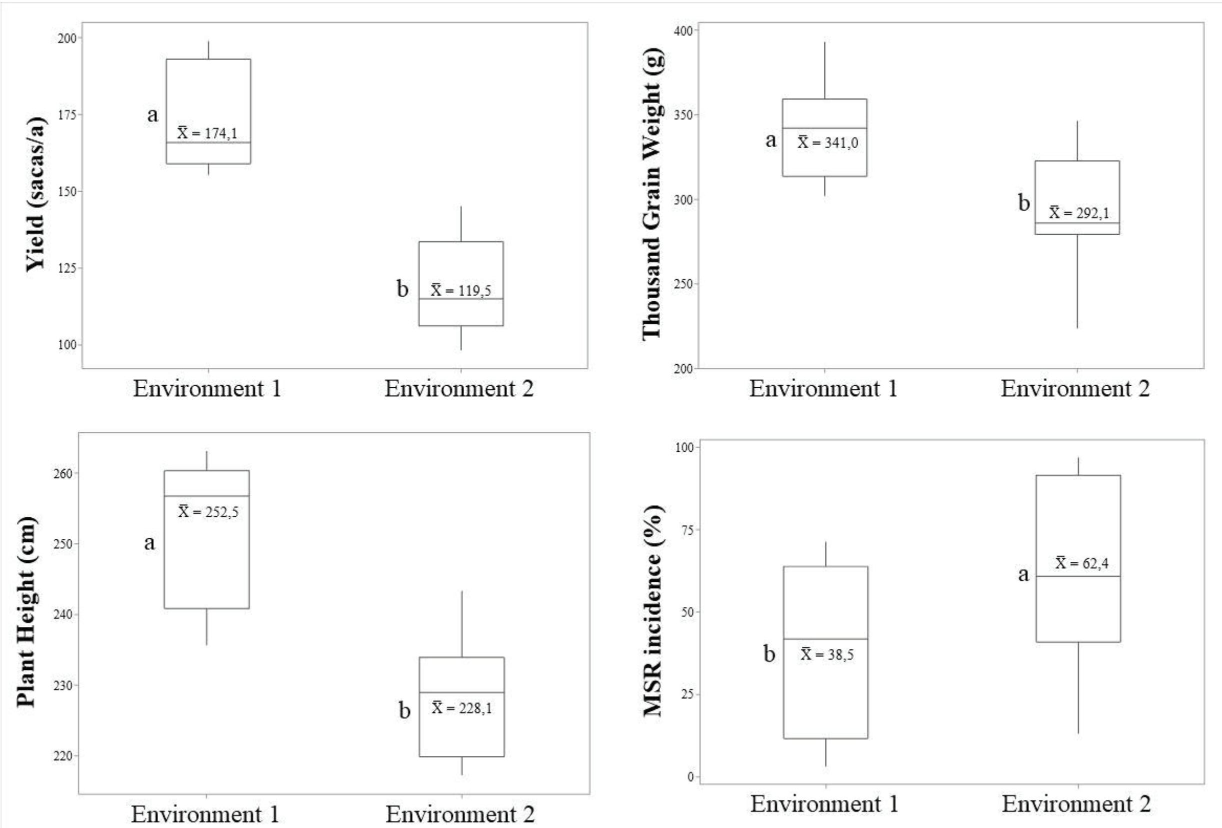

Figure 3. Yield boxplot (bags), thousand grain weight (g), plant height (cm), and incidence (%) of charcoal rot of 10 maize hybrids in two environments: Environment 1: Sowing in Latosol in 02/21/2022 (greater water availability); Environment 2: Sowing in Petric Plinthosol on 03/03/2022 (greater water restriction). Experiments conducted in the off-season in Paraíso do Tocantins, TO, 2022.

in the density of microsclerotia at high soil moisture levels could explain, to some extent, the reduced occurrence of the disease during rainy periods or after frequent irrigation. This study found that growing maize hybrids in a more arid environment resulted in an average increase in charcoal rot incidence of 60% (Figure 3).

