INFLUENCE OF FOLIAR APPLICATION OF FUNGICIDES TO CONTROL PHAEOSPHAERIA LEAF SPOT IN MAIZE

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ABSTRACT: The objective of this study was to evaluate the effectiveness of foliar application of fungicides to control *Phaeosphaeria* leaf spot and consequently on the features of the maize crop productivity in the crop year 2014/15. The experimental design was randomized blocks, in a 2x4 factorial scheme, with two hybrids, three treatments + a control in four replications. The treatments were based on the following fungicides (g i.a L^{-1}): epoxiconazole + pyraclostrobin (333 + 167); cyproconazole + picoxystrobin (80 + 200); azoxystrobin + benzovinliflupyr (300 + 150), which were applied in BG7051H and BG7060HR hybrids with knapsack/ manual sprayer, pressurized with CO_2 in the phenological stage of culture V8, 70 days after seeding (70 DAS). The experimental plot had four rows spaced at 0.50 m and length of 10.0 m, resulting in a total area of 20 m^2 and useful area of 8 m^2, which held the white spot severity assessments, grain yield (kg ha^{-1}) and thousand grain weight (g). Significant differences were observed for all fungicides compared to the control. The control alone had the highest severity index compared to the other treatments. Regarding the thousand grain weight and productivity (kg ha^{-1}), the azoxystrobin + solatenol treatment was superior to the control and other treatments with fungicides, with a higher production of grain compared to the control.

Key words: *Zea mays* L., chemical control, effectiveness of fungicides, *Phaeosphaeria maydis*.

INFLUÊNCIA DA APLICAÇÃO FOLIAR DE FUNGICIDAS NO CONTROLE DA MANCHA DE PHAEOSPHAERIA NA CULTURA DO MILHO

RESUMO: O objetivo deste trabalho foi avaliar a eficácia da aplicação foliar de fungicidas no controle da mancha de *Phaeosphaeria* e consequentemente sobre os caracteres de

produtividade da cultura do milho, no ano agrícola 2014/15. O delineamento experimental foi em blocos casualizados, em esquema fatorial 2x4, sendo, dois híbridos, três tratamentos + uma testemunha em quatro repetições. Os tratamentos basearam-se nos seguintes fungicidas (g i.a L^{-1}): epoxiconazol + piraclostrobina (333+167); ciproconazol + picoxistrobina (80+200); azoxistrobina + benzovinliflupir (300+150), que foram aplicados nos híbridos BG7051H e BG7060HR, com pulverizador costal/manual, pressurizado com CO_2, no estádio fenológico V8 da cultura, 70 dias após a semeadura (70 DAS). A parcela experimental apresentou 4 linhas espaçadas em 0,50 m e comprimento de 10,0 m, resultando em uma área total de 20 m^2 e área útil de 8 m^2, onde realizou-se as avaliações de severidade da mancha branca, produtividade de grãos (kg ha^{-1}) e peso de mil grãos (g). Foram observadas diferenças significativas para todos os fungicidas em relação à testemunha. A testemunha apresentou isoladamente o maior índice de severidade, quando comparada aos demais tratamentos. Em relação à massa de mil grãos e a produtividade (kg ha^{-1}), o tratamento azoxistrobina + benzovinliflupir foi superior à testemunha e aos demais tratamentos com fungicidas, apresentando uma produção de grãos superior em relação à testemunha.

Palavras-chave: *Zea mays* L., controle químico, eficácia de fungicidas, *Phaeosphaeria maydis*.

INTRODUCTION

Maize (*Zea mays* L.), it stands out for its wide distribution and climate adaptation, which can be grown in almost all over the world. It is considered in the context of agribusiness one of the highest socioeconomic value cereals, due to the role in human and animal food chain, either through consumption or through derivatives, which generates an annual increase in consumption of this grain (SANTOS *et al*., 2002; MIRANDA, 2012; JULIATTI *et al*., 2014).

The crop yield can be compromised by other determinants, such as soil fertility, plant density, cultivation system (rotating or monoculture), the seeding system (conventional or direct), incorrect use of irrigation, related factors to environmental conditions and sowing time (CAMPBELL; MADDEN, 1990; STEFANELLO *et al*., 2012; JULIATTI *et al*., 2014).

Among the foliar diseases, that come to generate losses between 50 and 70%, currently the *Phaeosphaeria* leaf spot is showing greater notoriety, due to damage and for its wide dissemination capacity (SANTOS *et al*., 2002; DENTI; REIS, 2003).

