






## Pathogenicity and virulence of *Bipolaris bicolor* on wheat, corn and sorghum<sup>1</sup>

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### ABSTRACT

The objective was to evaluate the pathogenicity of six strains of *Bipolaris bicolor* on wheat, corn and sorghum and its virulence when inoculated into healthy plant tissue of wheat under different light regimes at 25 °C. Pathogenicity was evaluated by inoculating mycelial discs in the corn leaves at 25 °C for six days in a completely randomized design and 6x3 factorial arrangement, with the six strains of *B. bicolor* and three crops (wheat, corn, and sorghum). Besides, virulence was evaluated in the same conditions, excepting as follow: 6x5 factorial arrangement (six strains of *B. bicolor* and five photoperiods). The evaluations were performed by measuring the lesioned leaf diameter daily, which was later transformed into the lesioned leaf area (LLA). The results showed the ability of *B. bicolor* to infect other plant species beyond wheat, that is, the strains proved to be pathogenic on corn and sorghum leaves. Regarding its physiology, the results showed that strains such as virulence differed when inoculated on wheat leaves. The 14 h light regimen showed the greatest significant reduction in disease severity, the point from which the increase in the frequency of luminosity led to an increase in the LLA up to the 20 h light regimen.

**Keywords:** physiology, photoperiod, virulence, symptomatology.

## INTRODUCTION

Wheat, corn and soybean crops represent an alternative for expanding agricultural activity in Brazil. This occurs due to the great potential offered by the “Cerrado” region of the Central Brazil in terms of climate, topography, area extension and availability of adapted cultivars.<sup>(1,2)</sup> Studies confirm the potential of this region, as it is presented wheat, corn and sorghum productivity of up to 5,302 kg/ha, 5,591 kg/ha and 3,027 kg/ha, respectively, under different water conditions.<sup>(3-5)</sup>

One crucial environmental factor that is often overlooked in epidemiological models is light. Filamentous fungi heavily rely on light as a vital source of information. The duration of light exposure (photoperiod or day length) and its quality (spectral distribution of light) impact the metabolic processes of fungi, such as nutrient absorption,<sup>(6)</sup> morphology, and conidiation.<sup>(7)</sup> Moreover, light can also postpone the development of appressoria and haustoria in fungal plant pathogens, functioning as a virulence determinant.<sup>(8)</sup>

Research has demonstrated that light directly affects fungi, which supports previous findings that white light impacts fungal conidiation and plant invasion by numerous fungal species. Molecular investigations have facilitated identifying and comprehending various light-responsive components that participate in light-mediated plant invasion in diverse species, such as *Aspergillus*, *Botrytis*, *Neurospora*, *Sordaria*, *Candida*, and *Fusarium*. These light-responsive factors serve specific roles in promoting the creation of conidia (asexual spores) and virulence, or the sclerotia (dormant structures), in the presence or absence of light by regulating the homeostasis of reactive oxygen species and secondary metabolism.<sup>(9-11)</sup>

Regarding the host, light fulfills multiple roles beyond being an energy source that regulates photosynthesis and gene expression in plants.<sup>(12)</sup> It also influences plant defense responses mediated by salicylic acid,<sup>(13)</sup> such as acquired systemic resistance and hypersensitivity response (a form of localized programmed cell death at the site of infection). While certain defense-related plant genes are induced by specific light wavelengths,<sup>(14)</sup> others are regulated in a circadian manner.<sup>(15)</sup> For instance, an extended period of light post-inoculation has been associated with increased disease resistance in *Arabidopsis* against *Hyaloperonospora arabidopsidis*<sup>(16)</sup> and *Pseudomonas syringae*.<sup>(17)</sup> This highlights the pathogens' use of light to synchronize pathogenesis

with their host's circadian patterns,<sup>(16)</sup> consequently avoiding the morning peak in plant defense gene expression.<sup>(17)</sup> Experiments designed to disrupt circadian rhythm through constant light application or phase-shifted light-dark cycles have led to modifications in the *Arabidopsis-Botrytis* interaction.<sup>(18)</sup>