In a seminal study, Olaya and Abawi (1996) explored the impact of water osmotic potential (Ψs) on the growth parameters of *M. phaseolina*. Their findings showed that microsclerotia's growth, production, and germination in Potato Dextrose Agar (PDA) increased as the matrix and osmotic potential decreased. These results were later confirmed by Goudarzi et al. (2008, 2011). These studies revealed that *M. phaseolina* could grow and produce large numbers of microsclerotia under low water potential conditions. These finds explain why this disease is prevalent in the tropics, where the growing periods often feature high temperatures and frequent dry spells.

There was a significant interaction between

Table 2. Incidence of charcoal rot, yield, height, and weight of a thousand grains of maize hybrids cultivated in two environments (Env. 1- Greater water availability; Env. 2 - Greater water restriction) in the off-season in Paraíso do Tocantins, TO, 2022.

Hybrids	Incidence (%)		Yield (bags ha^{-1})		Plant height (cm)		Thousand Grain Weight (g)
	Env. 1	Env. 2	Env. 1	Env. 2	Env. 1	Env. 2	
AS1822 PRO3	3.33 Cb	8.14 Ba	198.89 Aa	141.19 Ab	263.0 Aa	219.1 Bb	326.00
AS1820 PRO3	6.67 Ca	8.90 Ca	195.22 Aa	144.95 Ab	259.8 Aa	222.1 Bb	319.80
P3707 VYH	13.33 Ca	3.33 Ca	190.60 Aa	128.19 Bb	252.7 Aa	235.2 Ab	357.00
P3898	15.57 Cb	0.00 Ba	192.41 Aa	131.07 Bb	239.2 Ba	233.4 Aa	322.00
MG580 PW	37.04 Bb	5.57 Aa	160.32 Ba	106.43 Cb	241.3 Ba	229.1 Aa	326.00
AG7098 TER	46.67 Bb	6.67 Aa	156.02 Ba	105.11 Cb	257.9 Aa	220.1 Bb	293.50
20A38 VIP3	62.23 Ab	0.00 Aa	159.94 Ba	114.13 Cb	257.8 Aa	217.3 Bb	310.10
SHS7940 PRO3	62.82 Aa	6.67 Aa	166.64 Ba	98.36 Cb	255.6 Aa	243.2 Aa	309.80
NS45 VIP3	66.67 Aa	5.00 Ba	155.53 Ba	115.63 Cb	235.7 Ba	232.1 Aa	311.10
FS715 PWU	71.10 Aa	0.00 Aa	165.19 Ba	110.29 Cb	261.8 Aa	228.8 Ab	289.80
ENVIRONMENT 1	-	-	-	-	-	-	
ENVIRONMENT 2	-	-	-	-	-	-	

Means followed by the same letter (uppercase in the column and lowercase in the row) do not differ by the Scott-Knott test, with p ≤ 0.05%, CV = 9.5.

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the environment and the maize hybrids factors to plant height variable. In each environment, hybrids were separated into two distinct groups. In the first environment, the height of plants varied from 235.7 to 263 cm, with an average of 252.48 cm. The tallest hybrids in this environment had an average height of 258.3 cm, whereas the shortest had an average of 238.7 cm. In the second environment, which had higher water restrictions, the height of plants varied from 217.3 to 243.2 cm, with an average of 228.04 cm. The tallest group in this environment had an average height of 233.6 cm, and the shortest had an average of 219.65 cm. There was an approximately 25 cm (10 %) difference in the average height of plants between the two environments. This difference was due to the combined effects of increased water restriction and charcoal rot incidence. Thus, it was impossible to determine the separate effects of each factor on the growth of maize plants in this study.