Research shows that this disease is favored by relative humidity above 60%, average daytime temperatures between 18 and 21°C and night above 14ºC and rainfall of approximately 350 mm (ROLIM *et al*., 2007; JULIATTI *et al*., 2014).

Chemical control has shown positive results for controlling *Phaeosphaeria* leaf spot and spraying shoots of maize plants with fungicides has been a frequent practice (MALAGI

et al., 2011). Studies show the effectiveness of these products, according to (JANN *et al*., 2004) pyraclostrobin mixture + epoxiconazole showed effectiveness in the white spot control.

This study aimed to evaluate the effectiveness of foliar application of fungicides to control the *Phaeosphaeria* spot and consequently on the productivity characters of maize crop.

MATERIAL AND METHODS

The experiment was conducted in the municipality of Cruz Alta, Rio Grande do Sul, Brazil (28º 51 '49 "S, 53 31 '40 "W, 452 m), in 2014/15 season. The experimental design was randomized blocks in a 2x4 factorial scheme (2 hybrids x 3 fungicides + 1 control), with four replications, totaling 32 experimental plots occupying a total area of 640 m^2. The plots were established in an area of 20 m² (0.5 m x 10.0 m), with a useful area of 8.0 m^2 (1 m x 8.0 m). The soil preparation was carried out on September, 2014; it was consisted of an average harrowing and another light, a week before the implementation of the experiment. Seed treatment was made with the use of fungicides and insecticides Standak TOP - (Active Ingredients: Fipronil 250 g L^{-1}, Pyraclostrobin 25 g L^{-1}, Thiophanate Methyl 225 g L^{-1}) at the dose of 115 g a.i. 100 kg^{-1} seeds. The weed control and other agricultural practices during the experiment were carried out when necessary according to (EMBRAPA, 2013).

For this experiment, the hybrids BG7051H (susceptible) and BG7060HR (moderately resistant) were used, seeded on 17 September 2014, with the aid of a sower of four lines in conventional tillage, with spacing 0.50 m between lines and density of 4.8 seeds per meter groove, getting an average population density of 65.000 plants ha^{-1}.

At sowing, was applied 300 kg ha^{-1} of fertilizer 09-26-14 (N, P_2O_5 and K_2O) in the sowing furrow and 35 days after emergence were applied 300 kg ha^{-1} of ammonium sulfate in side dressing. All cultural practices and operations established during the experiment were suitable for the production of corn, following technology for high productivity (EMBRAPA, 2013).

Table 1. Treatments used in the experiment. Cruz Alta / RS, October 2014.

Treatments	Active ingredient	Concentration (g a.i. L^{-1})	Dose
Control	---	---	---
Abacus HC®	Epoxiconazole + Pyraclostrobin	(333+167)	380 mL ha^{-1}
Approach Prima®	Cyproconazole + Picoxystrobin	(80+200)	300 mL ha^{-1}
Elatus®	Azoxystrobin + Benzovinliflupir	(300+150)	150 g ha^{-1}

The treatments tested are listed in Table 1. The application of fungicides was performed at the phenological stage V8 (plants with 8 fully developed leaves), about 70 days after seeding (DAS), with the aid of a knapsack sprayer / manual. This equipment was pressurized with CO_2 at 04 bar fan type nozzles (RX 11002 / TEEJET) spaced 0.50 meters,

reaching application range 2.0 m, working pressure of 40 lb./pol^{-2}, constant flow of 300 L ha^{-1} and a traversal rate of 1 ms^{-1}.

The following variables were analyzed: severity of white spot (%), thousand grain weight (g) and productivity (kg ha^{-1}).

The evaluation of the white spot severity was carried out in two central rows of each plot based on the quantification of diseased leaf area in 10 plants in the useful area of each plot, using the white spot diagrammatic scale by Malagi *et al.* (2011) represented in Figure 1. Three assessments of disease severity were performed every 15 days, the first in pre-application, the second 15 days after application (85 DAS) and the third 30 days after application (115 DAS).

Figure 1. Diagrammatic scale for corn white spot severity. The values are percentages of leaf area with symptom of the disease. (MALAGI *et al.*, 2011).