In this way, the establishment of precision light treatments for the desired result has increased, and this could be possible with the increase in knowledge of phyllosphere-plant interactions at the molecular level, metabolic and hormonal, which provide the lowest pathogen infection rates and greater productivity of crops of economic interest.<sup>(19)</sup> In Brazil, light supplementation processes through technology, using the installation of artificial panels of high-performance (light emitting diode) in irrigated cereal production areas are becoming a reality in the country. This technology has allowed not only the irrigation of water in a conventional way but also the supplementation of light on all agriculture in a fully controlled way, which allows the extension of light hours on crops.<sup>(20)</sup>

Pathogenic variability is a consequence of the disease development scheme. Pathogenicity refers to a set of abilities that cause specific and nonspecific diseases.<sup>(21)</sup> In this context, studies conducted to characterize the pathogenic variability of *Bipolaris* strains from different geographical areas have shown significant differences in the host response. Additionally, specifically in Poaceae crops, studies indicate the existence of different abilities and levels of virulence and severity within the species of *Bipolaris* genus.<sup>(21-23)</sup> *B. bicolor* colonies show fast growth, reaching an area of 86.22 cm<sup>2</sup> of mycelium at a temperature of 25°C and a 12-hour photoperiod, within 5 days on Potato Dextrose Agar.<sup>(24)</sup> Lesions occur mainly in regions with temperatures ranging from 18 to 27°C, high humidity and dew formation.<sup>(25)</sup>

Preliminary studies of light supplementation have shown increased productivity by up to 66%, as seen in crops like soybeans, with just 40 days of supplementation. Furthermore, a visually significant reduction in disease incidence and the occurrence of pest insects throughout the crop's production cycle under the supplementation was observed. However, data are scarce since no experiments (disease and pest assessments) have been carried out for this purpose. Therefore, there is a great lack of information, considering that if this fact is proven, it could potentially reduce the production costs and the environmental impact of agricultural activities.<sup>(20)</sup> This study aimed to evaluate the

pathogenicity of six strains of *Bipolaris bicolor* on wheat, corn and sorghum and its virulence when inoculated into healthy plant tissue of wheat under different light regimes at 25 °C.

## MATERIALS AND METHODS

### *Strains of Bipolaris bicolor*

Six strains of *Bipolaris bicolor* (F-24-01, F-24-02, F-24-03, F-24-04, F-24-05, F-24-06) were used, obtained from wheat seeds previously identified by Menezes<sup>(26)</sup> and Muniz et al.,<sup>(27)</sup> and stored in the microbial culture collection of the State University of Goiás, Ipameri Academic Unit, Goiás, Brazil. In order to ensure their purity, the cultures were preserved in Potato Dextrose Agar (BDA) medium at a temperature of 5 °C according to the Castellani method. The strains were reactivated for the subsequent experiments in BDA medium from the samples kept at a low temperature in a freezer at UEG's Phytopathology Laboratory.

### *Obtaining plant material for pathogenicity and virulence assays*

Seeds of wheat cv. 'BRS264', sorghum cv. 'K-200', and corn cv. "AG-1051" were sown in 10 pots (8 L) containing dystrophic Red-Yellow Latosol.<sup>(28)</sup> The plants were cultivated in a greenhouse at the Ipameri Academic Unit of the State University of Goiás by the technical guidelines provided by Alves et al.<sup>(29)</sup> and Souza & Fronza<sup>(30)</sup> for the production of plant material for the pathogenicity experiments. The plants were irrigated daily with 300mL of water and fertilized according to the technical recommendations for the culture.