Several studies have shown that maize plants experience reduced growth under water stress conditions (Chaves et al., 2003; Bu et al., 2010; Bonfim-Silva et al., 2011; Melo et al., 2018). Lack of soil moisture hinders the metabolic processes of maize plants, leading to reduced biomass accumulation and a lower photosynthetic rate, resulting in smaller and less productive plants (Bu et al., 2010). The extent of this effect varies based on the duration and severity of the water stress (Chaves et al., 2003). Bonfim-Silva et al. (2011) found that maize plant growth was reduced by approximately 50% when field capacity decreased from 60% to 30%. Another study by Melo et al. (2018) showed that 12.5% of the genotypes had higher plant height under water stress than the control. In this study, 40% of the hybrids showed no significant differences in plant height between the two environments. However, all hybrids had lower yields under water stress conditions; therefore, it cannot be concluded that these hybrids are more resistant to water stress or charcoal rot.

The yield of the maize hybrids was analyzed in two environments. In Environment 1, 40% of the hybrids were categorized into the most productive group, whereas in environment 2, this group comprised only 20% of the hybrids. The hybrids AS1822 and AS1820 were the most productive in both environments (Table 2). The weight of 1000 grains was also analyzed and showed a difference between the two environments, with higher averages in the environment with greater water availability (Table 2). However, it was impossible to separate the effects of water restriction and charcoal rot incidence on maize hybrids' yield and plant height, as warmer and drier environments are highly conducive to disease development.

Water restriction has a direct effect on maize yield parameters in addition to disease (Wang et al., 2017). These combined parameters strongly reduced the maize growth and yield compared with the disease alone. Maize requires a significant amount of water throughout its growth cycle (Albuquerque & Resende, 2002), and a water deficit during its vegetative, flowering, and grain-filling stages can significantly decrease the number and weight of grains produced (Wang et al., 2017), reduce evapotranspiration (Wang et al., 2019), and shorten the crop cycle (Shi et al., 2021). Shi et al. (2021) showed that a 50% water deficit during the vegetative and flowering stages could reduce the effective grain-filling phase duration by 24%. The greater water availability can partially explain the higher yields obtained in Environment 1 during the vegetative stage and close to flowering compared to the conditions in Environment 2 (Figure 2).

Although it is not accurate to solely attribute the negative impact on maize yield under water-restricted conditions to charcoal rot incidence, the disease was shown to affect the performance of maize hybrids significantly. This situation can be seen in the strong negative correlations between yield and charcoal rot incidence in both environments of -0.89 and -0.76, respectively (Figure 4). These solid negative correlations indicate that the higher the charcoal rot incidence, the lower the maize yield. This result is consistent with the findings of Costa et al. (2019), who studied the impact of charcoal rot on yield losses in off-season maize crops and found that all maize yield parameters were lower in infected plants than in healthy ones. Zanella et al. (2020) reported similar results when examining the severity of charcoal rot in different bean cultivars.

The incidence of charcoal rot increased in most maize hybrids as water restriction increased. On average, the incidence of charcoal