To determine the yield (kg ha^{-1}) and thousand grain weight (g), manual harvesting was carried out (14 February 2015 – 163 DAS) of the ears from the two central rows of each plot (to exclude the border effect), subsequently the corn grains were removed from the cob, the grains were weighted and the grains' moisture content established, being that the grains yield data were corrected to a moisture content of 13 %.

For efficacy evaluation of each fungicide it was used the formula proposed by Abbott (1925):

$$E(\%) = \frac{T - F}{T} x100$$

Where:

T = average severity in the control;

F = average severity in the treatments;

E% = Percentage of effectiveness of each assessed treatment.

To estimate the area under the disease progress curve (AUDPC), the data of disease severity obtained in all assessment times were used according to equation cited by Shaner and Finney (1977).

$$AUDPC = \sum [(Y_{i+n1} + Y_i)/2][T_{i+1} + T_i]$$

Where:

n = the number of observations;

Yi = the severity of disease at the i^{th} observation;

Ti = is the time in days at the i^{th} observation.

Initially the results were submitted to the normality and homogeneity tests. Then individual analysis of variance (ANOVA) was performed, subsequently the combined analysis involving both hybrids within each treatment. The means of the variables significant in F test were compared by Tukey test, adopting a level of 5 % error probability ($p < 0.05$), using the statistical software SISVAR version 5.1 (FERREIRA, 2011).

RESULTS AND DISCUSSION

According to Table 2, the variation coefficients were from 3.07 to 16.79, having no significant external interference that prevented the continuation of the trial. In Figure 1 is shown the progression of severity in relation to their respective treatments for the two hybrids studied, in which it was observed a discordant disease progression between the control and the treatments with foliar application of fungicides in the phenological stage V8 (70 DAS), being more pronounced in the hybrid BG7060HR.

Table 2. Treatments used in the experiment. Cruz Alta / RS, November 2014. Summary of Variance Analysis of characteristics: SEV1 - 1st severity assessment (17/11/2015 – 70 DAS) SEV2 - 2nd severity assessment (12/02/2014 – 85 DAS) SEV3 - 3rd evaluation severity (17/12/2014 – 115 DAS), PROD - Productivity in kg ha^{-1}, PMG - thousand grain weight in grams (harvest 2014-15).

Source of variation	GL[1]	QM[2]					
		SEV1	SEV2	SEV3	PROD	PMG	AACPD
Hybrid (H)	1	0.02	0.11	0.08	7698.67NS	1568.46NS	15.80NS
Treatment (T)	3	0.08	2.12	14.81	8826.21*	6822.22*	2755.92*
H*T	3	0.57	0.09	0.02	3180.76NS	7207.38*	5.73NS
Error	24	0.02	0.06	0.06	3807.04	4415.04	24.44
CV (%)		3.07	5.27	4.60	12.15	16.79	3.57
General Average		4.22	4.56	5.10	5078.51	449.14	183.33

[1] degree of freedom; [2] Average square, * significant at 5 % and (NS) not significant.

There were significant differences at 5 % probability, from the second evaluation, between treatments with fungicide application and the control without application, approximately 15 days after application (85 DAS) (Table 3), highlighting the superiority of all treatments compared to the control. As regards efficacy of the fungicides applied, it was found that all treatments with fungicide application showed efficacy above 78.92 % compared to the control. The best treatment verified for both hybrids was Elatus®

(azoxystrobin + benzovinliflupyr) with average efficacy of 94.44 % for the hybrid BG7051H and 95.10 % for the hybrid BG7060HR (Table 3).

Table 3. White spot severity in corn plants, percentage of effectiveness of treatments for each evaluation and area under the disease progress curve (AUDPC). Tukey test (P <0,05). Cruz Alta / RS, November 2014.

Hybrid	Treatments							
	Control		Abacus HC®		Aproach® Prima		Elatus®	
	SEV	E. (%)	SEV	E. (%)	SEV	E. (%)	SEV	E. (%)
1st Evaluation								
BG7051H	4.20Aa	---	4.20Aa	---	4.20Aa	---	4.20Aa	---
BG7060HR	4.50Bb	---	4.16Aa	---	4.18Aa	---	4.15Aa	---
2nd Evaluation								
BG7051H	5.55Bb	0.00	4.35Aa	88.89	4.30Aa	92.59	4.28Aa	94.44
BG7060HR	5.11Ba	0.00	4.31Aa	94.12	4.30Aa	78.92	4.28Aa	95.10
3rd Evaluation								
BG7051H	7.10Ba	0.00	4.59Aa	86.64	4.39Aa	93.56	4.33Aa	95.69
BG7060HR	7.18Ba	0.00	4.54Aa	85.98	4.29Aa	77.10	4.41Aa	90.19
AACPD								
BG7051H	168.00Ba		131.16Aa		128.91Aa		128.06Aa	
BG7060HR	164.25Ba		129.94Aa		127.97Aa		128.34Aa	

* Averages followed by the same letter, capital in the line and lowercase in column do not differ statistically by Tukey test at 5 % probability.