### *Pathogenicity of Bipolaris bicolor on wheat, corn and sorghum*

Segments (10 cm) of young and healthy leaves from wheat plants (60 days old), sorghum (45 days old), and corn (56 days old) were washed under running water and allowed to air dry in a laminar flow chamber for 10 minutes. To carry out the inoculation process, five holes were made at the center of the leaf blade using a sterile needle, and an agar disc (5 mm Ø) containing the mycelium of each of the six strains was placed on the abaxial wheat, corn and sorghum leaf surface.<sup>(31,32)</sup> The inoculated leaves were subjected to controlled humid chamber conditions within transparent acrylic Gerbox-type boxes (11 x 11 x 3.5 cm) containing a germination paper sheet to maintain a

consistent humidity level exclusively on the paper surface. The experimental design followed a completely randomized (CRD) 6x3 factorial arrangement, which included six strains of *B. bicolor* and three different crops (wheat, corn and sorghum). Only the 12-hour light regime was used to compare strain behavior among the three evaluated crops. Each strain had five replications, and one leaf (wheat, corn or sorghum) per gearbox was used. The control treatment consisted of applying agar disc (5 mm Ø) without the pathogen on the the holes in the leaf blade.

### *Virulence of Bipolaris bicolor on wheat under different light regimes at 25 °C*

Segments (10 cm) of young and healthy plant wheat leaves (60 days old) were washed under running water and allowed to air dry in a laminar flow chamber for 10 minutes. To carry out the inoculation process, five holes were made at the center of the leaf blade using a sterile needle, and an agar disc (5 mm Ø) containing the mycelium of each of the six strains was placed on the abaxial wheat leaf surface.<sup>(31,32)</sup> The inoculated leaves were subjected to controlled humid chamber conditions within transparent acrylic Gerbox-type boxes (11 x 11 x 3.5 cm) containing a germination paper sheet to maintain a consistent humidity level exclusively on the paper surface. The experimental design followed a completely randomized (CRD) 6x5 factorial arrangement, which included six strains of *B. bicolor* on wheat leaves and five different photoperiods (12 h, 14 h, 16 h, 18 h, and 20 h), which were performed separately. Each strain had five replications, and one wheat leaf per gearbox was used. The control treatment consisted of applying agar disc (5 mm Ø) without the pathogen on the the holes in the leaf blade. The experiments were carried out in a Fanem 347 BOD chamber.

### *Obtaining the the lesioned leaf area (LLA)*

For the evaluations, daily measurements of lesions on the leaf's abaxial surface were conducted until the 6th day post-inoculation (DAI). A digital caliper was utilized to capture the average of two diametrically opposite diameters. The lesion's diameter measurements (cm) were employed in the calculation of the lesioned leaf area (LLA) (cm<sup>2</sup>) through the circumference formula ( $LLA = \pi * r^2$ ).

### *Statistical analysis*

The data collected from the pathogenicity experiments (wheat, corn and sorghum) were subjected to analyses of

variance and the Scott-Knott ( $p \leq 0,05$ ) to verify the differences among the strains and across the three crops.

The data related to the virulence assay (wheat) were subjected to regression analyses to derive the epidemiological models for disease severity in wheat in response to photoperiods (12 h, 14 h, 16 h, 18 h, and 20 h).

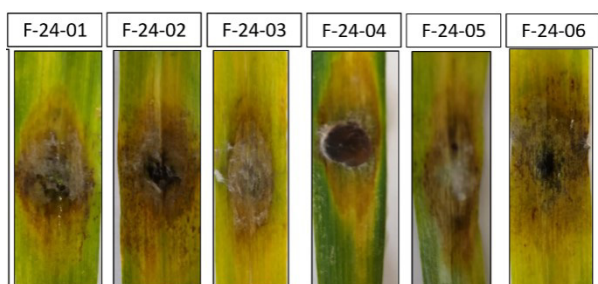
All the statistical analyses were conducted using the Sisvar 5.6 program.<sup>(33)</sup>

## RESULTS

### *Pathogenicity of Bipolaris bicolor on wheat, corn and sorghum*

The six strains of *Bipolaris bicolor* demonstrated pathogenicity towards wheat leaves. The initial symptoms were observed as brown necrotic lesions on the surface of the leaf blade, which then turned into an elongated oval shape with a dark brown color. The lesions were surrounded by a narrow yellow halo in nearly all cases. In the final assessment stage at 6 DAI, the center of the lesions turned dark brown to almost black, with the presence of fungal fruiting bodies and the emission of spores (Figure 1). The control treatment presented no symptoms.

