Efficiency of fungicide chemical group in the preventive and curative control of *Puccinia sorghi* in corn and *Cercospora zeae-maydis* sporulation in different culture media

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ABSTRACT
The chemical control of diseases is one of the most used measures, especially for a rapid and precise control. The objectives of this work were to verify the behavior of three chemical groups of fungicides applied in a preventive and curative way aiming the control of common corn rust and the sporulation of Cercospora zeae-maydis in different culture media. Two experiments were installed, one for chemical control and the other for sporulation. For the chemical control experiment, propiconazole, azoxystrobin, ciproconazole + azoxystrobin and benzovindiflupir + azoxystrobin were the fungicides preventively and curatively applied for the control of common corn rust in a random block experimental design with three replicates. In relation to cercosporiosis sporulation experiment, five culture media were used: potato sucrose agar (PSA), V8 agar juice (V8), (LCHA), corn leaf extract (CL) and seasoned tomato extract (STE) were used. Discs with Mycelial of the fungus were placed on the media and submitted to the 12-hour light/12-hour dark continuous dark in a double factorial (culture media and light treatments) experimental design with four replicates. The fungicides azoxystrobin and ciproconazole + azoxystrobin were efficient for up to 21 days after inoculation of P. sorghi, the fungicide azoxystrobin was efficient for the curative control of P. sorghi. The greatest sporulation of the fungus was verified in the medium with seasoned tomato extract submitted to the photoperiod.

Key words: Cercosporiosis; Chemical control; Common rust; Conidia.

1. Introduction

Corn (*Zea mays* L.) is grown in all parts of the planet, and the world's largest producers are the United States, China and Brazil, which ranks the third place with a current average production of approximately 88.9 million tons (Conab 2018). The productivity of this crop is affected by several factors, among which, the diseases, which cause great losses, impairing the development and reducing the photosynthetic area, inhibiting the translocation of assimilates from the source of production to the areas of growth and deposition of yield material (Gomes et al. 2011).

Among the diseases that occur in corn, common rust and cercosporiosis stand out. Common rust has *Puccinia sorghi* Schw. as its etiological agent and deserves attention because it is classified among the

main foliar diseases in the corn crop, which can cause direct damage to the plant by reducing the photosynthetic area, which can culminate in a reduction in grain yield (Von Pinho et al. 1999). In many cases chemical control of plant diseases is the only efficient and economically feasible measure capable of ensuring high productivity and quality of production. The use of fungicides is more intense in the most economically developed countries, where agriculture uses more advanced technology, greater application of inputs and prospects for better harvests. The application of fungicides in corn crop has shown maintenance of crop productivity since chemical products control efficiently when correctly applied (Lago and Nunes 2008).

The triazole fungicides group, named demethylation inhibitors (DMI), acts on the pathogen by inhibiting the C-14 demethylation reaction (Linhares and Ghini 2001). Strobilurins interfere in mitochondrial respiration, blocking the transfer of electrons through the cytochrome bc1 complex, through the inhibition of ubihydroquinonacytochrome c oxidase reductase (Ghini and Kimati 2000). Cytochromes are ferroproteins that act sequentially, transferring electrons from CoQH2 to molecular oxygen. The interference exerted by the fungicide prevents energy release and ATP formation (Forcelini et al. 2001).

Carboxamide, the last chemical group to be launched in the market, has high systemicity and a wide spectrum of action in ascomycetes, basidiomycetes and deuteromycetes (mitosporic), adding consistency in the control residual. In addition, they exhibit protective and curative action, even controlling resistant pathogens to strobilurins. These indicate a possible effect on the biosynthesis of proteins, lipids, DNA and RNA, in addition to a greater transformation of glucose or acetate into succinate and a decrease in the transformation of citrate, malate and fumarate. The use of mixtures with fungicides with different mode of action increases the fungus control spectrum, the residual period of applications and helps to avoid the development of the sensitivity reduction of the fungus to the fungicide (Reis et al. 2010).