In the third evaluation, the treatments showed similar behavior, being equal among each other, however, were higher than the control. In the third evaluation, it was noticed an increase in disease severity (Figure 2) for the control group and superiority in efficacy for the Abacus® HC treatments (380 mL ha^{-1}), Aproach® Prima (300 mL ha^{-1}) and Elatus® (azoxystrobin + benzovinliflupyr) (150 g ha^{-1}), with efficiency up to 77.10 % compared to the control.

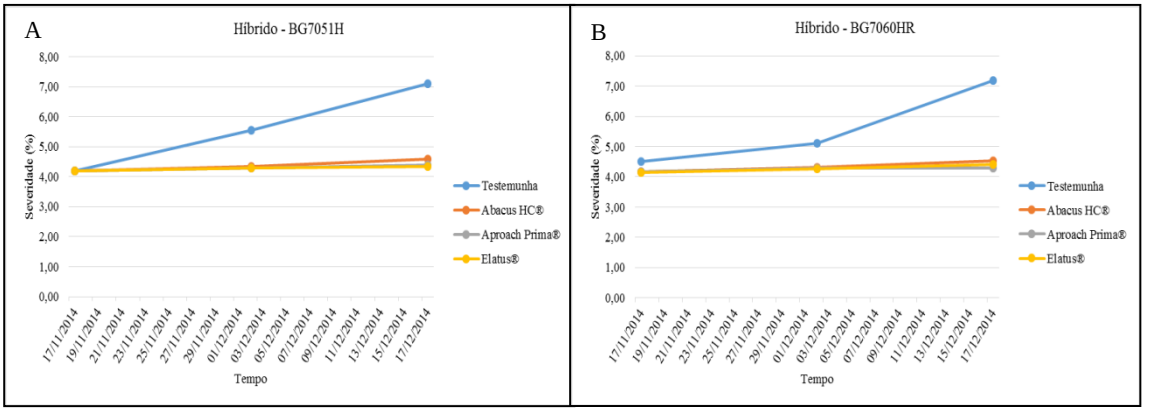

Figure 2. Progression of severity chart. Cruz Alta / RS, November, 2014.

All the fungicides were better than the control when they were applied in the V8 stage, since it has already been observed the externalization of the first symptoms of the disease in

the field. The results of this study show the efficacy of fungicides formulated from the mixture of active principles of chemical strobilurin and triazole groups for the control of *Phaeosphaeria* leaf spot. According to (PEREIRA *et al*., 2005), the fungicides of the strobilurin chemical group had effective control of the disease.

The effectiveness of prefabricated mixtures of chemical groups of Strobilurin and triazoles were also reported by (MANERBA *et al*., 2013), that in assessing the severity of corn's white spot in two application periods (V8 and pre-bolting) of different fungicides, found that the application of pyraclostrobin + epoxiconazole resulted in a lower disease severity. (COSTA *et al*., 2012) also observed the efficacy of pyraclostrobin + epoxiconazole treatment in reducing the severity of this disease.

When analyzed the area under the disease progress curve (Table 3), obtained from the severity data collected in the three assessments. In both hybrids, the same behavior was verified, all the treatments, where there was a foliar fungicide application, statistically differentiated from the control, however they did not differ among themselves. Probably, due to the fact that none of the hybrids have responded physiologically in the construction of a cell wall with higher levels of lignin, which proves the greater reduction of the progress curve of the disease. When conducting joint analysis of hybrids within each treatment, there was also no difference amongst them, irrespective of presenting different levels to the white spot leaf disease.

Similar results were described by Manerba *et al*. (2013), that by evaluating AUDPC under application of different fungicides the V8 stage to control the white spot, observed high efficacy of the fungicide pyraclostrobin + epoxiconazole (750 mL p.c ha^{-1}). Juliatti *et al*. (2014) evaluated the effect of foliar application of fungicides on AUDPC for maize white spot, found that among the fungicides used, pyraclostrobin + epoxiconazole obtained intermediate results in reducing AUDPC. Szeuczuk *et al*. (2012) evaluated the AUDPC of common rust in maize, found significant differences between the different fungicides evaluated when compared to the control.