In addition to the observed lesions on wheat cv. 'BRS264', the six strains of *B. bicolor* showed pathogenicity on leaves of maize cv. 'AG1051' and sorghum cv. 'K200'. Analyzing the lesioned leaf area caused by the strains individually in the wheat crop, strains F-24-02 and F-24-06 displayed the highest values of LLA (Table 1). In the maize crop, strains F-24-03, F-24-04, and F-24-05 exhibited the highest mean LLA values, whereas strains F-24-01, F-24-02, and F-24-06 showed smaller areas, primarily due to the host's hypersensitivity reaction (Figure 2). There was no significant difference in LLA among the strains in the sorghum crop, all of which displayed minimal values.



**Figure 1.** Symptoms and signs of *Bipolaris bicolor* strains (F-24-01, F-24-02, F-24-03, F-24-04, F-24-05 and F-24-06) on the abaxial surface of wheat leaves at 6 DAI.

This outcome resulted from the plant's hypersensitivity reactions, which confined the pathogen to tiny areas and prevented the expansion of lesions on the leaves (Figure 3).

Necrotic brown lesions were noted on the maize leaves, appearing on the leaf blade's surface. These lesions progressed and assumed an elongated oval shape in a dark brown color. Almost all cases, the lesions were surrounded by a chlorotic halo (Figure 2). By the 6th DAI, spore formation of the strains took place on these lesions.

In sorghum leaves inoculated with *B. bicolor* strain discs, small lesions with mycelial growth developed on the leaf blade's surface. Reddish halos encircled these lesions, indicating the plant's hypersensitivity reaction to the strains (Figure 3).

### *Virulence of Bipolaris bicolor on wheat under different light regimes at 25 °C*

The interaction among the six *Bipolaris bicolor* strains and the five photoperiod conditions to which they were exposed revealed a significant difference in the severity levels of the strains at 6 DAI. Concerning the strain factor under different light regimes, it is evident that strain F-24-02 stood out, displaying the highest lesioned leaf area at 6 DAI, ranging from 4.50 to 8.18 cm<sup>2</sup> of lesioned leaf area (LLA) when subjected to 14 and 12 hours of light, respectively, as well as the highest average. Interestingly, the 14-hour light photoperiod showed the lowest mean rates of LLA (3.65 cm<sup>2</sup>) (Table 2). The control treatment presented no symptoms.

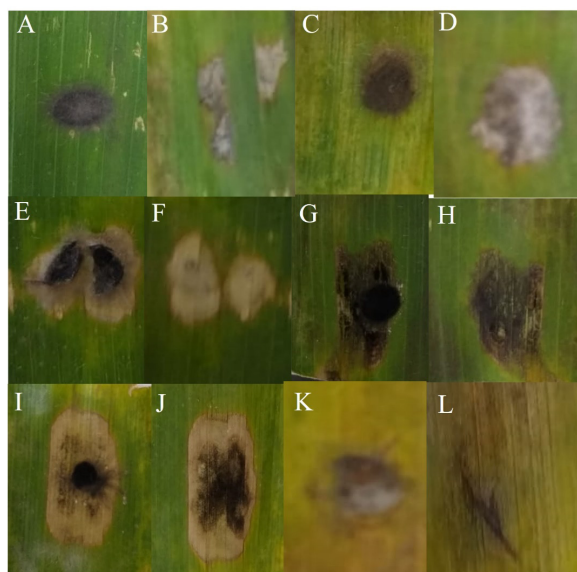
The patterns observed in the curves representing the extent of lesioned leaf area concerning the light exposure hours differed among the six strains of *B. bicolor*. These trends were fitted using quadratic polynomial models for

**Table 1.** Lesioned leaf area (LLA) by *Bipolaris bicolor* on wheat, corn, and sorghum at 6 DAI

Strain	LLA (cm <sup>2</sup> ) <sup>(1)</sup>		
	Wheat	Corn	Sorghum
F-24-01	5,53 bA	0,07 bB	0,46 <sup>ns</sup> B
F-24-02	8,18 aA	0,28 bB	0,40 B
F-24-03	5,03 bA	2,22 aB	0,66 C
F-24-04	3,34 cA	1,97 aB	0,33 C
F-24-05	3,56 cA	2,12 aB	0,33 C
F-24-06	6,74 aA	0,02 bB	0,48 B
Average	5,40A	1,11 B	0,44 C
C.V (%)	10,68	26,25	14,69

<sup>(1)</sup> Means followed by the same letter in the column are not significantly different from each other according to the Scott-Knott test ( $p < 0.05$ ).