The fungicides present protective, eradicating, curative and anti-sporulating action. The protective action is expressed when it is applied before the pathogen infects the tissues of the plant. The curative action consists in the ability of the fungicide to limit the development of the pathogen inside the tissues, when applied in the latent period, that is, in the interval between the penetration and the appearance of the first symptoms. Among the factors that may influence the curative action of a fungicide, host susceptibility, disease pressure, meteorological conditions and the moment of application of the fungicide in the plant stand out (Genet et al., 2000). Factors such as syrup volume, number and interval of applications and the application technology used may directly infer on the protection potential of a fungicide (Töfoli 2006). So, the knowledge of the action of each chemical group is important as it may interfere with the control efficiency in the field.

Another important disease is cercosporiosis, which has fungus *Cercospora zeae-maydis* Tehon & E.Y. Daniels 1925 as its etiological agent. According to Ward *et al*. (1993), the disease is able to reduce grain production by 20 to 60%, depending on the susceptibility of the corn hybrid. This disease has caused significant losses in the major corn producing regions in the Brazilian crops in 2000 and 2001, no longer being considered secondary and becoming part of the most important diseases in corn crop (Pereira et al., 2005). One of the greatest difficulties is the sporulation of this fungus in laboratory for later inoculation in plants for research works.

The composition of the culture medium determines the mycelial growth and the sporulation of phytopathogens. Most fungi require a source of carbon and nitrogen, as well as other elements at lower amount, such as potassium, phosphorus, sulfur, iron, magnesium, zinc, manganese and vitamins. Carbon is the most important element for the development of the fungus, since it is essential for the synthesis of enzymes besides being the main source of energy. Nitrogen is a fundamental part of amino acids, which in turn makes up proteins, which can be from organic sources such as casein and peptone or from inorganic sources, such as sodium and potassium nitrate (Alfenas and Mafia 2007).

In addition to the components of the culture medium, another factor that interferes with the development of pathogens is the light treatment. According to Teixeira et al. (2001), light can induce, inhibit or have a neutral effect on fungal growth and sporulation. Light stimulates asexual and sexual reproduction in most fungi, and light-sensitive fungi spores when exposed to this condition, but others require a period in the dark or continuous dark to initiate sporulation.

The objectives of this work were to verify the behavior of different chemical groups of fungicides when applied in a preventive and curative way aiming to control the common rust in corn and to determine the sporulation of *Cercospora zeae-maydis* in different culture media, submitted to light treatments, 12-hour-light/ 12 -hours dark photoperiod and continuous dark.

2. Material and methods

The experiments were conducted in Laboratório de Fitopatologia and in a greenhouse at Faculdade de Agronomia e Medicina Veterinária at Universidade de Passo Fundo, Passo Fundo-RS, Brazil.

2.1 Experiment 1 – Preventive application of different chemical groups for *Puccinia sorghi* control

Plant growing and fungicide application

The hybrid Pioneer 30F53, susceptible to common rust was sown in pots with 10 kg of horticultural soil, and kept in greenhouse with 12-hour photoperiod. Three plants per pot were used. When plants reached the V4 stage (fourth expanded leaf with visible collar, ligula and auricle) (RITCHIE, 1993), they were sprayed with the different treatments (Table 1) using CO_2 pressurized backpack spray, calibrated for syrup volumes of 100 L/ha.

Table 1. Fungicides used for *Puccinia sorghi* control in corn.