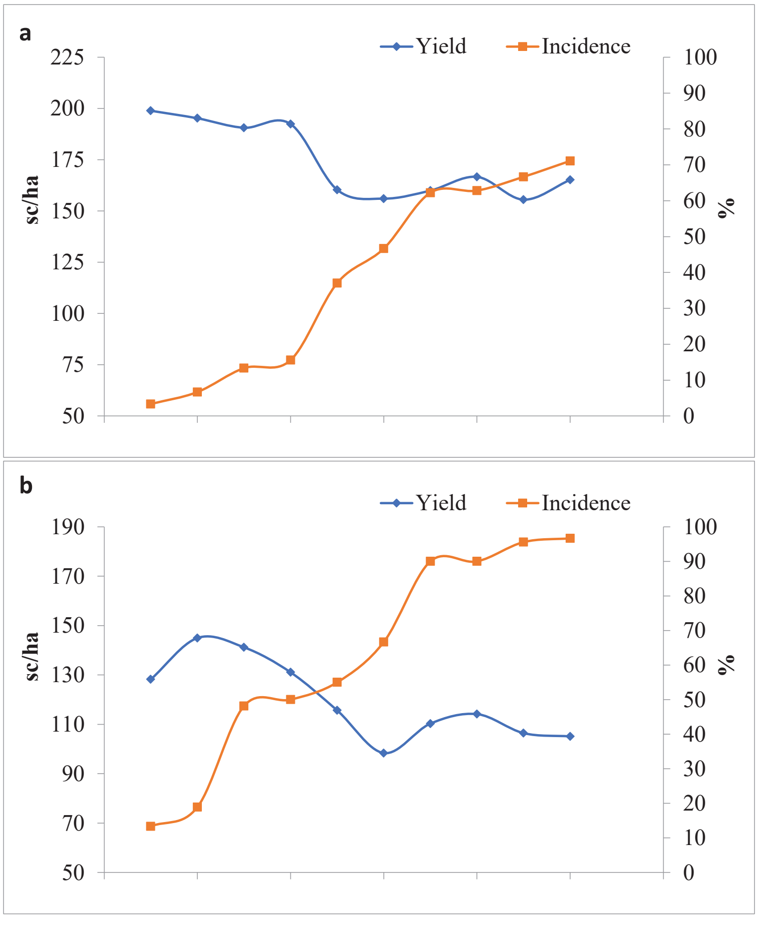

Figure 4. Correlation between yield and charcoal rotincidence of 10 maize hybrids grown in two environments: Environment 1 (a) and Environment 2 (b).

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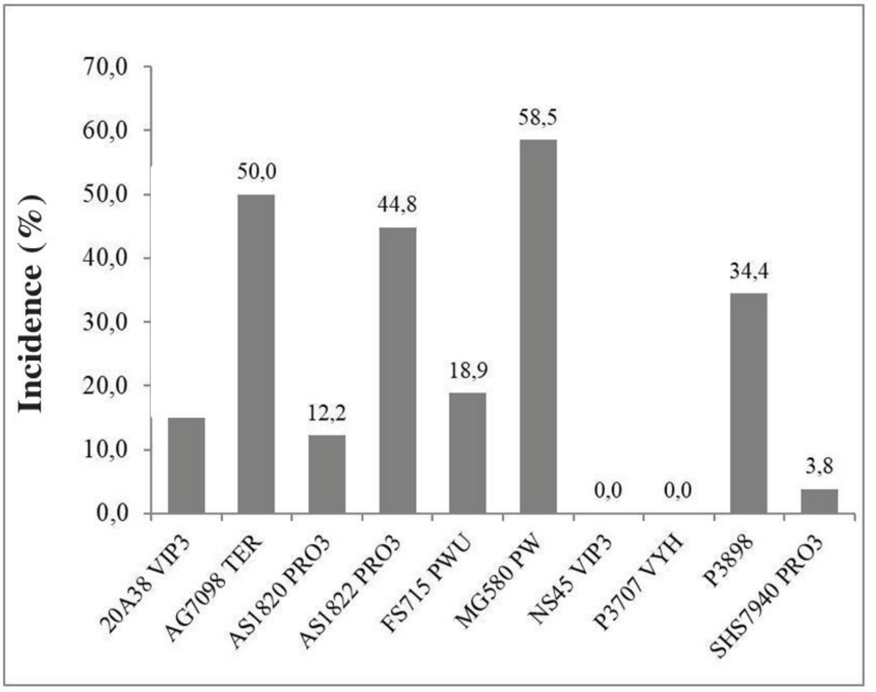

Figure 5. Difference in the charcoal rot incidence in maize hybrids in Environment 1 and Environment 2 conducted in the off-season, in Paraíso do Tocantins, 2022.