**Figure 2.** Brown lesions on the leaf blade (F-24-03, F-24-04, and F-24-05) and hypersensitivity reactions (F-24-01, F-24-02, and F-24-06) to *Bipolaris bicolor* strains on the adaxial and abaxial surfaces of maize leaves at 10 DAI. **A-B)** F-24-01; **C-D)** F-24-02, **E-F)** F-24-03, **G-H)** F-24-04, **I-J)** F-24-05, **K-L)** F-24-06.

strains F-24-01, F-24-02, F-24-05, and F-24-06, while linear models were applied for strains F-24-03 and F-24-04. The coefficients of determination ( $R^2$ ) ranged from 0.26 to 0.89, enabling the estimation of disease progression patterns (Table 3).

It was observed that strains that displayed a polynomial pattern in the development of lesions showed a significant reduction in the severity of the disease as the incidence of lightness increased until the 14-hour point light (as shown in Table 2). After this point, an increase in light frequency resulted in a rise in the LLA until the 20-hour regime, which had similar levels of virulence as the 12-hour regime. However, an exception was found in the F-24-04 strain, which showed an increase in the area of leaf lesions with an increase in light incidence (Table 2).

## DISCUSSION

### *Pathogenicity of Bipolaris bicolor on wheat, corn and sorghum*

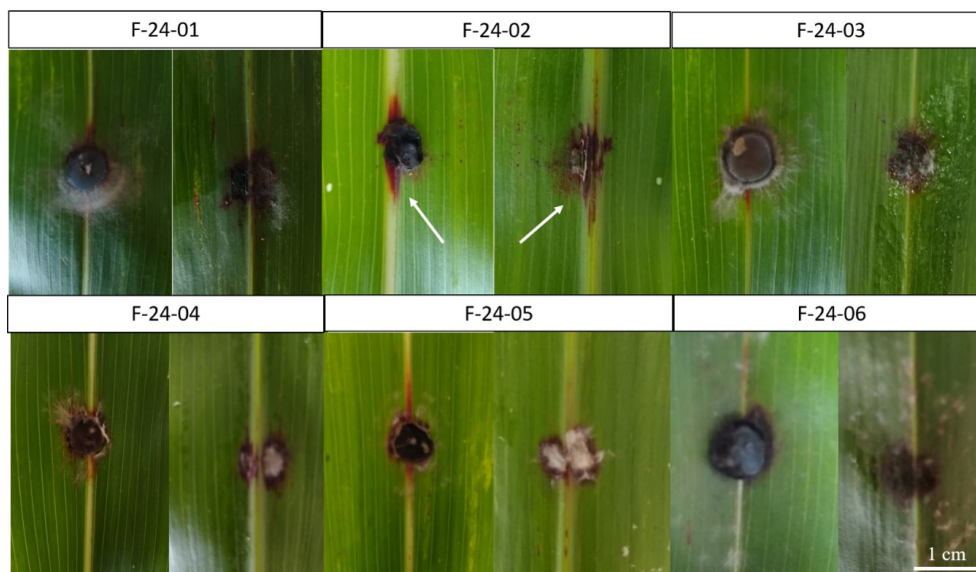
The fungus *B. bicolor* is known to infect wheat and sorghum seeds, causing necrotrophic damage.<sup>(34,35)</sup> While it is less common, there have been reports of *B. bicolor* causing foliar infections in sorghum and maize as well.<sup>(36)</sup> Our research found that strains of *B. bicolor* originating from wheat seeds were highly pathogenic when it came to maize cultivation. They caused lesions that were similar

in size to those caused by more prevalent species like *B. maydis*.<sup>(25)</sup> These findings have significant implications since *B. bicolor* populations from the Brazilian Central Plateau have high levels of virulence on different hosts. Additionally, the fungus is hemibiotrophic, which means it can survive on plant residues between cropping seasons and can be responsible for cross-transmission and the generation of disease epidemics.<sup>(37)</sup> This could lead to increased challenges in field control and significant losses in grain production.<sup>(38)</sup>