Commercial name	Chemical group	Active ingredient	Dose [3 4] (g a.i. /ha)	Dose [3 4] (L or kg p.c/ha)
Tilt	Triazole	Propiconazole	100	0,4
Priori	Strobilurin	Azoxystrobin	75	0,3
Priori Xtra[1]	Triazole + Strobilurin	Ciproconazole + azoxystrobin	24 + 60	0,3

Elatus[1]	Carboxamide + Strobilurin	Benzovindiflupir + azoxystrobin	30 + 60	0,2

[1]Adjuvant added Nimbus 600 mL/ha; [2]Active ingredient. [3]Commercial product;

Inoculum production and plant inoculation

The inoculum was multiplied in hybrid Pioneer 30F53 plants and kept in greenhouse for later application. For the preparation of the suspension, leaves with intense sporulation were collected and placed in a container for agitation with water and polyoxyethylenesorbitane spreader (Tween 20) for spore release. Then, the spores were quantified in a Neubauer chamber (Alfenas and Mafia 2007), and the suspension was calibrated to $20x10^3$ spores / mL.

The plants were inoculated with the fungus *Puccinia sorghi* and then covered with plastic bags for 24 hours to maintain leaf wetness. The inoculated leaves were marked with permanent marker for later identification, because only the leaves that received fungicide were evaluated. Inoculations were done on days 1, 4, 7, 10, 15 and 21 after application of the fungicides (Table 1).

Evaluations

After 30 days of inoculation, the marked leaves were collected and the severity and number of uredia/cm^2 were evaluated. For the severity, the leaf area attacked by the disease was estimated and severity scores from 0 to 100% were assigned based on severity data. Based on severity data, control efficiency was evaluated using the ABBOT formula (1925) (Efficiency (E%) = (severity of the control - severity of the treated plot) / severity of the control * 100).

For the number of uredia/cm^2, a circular area of 0.81 cm^2 of each leaf was extracted with two replicates per leaf, and the uredia were counted with the aid of a magnifying glass. The experimental design was a randomized block design with four replications, each replicate was composed of a plot with three plants.

2.2 Experiment 2 – Fungicide curative application for *Puccinia sorghi* control

The Pioneer 30F53 hybrid corn susceptible to common rust was sown in a pot with 10 kg of horticulture soil and kept in a greenhouse in a 12-hour photoperiod. When the plants reached the V4 stage, they were sprayed with the same fungicides from the previous experiment (Table 1); the applications were carried out on days 1, 3, 6, 9, 13, 16 and 20 after inoculation of the plants with the fungus, using O_2 pressurized backpack sprayer, calibrated for 100 L/ha syrup volumes.

Inoculum multiplication, inoculation and incubation of the plants and evaluations were performed using the same procedures for the preventive application experiment with this same fungus.

Firstly, the fungus was inoculated and then the fungicides were applied, and after thirty days, the evaluations mentioned in experiment 1 were carried out with preventive application of fungicides to control this fungus. The design was a randomized block design with four replications.

2.3 Experiment 3 –*Cercospora zeae-maydis* sporulation

The experiment was carried out in the Phytopathology Laboratory and in a growth climatized chamber at Faculdade de Agronomia e Medicina Veterinária at the University of Passo Fundo, Passo Fundo/RS/Brazil. The isolates of *C. zeae-maydis* were grown in V8 culture medium (Tuite, 1969). After the monosporic isolation, different culture media were evaluated in order to determine the best sporulation of the fungus, which were: 1) potato sucrose agar - PSA (Tuite 1969): (200 g potato extract, 20 g dextrose and 16 g agar and water up to reach 1000 ml); 2) V8 juice agar (19) - (200 ml of Campbell Soup Co. V8 vegetable juice, 16 g agar, 3.2 g CaCO3 and 800 ml distilled water); 3) lactose casein hydrolyzed agar - LCHA (19): in 2000-ml Erlemeyer, the following were added: i) 3 g/L hydrolyzed casein; ii) 10 g/l agar; iii) lactose 37.5 g/L; iv) $MgSO_4.7H_20$ 0,5 g/L; v) $ZnSO_4.7H_20$ 0,43 g/L, vi) $MnSO_4.4H_2O$ 0,2 g/L, vii) $Fe(NO_3)3.9H_2O$ 0,72 g/L, at a final volume of 1000 mL, after the dilution, the pH was corrected to 6; 4) Corn leaf extract - CL (1): (80 g chopped corn leaf extract and heated until boiling, 16 g agar and water up to 1000 mL); 5) seasoned tomato juice - STJ (Tuite, 1969): 200 mL of Super Bom® tomato juice; 16 g agar; 3.2 g $CaCO_3$, 0.2 g streptomycin and 800 ml distilled water.