The grain yield was influenced only by fungicide factor. In the hybrid BG7051H, the control performed by the fungicide Elatus® (azoxystrobin + benzovinliflupyr) resulting in an increase of 64.06 % in grain production, reaching an average yield of 6.543 kg ha^{-1}. The same behavior was observed for the hybrid BG7060HR in which the Elatus® fungicide (azoxystrobin + benzovinliflupyr) controlled more and generated an increase of 63.48 % in grain production, with an average yield of 6.465 kg ha^{-1}. These productivity values are according to average of the Rio Grande do Sul State, which are less than 6.560 kg ha^{-1} for the same harvest 2014/15 (CONAB, 2015).

These results are similar to those cited by Juliatti *et al.* (2014), which observed the higher efficacy of the strobilurins in blends with triazole, in the control of white spot and increasing productivity, even as the most productive treatment was azoxystrobin + cyproconazole + Nimbus (12.365 kg ha^{-1}). The use of mixtures of active ingredients assists

in better control of fungi once an action occurs in more than one loco in the cellular structure of the pathogen.

Manerba *et al.* (2013) also observed the influence of fungicide application on grain yield, since all treatments with fungicide application were higher than the control, especially systemic fungicide pyraclostrobin + epoxiconazole, that promoted an increase in productivity of 90 % compared to the control and 50 % compared to other treatments. By having a greater upward mobility in the leaf, the systemic fungicides increase the leaf area protected against the pathogens, resulting in greater control and return in productivity.

On the other hand, (VILELA *et al*., 2012) also found that, despite the foliar application of fungicides pyraclostrobin + epoxiconazole and azoxystrobin + cyproconazole there was a reduction the incidence of foliar diseases in maize, this behavior did not reflect in the increased productivity, due to the action of the fungicides in preventing the spores germination of pathogens (KIRK *et al.*, 2008). Costa *et al*. (2012) in studies observed no significant difference in productivity regarding to the application of fungicides compared to the control.

Tabela 4. Average productivity of treatments and thousand grain weight. Cruz Alta / RS, September, 2014.

Hybrid	Treatments			
	Control	Abacus HC®	Aproach® Prima	Elatus®
	Productivity (Kg ha^{-1})			
BG7051H	3988.22Ca	5344.05ABa	5058.97BCa	6543.23Aa
BG7060HR	3954.82Ba	4449.95Ba	4823.67Ba	6465.19Aa
	Thousand grain weight (g)			
BG7051H	356.50Bb	406.46Ba	375.15Ba	569.92Aa
BG7060HR	485.02ABa	392.72Ba	404.70Ba	602.70Aa

* Averages followed by the same letter, capital in the line and lowercase in column do not differ statistically by Tukey test at 5 % probability.

Regarding the thousand grain weight (Table 4), the statistically superior treatment to the control were azoxystrobin + solatenol with averages of 569.92 g and 602.70 g for hybrids BG7051H and BG7060HR respectively. The results obtained are superior to those reported by (JULIATTI *et al*., 2014), who evaluated the effect of the combination of strobilurins and triazoles in the management of white spot of corn and the corresponding effect on production components. They reported that the best treatments were epoxiconazole + pyraclostrobin and azoxystrobin + cyproconazole + Nimbus with averages of 344.3 and 353.4 g thousand $grain^{-1}$, respectively.

When analyzing the thousand grain weight of hybrids in the control treatment, we determined the genetic difference between them, since this treatment was no statistical difference between both, with the hybrid BG7060HR showing the highest thousand grain weight. According to BioGene (2015), the company responsible for the production of seed of this hybrid, the same rate as moderately resistant to disease caused by the fungus

Phaeosphaeria maydis, while the hybrid BG7051H classified as susceptible, had lower thousand grain weight.

CONCLUSION

The control alone had the highest severity index compared to the other treatments.

Fungicide application in the V8 stage (70 DAS) was effective in controlling white spot and provided an increase in the production of grain when compared to control for the treatment Elatus® (azoxystrobin + benzovinliflupir).

The Elatus® treatment (azoxystrobin + benzovinliflupir) was superior to the control in the thousand grain weight variable (MMG) and productivity.

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