The pathogenicity of *B. bicolor* strains in the three crops tested has raised concerns regarding the disease's epidemiology. The *Bipolaris* genus is known to infect a wide range of hosts, including both grass and non-grass species.<sup>(39)</sup> However, only a handful of economically significant species have been thoroughly studied in terms of biology and host range.<sup>(40)</sup> For instance, *Bipolaris* species like *B. sorokiniana*, *B. maydis*, and *B. oryzae* have been found on various hosts. In contrast, several other *Bipolaris* species, such as *B. clavata*, *B. microstegii*, and *B. gossypina*, have only been reported to infect a single host.<sup>(39)</sup>

These capacities for high virulence or avirulence and adaptability within the host spectrum may be linked to genomic changes in the pathogen's genome over time. These changes may include hybridization, horizontal gene transfer, point mutation, partial or complete gene deletion, and substitution of nucleotides and/or amino acids, which could result in the pathogen infecting new hosts.<sup>(41)</sup> For example, the emergence of *Magnaporthe oryzae* in wheat was due to the loss of function of a single avirulence gene. Another example is *Blumeria graminis* f. spp. *triticales*, which is a hybrid of two *B. graminis* subspecies that specialize in wheat and rye.<sup>(42)</sup> However, the genetic factors that cause the pathogen to infect more hosts are still not fully understood.<sup>(41)</sup>

According to a recent study by Ramesh et al.,<sup>(37)</sup> *B. setariae* was first discovered in 1987 infecting various plant species including *Echinochloa* spp., *Eleusine coracana*, *Eragrostia* spp., *Panicum* spp., *Pennisetum* spp., and *Setaria italica*. However, the study's results showed that *B. setariae* did not infect certain plants such as *E. coracana*, *E. frumentacea*, *P. sumatrense*, *P. miliaceum*, and *P. scrobiculatum*. This suggests that there is an incompatible relationship between the pathogen and resistant/tolerant accessions. In simpler terms, for the disease to occur, the pathogen must recognize and infect its host, which could explain the low severity of strains F-24-01 and F-24-06 in corn and the six strains in sorghum.



**Figure 3.** Hypersensitivity reactions of sorghum cv. 'K200' to *Bipolaris bicolor* strains (F-24-01, F-24-02, F-24-03, F-24-04, F-24-05 and F-24-06) on the adaxial and abaxial surfaces of the leaf blade at 6 DAI. Arrow indicates superficial colonization of hyphae on the leaf blade's surface and localized cell death reactions within the leaf tissue.

**Table 2.** Lesioned leaf area (LLA) caused by *Bipolaris bicolor* strains on wheat leaves cv. 'BRS264' six days after inoculation (DAI) under different photoperiods

Strain	Light regime					Average
	12 h	14 h	16 h	18 h	20 h	
F-24-01	5,53 cA	2,57 bB	5,44 aA	4,86 <sup>ns</sup> A	5,63 <sup>ns</sup> A	4,81 b
F-24-02	8,18 aA	4,50 a C	6,54 aB	6,02 B	6,06 B	6,26 a
F-24-03	5,03 cA	4,87 aA	5,54 aA	5,42 A	5,42 A	5,26 b
F-24-04	3,34 dB	3,91 aB	3,87 bB	5,09 A	5,22 A	4,28 c
F-24-05	3,56 dA	2,17 bB	3,78 bA	4,12 A	5,07 A	3,74 c
F-24-06	6,74 bA	3,88 aB	4,18 bB	4,64 B	5,59 A	5,01 b
Average	5,40 A	3,65 B	4,89 A	5,02 A	5,50 A	
CV (%)	21,79	24,31	14,85	27,25	22,38	22,67

<sup>(1)</sup> Means followed by the same letter in the column are not significantly different according to the Scott-Knott test at  $p < 0.05$ .