After the preparation, the culture media were autoclaved at 121°C for 20 minutes and aseptically poured into polyethylene petri dishes (60 x 15 mm). Thereafter, eight plates were prepared for each of the culture media. In the center of each plate, a 4.68 mm diameter mycelial disc of the fungus grown for twenty-five days was deposited in PSA culture medium (Tuite, 1969). Then, four plates of each medium were submitted to the photoperiod (12 hours of light and 12 hours of dark) and the continuous dark. Treatments were randomly assigned to the growth chamber with OSRAM® Universal 40-watt fluorescent lamps at 25°C for 25 days. After that, the sporulation was quantified, so 5 mL of distilled water were added to the petri dish and with the help of a brush, the conidia were removed. From this suspension, 1 mL was collected, and then the number of conidia counted in Neubauer-type hemacytometer (Alfenas & Mafia, 2007). The number of conidia.cm^{-2} was calculated on the basis of number of conidia.mL^{-1}.

The experimental design was completely randomized with a 5 x 2 double factorial arrangement (culture medium x light regimes) with four replicates. The number of conidia.cm^{-2} was submitted to analysis of variance and the means were compared by the test of Tukey at a 5% probability of error. The Assistat software was used in this experiment.

4. Results and discussion

4.1 Fungicide preventive application for *Puccinia sorghi* control

Twenty-one days after fungicide application, the fungicides benzovindiflupir + azoxystrobin (mixture of carboxamide and strobilurin) and ciproconazole + azoxystrobin (mixture of triazole and strobilurin) showed the lowest value for this variable and control of 90.99% and 80.81% respectively. For the number of uredia/cm^2, the benzovindiflupir + azoxystrobin and ciproconazole + azoxystrobin presented the lowest values and control of 86.25% and 76.34%, respectively (Table 3).

For *P. sorghi* severity, it was verified that the fungicide benzovindiflupyr + azoxystrobin presented a 15-day residual whereas the fungicide propiconazole (triazole) showed an 18-day residual. The fungicides azoxystrobin and cyproconazole + azoxystrobin, the chemical group of strobilurins and mixture with

triazole were efficient for up to 21 days after inoculation (Figure 1A). When the number of uredia.cm^2 was evaluated, all the fungicides but fungicide propiconazole presented control of the disease (Figure 1B).