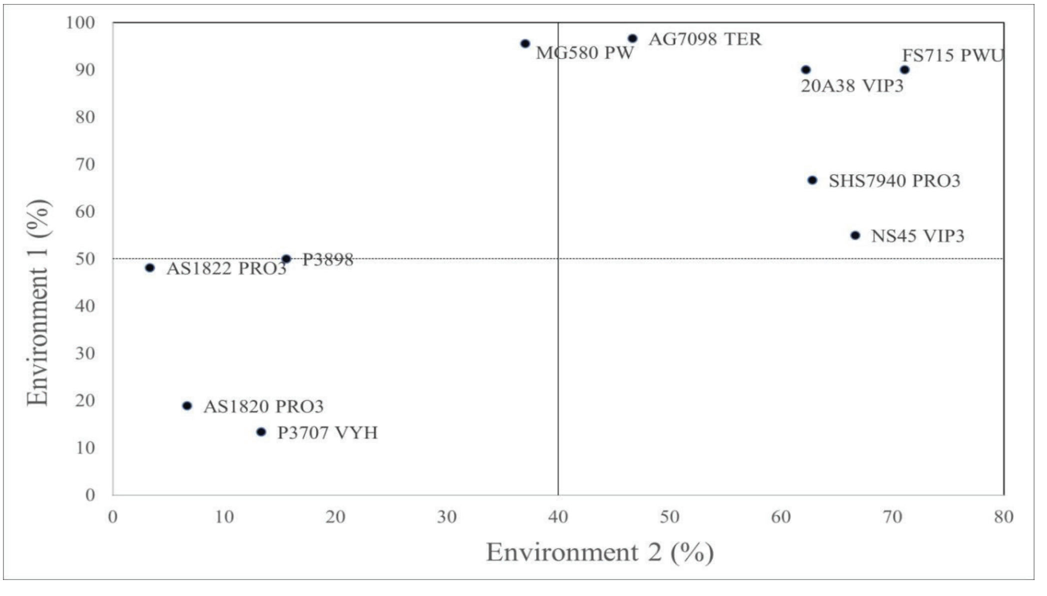

Figure 6. Graph of quadrants of charcoal rot incidence in maize hybrids in two environments in Paraíso do Tocantins, TO, 2nd Crop season. The 1st upper left quadrant corresponds to the hybrids with the highest incidence of charcoal rot in Environment 2; the 2nd upper quadrant on the right corresponds to the hybrids with the highest incidence of charcoal rot in both environments; the 3rd lower left quadrant refers to the hybrids with the lowest incidence of charcoal rot in both environments; and the 4th quadrant refers to the hybrids with the highest incidence of charcoal rot in Environment 1.

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rot increased by 24.9% (Figure 5), ranging from 0.0% to 58.5%. However, half of the hybrids did not show statistically significant differences in charcoal rot incidence between the two environments with varying water restrictions (Table 2). There were no hybrids with high charcoal rot incidence in one environment or low incidence in another (Figure 6). Some hybrids showed high charcoal rot scores in both environments, whereas two hybrids, AS1820 and P3707, showed lower averages. Costa et al. (2020) evaluated 20 commercial maize hybrids for susceptibility to *M. phaseolina* via artificial inoculation. The authors found variability in the reactions of the hybrids to the fungus, with the majority classified as susceptible. However, the hybrid P3707 was classified as one of the most susceptible, which contradicts the results of the present study. This discrepancy may be due to differences in the evaluation methods (natural vs. artificial inoculation) or the populations of pathogens used in each study.

The results presented in this study are crucial for the choice of hybrids to be cultivated in environments with high soil water restrictions, as the prevalence of the fungus is directly related to plant genotype and climatic conditions.

Conclusions

Soils with higher water deficit favored the growth of charcoal rot of maize. Nevertheless, a relatively high incidence of charcoal rot was also noted when more water was available, particularly in cultivars more susceptible to the disease. There were variations in disease incidence and yield among the different maize hybrids. Based on the joint analysis of the environments, maize hybrids AS1820, AS1822, and P3707 showed the lowest average incidence of charcoal rot and the highest yields.

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