**Tabela 3.** Epidemiological models depicting the behavior of symptom severity on wheat leaves caused by *Bipolaris bicolor* strains in response to different light regimes

Strain	Regression Models	R <sup>2</sup>	P≤
F-24-01	$y = 0,255x^2 - 1,273x + 5,908$	0,26	0,05
F-24-02	$y = 0,3257x^2 - 2,2563x + 9,508$	0,40	0,05
F-24-03	$y = 0,186x + 4,58$	0,34	0,05
F-24-04	$y = 0,494x + 2,804$	0,89	0,05
F-24-05	$y = 0,1864x^2 - 0,6216x + 3,634$	0,64	0,05
F-24-06	$y = 0,5986x^2 - 3,7454x + 9,598$	0,88	0,05

The observation of hypersensitivity reactions in sorghum leaves may be linked to the plant's defense mechanisms against the specific pathogen species.<sup>(43)</sup> Reports of

sorghum resistance to *B. bicolor* have been documented in several cultivars.<sup>(44)</sup> In this study, it was noticed that the pathogen initiated the pathogenesis process, with symptom

and sign development on the leaves until 4 DAI. However, the plant appeared to begin blocking the infection process, as evidenced by the presence of necrotic areas formed around the inoculated mycelium disk, exhibiting a reddish/brown coloration. This plant-triggered reaction can be explained by the fact that after the pathogen arrives in a resistant or tolerant plant, infected cells quickly lose turgor, typically becoming brown (due to phenol oxidation) and eventually dying.<sup>(45)</sup> This process can be highly beneficial for the plant as it isolates the pathogen inoculum in a small leaf area.<sup>(46)</sup> However, it's worth noting that while this situation favors the sorghum plant at this stage, it might later become deleterious because the pathogen *B. bicolor* is hemibiotrophic, initially exhibiting biotrophic actions during its early interactions with the host but subsequently transitioning to a necrotrophic lifestyle. In such cases, the hypersensitivity reaction can be beneficial to the host at the beginning but not at the end of the interaction.<sup>(47,48)</sup>

The varying levels of symptoms caused by different strains in maize and sorghum in this study support previous research on cross-pathogenicity between these crops. In a study by Bruckart et al.,<sup>(49)</sup> where they evaluated the virulence and aggressiveness of *B. microstrip* and *B. drechsleri* in maize and sorghum, they discovered that distinct pathogenic reactions occurred among *Bipolaris* sp. populations and accessions of economically important crops. These reactions included the formation of hypersensitivity reactions and tolerance levels, as observed in the current study.

### ***Virulence of Bipolaris bicolor on wheat under different light regimes at 25°C***

The incidence of *B. bicolor* has already been widely recorded in wheat seeds in Brazil.<sup>(35,50)</sup> However, the pathogenicity of this species in wheat crop leaves needs to be better reported in the country. The symptoms observed on wheat leaves caused by the six strains of *B. bicolor* in all the photoperiods tested were similar to those described for other species of *Bipolaris* sp. Pereira et al.<sup>(44)</sup> and Al-Sadi et al.<sup>(51)</sup> characterize the lesions resulting from *Bipolaris* sp. as brown necrotic lesions forming on the leaf blade's surface. These lesions advance into elongated oval patterns with a dark brown color and are accompanied by a yellow halo, similar to those noticed in the current study.