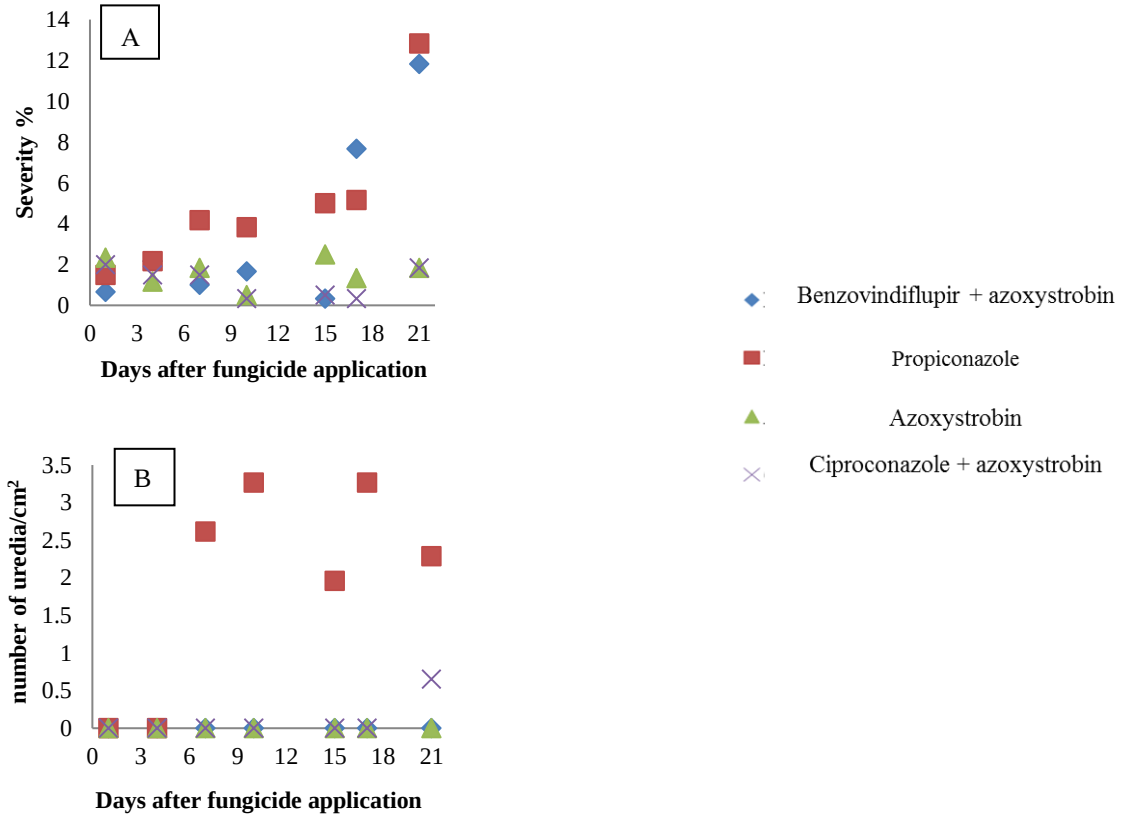

Figure 1. Severity (%) (A) and number of uredia.cm^2 (B) relationship in the interval in days between fungicide preventive application and Puccinia *sorghi* inoculation in the corn.

Table 3. Severity, number of uredia and spores produced per cm² of leaf area 21 days after fungicide preventive application to *Puccinia sorghi* control.

Fungicide	Severity (%)	Control (%)	Number of uredia /cm^2	Control (%)
Control	20.33 a[1]	-	19.28 a	-
Propiconazole	12.83 b	36.98	13.76 b	28.62
Benzovindiflupir+ azoxystrobin	1.83 c	90.99	2.65 c	86.25
Azoxystrobin	11.83 b	41.81	14.23 b	26.19
Ciproconazole + azoxystrobin	3.90 c	80.81	4.56 c	76.34
CV (%)	15.6		17.0	

[1]Means followed by the same letter are not different from each other by the test of Tukey at 5% probability.

4.2 Fungicide curative application for *Puccinia sorghi* control

In the curative applications of disease severity, it was observed that all the fungicides showed efficient control until 16 days after inoculation of the fungus. Twenty days after inoculation, the fungicide propiconazole (triazole) showed an increase in disease severity (Figure 2A). For the number of uredia.cm^2 and number of spores.cm^2 at 16 days after inoculation, the fungicides propiconazole (triazole) and benzovindiflupyr + azoxystrobin (mixture of carboxamide and strobilurin) showed the greatest values for these variables (Figure 2B).

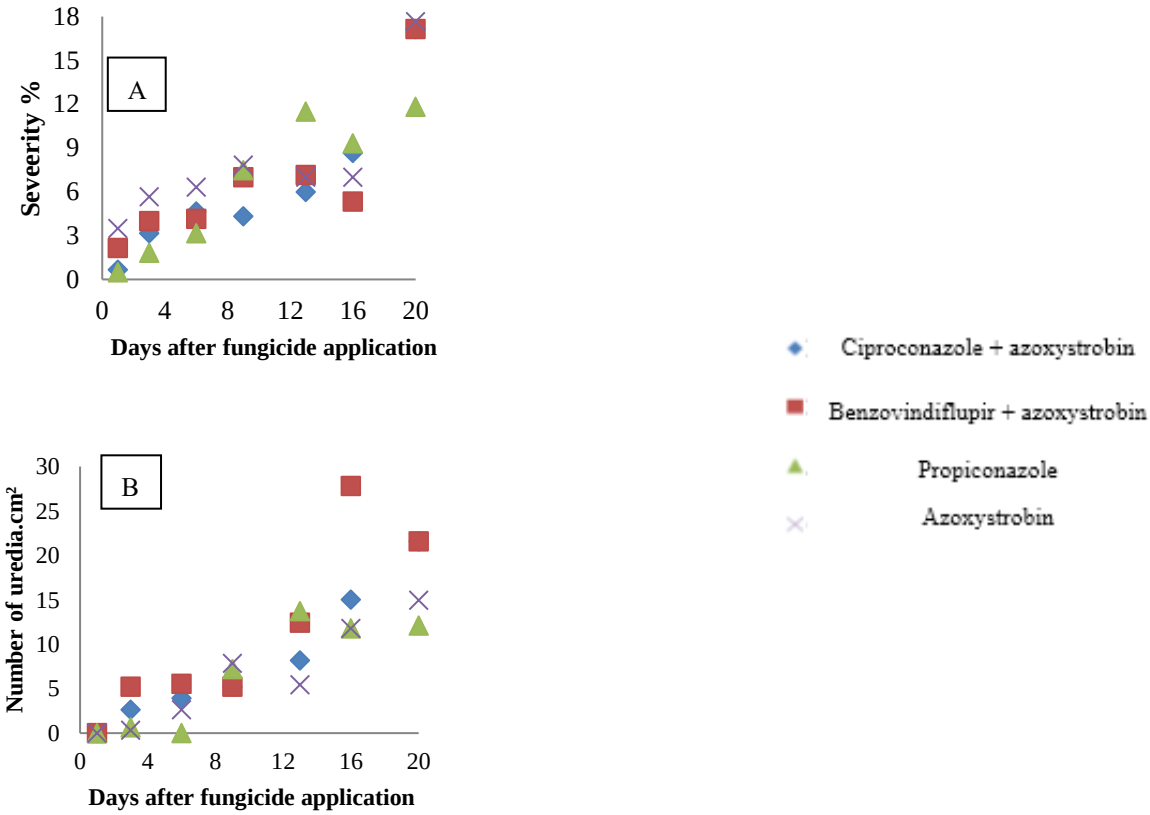

Figure 2. Severity (%) (A) and number of uredia.cm^2 (B) relationship in day intervals between *Puccinia sorghi* corn inoculation and application of the different fungicides.

For severity and number of uredia/cm^2 of *P. sorghi* at 20 days after fungicide application, all treatments were greater than control with values ranging from 41.68% to 51.50% (Table 4).

Table 4. Estimated severity, number of uredia and spores produced per cm² leaf area 20 days after fungicide curative application for *Puccinia sorghi* control.

Fungicide	Severity %	Control (%)	Number of uredia /cm²	Control (%)
Control	32.29 a	-	31.45 a	-
Ciproconazole + azoxystrobin	17.16 b	46.86	20.56 b	34.63
Benzovindiflupir + azoxystrobin	17.00 b	47.35	20.01 b	36.38
Propiconazole	15.66 b	51.50	18.09 b	42.48
Azoxystrobin	18.83 b	41.68	20.94 b	33.42
CV (%)	23.56		9.98	

[1]Means followed by the same letter are not different from each other by the test of Tukey at 5% probability level.

Chemical control is one of the chief methods for controlling plant diseases, both for ease of use and for the results obtained. However, its constant indiscriminate use may promote the selection of resistant fungi, putting at risk the efficiency of the method. This is undesirable to the chemical industry, to the producers and to the consumers, as approached by Ghini and Kimati (2000).