Photoperiodism is a critical variable for plant development, strictly related to grain production, and consequently reflected in the pathogenesis and management of foliar diseases.<sup>(20)</sup> In addition, light is an energy source that regulates

photosynthesis and gene expression in plants.<sup>(12)</sup> For wheat, the plant's response to the photoperiod (day length) begins immediately after emergence and continues until the end of the reproductive phase. Wheat is a long-day plant, and its development accelerates as the photoperiod increases, up to a maximum of 20 hours per day.<sup>(2)</sup> In the pathosystem studies of *B. bicolor* vs. wheat in different photoperiods, a difference in disease severity caused by *B. bicolor* strains was observed. With the increase in the hours of light exposure on wheat leaves, there was a decrease in the LLA until the 14-hour regime, beyond which a moderate elevation was observed while maintaining similar epidemiological rates. Supporting this fact, Macioszek et al.<sup>(52)</sup> analyzed the pathogenicity of *Alternaria brassicola* in *Brassica juncea*. Their findings indicated a reduction in necrotic lesions induced by the pathogen as cultivated plants were subjected to extended periods of continuous light.

In these scenarios, extending the duration of light exposure beneath the infected plant diminished disease progression. It probably impeded fungal growth, an occurrence evident in our research, especially under 14 hours of light exposure. This occurrence is linked to the influence of the circadian rhythm on plant immunity, even though the plants appear to be more susceptible to the pathogen attack during the light period due to photosynthetic activity and open stomata. Nonetheless, there is a peak of jasmonic acid (JA), a hormone essential for plant defense, in addition to activating salicylic acid-mediated defense pathways that exhibit efficacy against necrotrophic fungi like *B. bicolor*. This phenomenon has been observed through an extended light period in *Arabidopsis*.<sup>(13,16,53)</sup>

Furthermore, some authors suggest that decreasing the length of the plants' dark period can increase their ability to resist *B. bicolor* by promoting extensive production of metabolites like phenolic compounds that have antifungal properties.<sup>(54,55)</sup> The biosynthesis of these antifungal compounds is light-regulated, and it has been demonstrated that exposure to darkness reduces their content.<sup>(55)</sup> This reduction in *B. bicolor*'s pathogenic activities could also be linked to pathogen stress. The presence of a circadian rhythm in pathogenesis has been reported for several phytopathogenic fungi, such as the necrotroph *Botrytis cinerea*, with light influencing their development and stress responses,<sup>(56)</sup> for instance. To illustrate, in species within the *Alternaria* genus, extended periods of light inhibit growth, sporulation, and even toxin production.<sup>(57)</sup> Therefore, the increase in continuous illumination and reductions

in the dark period could act as a stressor for both the host plant, prompting it to activate defense pathways and the pathogenic fungus, reducing its phytopathogenic activity. However, such widespread stress can also be detrimental to the host, resulting in reduced efficiency of the photosynthetic apparatus and alterations in leaf positioning under high-light conditions in certain plants.<sup>(52)</sup>

It is worth mentioning that the *B. bicolor* strains exhibited varying levels of virulence under different photoperiods. There have been reports of pathogenic variabilities encompassing distinct levels of virulence and aggressiveness in species within the *Bipolaris* sp. genus. This observed variability reaffirms the presence of diverse abilities within local populations undergoing evolutionary processes, demonstrating differences among them and indicating that the *Bipolaris* sp. genus undergoes continuous adaptations in its pathosystems.<sup>(22,23)</sup>

It should be noted that, despite a 16-hour exposure to light, no reduction was observed in the damaged leaf area during the study. Nonetheless, the virulence and aggressiveness patterns remained consistent with those observed after 12 hours of light. Thus, a more in-depth study of the crop's productive feasibility is necessary, involving an analysis of agronomic traits to validate the investment potential, such as recommending light supplementation.<sup>(20)</sup>

## CONCLUSION

While the six different strains of *B. bicolor* can infect other plants in the same family, they seem to prefer wheat. Interestingly, each strain of the fungus behaves differently when it infects a particular host, which can affect the severity of the infection. When the sorghum plant with the 'K-200' variety is infected with *Bipolaris bicolor*, it displays hypersensitivity reactions and only a small portion of its leaves are affected.



When plants are exposed to too much light each day, it can disrupt the way they interact with disease-causing agents. In particular, when wheat plants with the 'BRS264' variety are exposed to 14 hours of light per day, they experience the least severe symptoms from the *B. bicolor* fungus.




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