According to Boller et al. (2007) the effectiveness of the control depends on the age of the infection. A fungicide application on newly established infections results in the death of the pathogen. Azevedo (2007) reports the curative/eradicating effect in most crops is most pronounced of fungicides up to 48 to 72 hours after infection of the pathogen. In older infections (longer than half latent period), the energy no longer used for growth is reallocated to reproduction, causing wounds and forming spores, viable or not. This process lasts for three to four days, sometimes more if the air temperature is low. At first sight, this fact surprises the farmer, who perceives more wounds after the application of the fungicide and may interpret it as a failure in the control. The cause, however, is the high number of non-visible infections at the incubation stage, which reinforces the need to an earlier application, preventively or when the first symptoms appear, at the very most (Boller et al. 2007).

When applied for the control of other patossystems such as the yellow spot in wheat, fungicides are efficient only when applied in the first days after infection. After 10 or 12 days, the action of the fungicide no longer influences the existing infections (Ranzi and Forcelini 2013). Similar results were obtained by Menegon et al. (2005) with leaf spot on barley. This fact reinforces the importance of the moment to start the application of fungicides, as already pointed out by Reis and Casa (2007), as one of the factors that most affects the effectiveness of chemical control of diseases, emphasizing that the application of fungicides should be preventive or, at the most, right after the initial symptoms were detected.

Works on fungicides that evidence the control of P. sorghi are scarce in the literature. It was only found for other rust species. Godoy and Canteri (2004), when testing the effect of fungicides also verified that the application at two days after the inoculation of *P. pachyrhizi* reduced the amount of infections,

however, it did not act on the eradication. Azevedo (2007), on the other hand, reports that the curative and eradicating effect of systemic fungicides is more pronounced in the first 48 to 72 hours after pathogen infection. Ugalde (2005) believes that this behavior is attributable to the inhibition of foliar tissue colonization, or even a fungistatic effect upon uredinospores.

The triazoles are more systemic, which gives a characteristic of greater mobility, occupying the sites that are infected or more quickly susceptible of infection. Whereas strobilurins present a slower systemic action, a property that confers greater residue and, therefore, greater efficiency in preventive applications (Hewitt 1998).

According to Vieiro (2008), in the wheat leaf rust patosssystem, the triazole + strobilurin mixture presented better performance, especially in relation to the number of spores formed in each wound, which evidences two aspects: a lower sensitivity of the fungus to the triazole fungicides and an anti-sporulating action of strobilurin.

4.3 *Cercospora zeae-maydis sporulation*

Regarding the culture media, a significant interaction (Table 6) occurred. The largest sporulations of the fungus *C. zeae-maydis* were in media V8, PSA and LCHA, which were statistically equal among each other, regardless of the light regime. The STE medium presented a greater value in the photoperiod, as well as CL in the continuous dark, and no statistical difference was found between them. The lowest values were for the CL medium in the photoperiod and STE in the continuous dark. In addition, they were statistically inferior to the other media.

For the light treatments, when the plates with culture media were submitted to the photoperiod, the STE medium presented greater fungus sporulation, statistically differing from the other media, whereas the smaller sporulations were in the CL and LCHA media, which were statistically inferior to the others. For the continuous dark, the highest sporulations were in the STE, V8, PSA and CL media, all of them statistically superior to the LCHA, which presented the lowest sporulation.

Table 6. Sporulation of *Cercospora zeae-maydis* in different culture media and light regimes.

Culture medium	**Sporulation (conidia.cm^{-2})**	
	Photoperiod [1]	**Dark [2]**
Seasoned tomato extract (STE)	A 1153.0 a[3]	B 154.5 a
V8 juice agar (V8)	A 174.2 b	A 80.7 a
Potato sucrose agar (PSA)	A 109.2 b	A 52.1 a
Corn leaf extract (CL)	B 19.6 c	A 170.3 a
Lactose casein hydrolysate agar (LCHA)	A 17.7 c	A 9.8 b
CV (%)	13.92	

[1]Photoperiod: 12 hours of light and 12 hours in dark.

[2]Continuous dark.

[3]Means followed by the same lower-case letter in the column and upper-case letter on the row are not different from each other by the test of Tuckey at 5% error probability.

Sporulation of *Cercospora* species in culture medium is difficult and experiments where abundant sporulation has been *in vitro* induced (Calpouzos 1954) are scarce. One of the characteristics of this genus is the slow growth and scarcity of sporulation in artificial media. According to some authors, the V8 culture medium is reported as good sporulation inducer in several species of the genus *Cercospora* (Beckman and Payne 1983; Brunelli et al., 2006; Hanada 2002). This medium presents higher nutritional richness and greater amount of complex carbohydrates (Tehon and Daniels 1925), and these characteristics promotes sporulation of mitosporic fungi (Lukens 1963). The STE which is a variation of V8 and was also cited as a proper sporulant for *Passalora sojina* (Hara) H.D. Shin & U. Braun 1996) (Camera et al. 2014), *C. zeae-maydis* (Brunelli 2006) and *Cercospora nicotianae* Ellis & Everh (Queiroz and Menezes 2009). In addition, STE can be a more economical alternative as tomato is available in the local market and is a product of easy access and domestic production, besides its practicality.

Castro and Coelho (2000) verified that *Cercospora* conidia production did not start on day five, with sporulation peak occurring on the eighth day of incubation under continuous light treatment in Carrot-Dextrose-Agar medium. According to Koshikumo (2011), the culture medium PSA provided a greater sporulation of *Cercospora*.

Regarding the genus *Cercospora*, there are reports indicating the positive effect of light on fungal sporulation, as well as on light and dark alternating treatments, which is verified for *Cercospora arachidicola* Hori (Moraes and Salgado 1997), *C. zeae-maydis* (Beckman and Payne 1983), *Cercospora kikuchii* (Matsumoto and Tomoyasu) Gardner] (Dela-Cueva 1995) and *P. sojina* (Camera et al., 2014). Total or partial inhibition of sporulation under continuous dark conditions is also observed for *Cercospora nicotianae* Ellis & Everh and *Cercospora arachidicola* Hori (Kilpatrick and Johnson 1956; Stavely and Nimmo 1969).

According to Brunelli (2006), only the V8 and STE media induced abundant sporulation of *C. zeae-maydis*, both in the photoperiod and under the sequential light treatment (144 h light and 72 h dark). In addition, a small number of conidiophores were produced in PSA, but not of conidia, and for CL and CL with CaCO3, no formation of these structures occurred. Numerically speaking, the STE medium provides smaller growth in different species of *Cercospora* and explains that it is a medium with greater nutritional richness, containing vitamins, amino acids and other nutrients that stimulate spore production, which starts at the edges of colonies, and inhibits mycelial growth.

It was verified in the present work that the sporulation of the fungus *C. zeae-maydis* is related both to the composition of the culture medium and to the light regime, since the STE medium presented higher sporulation when the plates were submitted to photoperiod and lower in the continuous dark; kowever, this pathogen presents low sporulation when compared to other fungus genotypes.

5. Conclusions

The fungicides benzovindiflupir + azoxystrobin of the carboxamides + strobilurins chemical group and cyproconazole + azoxystrobin from the chemical group of triazoles + strobilurins present satisfactory control in preventive applications for the control of *P. sorghi*.

For curative control of P. sorghi, the fungicides ciproconazole + azoxystrobin, benzovindiflupir + azoxystrobin, propiconazole and azoxystrobin do not present satisfactory control at 21 days after inoculation of the fungus. So, these fungicides must always be used in in a preventive way, that is, they should be applied before the entry of disease in the field.

The greatest sporulation of *C. zee-maydis* is verified in tomato juice medium (STE) in the 12-hour light/12-hour dark photoperiod. In addition, this medium can be used in further studies with this pathogen.

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