International Journal for Innovation Education and Research

ISSN: 2411-2933

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Abstract

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Published Date: 8/1/2020 **Page.519-529** **Vol 8 No 08 2020**

DOI: https://doi.org/10.31686/ijier.vol8.iss8.2555

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Gilmar Seidel, Caroline Wesp, Jana Koefender, Diego Pascoal Golle, André Schoffel, Elaine Deuner, Juliane Nicolodi Camera
Universidade de Cruz Alta
Brazil

ABSTRACT

In Brazil, corn planted area increased by 30%. Therefore, there was also an increase in the incidence of pathogens in the crop. The objective of this work was to evaluate the influence of fungicide applications on corn crops in the different growth stages for the control of the diseases and its effect on the occurrence of mycotoxins in the grain. The experiment was carried out in the 2017/2018 summer crop, in the municipality of Cruz Alta, state of Rio Grande do Sul/BR. This study used P 1630 hybrid, in a randomized block design with eight treatments (control, V8, PT, V4 + V8, V4 + V8 + PT, V8 + PT, V8 + PT + 15, PT + 15) and three replicates. The fungicide fluxapiroxade + pyraclostrobin + epoxiconazole at the dose of 1.0 L/ha^{-1} was used. The diseases that affected the crop were the white spot and helmintosporiosis, therefore, reducing the incidence of diseases. Also the highest yields were obtained where the applications started at the phenological stage V4 and V8. The levels of mycotoxins detected in this work remained within the limits of the legislation, with a significant reduction in the accumulation of fumonisin and aflatoxin with fungicide applications.

Keywords: helmintosporiosis; productivity; toxins; white spot; *Zea Mays*.

INTRODUCTION

According to data from Conab (2018), Brazil is consolidated as the third largest corn producer in the world and the second largest exporter, with a high domestic consumption of cereal as it is one of the world's leading producers of animal protein. Over the last five years, the dynamics of the corn production chain have changed significantly in the country, since the grain was no longer just a product aimed for animal feeding, but also an exportable commodity. In addition, it has established itself in the last two years as an energy matrix in ethanol production. In Brazil, the cropped area has increased by 30%, and it has changed from being only an accessory culture, a simple component of a rotation system, to a strong culture, through intense professionalization process, with the adoption of new technologies (mainly the use of transgenic) and consequent increase in the productivity (Peixoto, 2014).

As the cropped area was increased, so did the occurrence of diseases in the crop. In Brazil, there are at least 20 pathogens occurring in the culture and may cause significant damage (Reis & Casa, 1996). This increase in diseases observed in the field may be due to the long period of time in which the crop remains in the field, with two growing seasons, crop and off-crop (*safrinha*).

Corn diseases occur on leaves, ears and stems. In the leaves, they reduce leaf area through necrosis, which reduces the photosynthetic active leaf area, thereby reducing productivity. In the ears, besides causing direct damage to harvested grains, known as burnt grains, secondary metabolites denominated mycotoxins are produced. They do not directly reflect on productivity, but are important for animal and human health, as they can cause disease through its ingestion (Guterres-Wesp *et al*., 2017). Mycotoxins reduce animal productivity as well as the contamination of the final product such as meat or milk. They generally cause lower food intake, reduce reproductive performance, leading to miscarriages, reduction in female ovulation rate, milk production and immunity of animals. They can also cause tumors and even deaths. Thus, disease management becomes a determining factor for the maintenance of crops with high productive potential (Fancelli, 2013).

Fancelli and Dourado-Neto (2004), report that during the vegetative phase, the beginning of the definition of the productive potential occurs, with the definition of the number of rows of grains in the ear, size of ear and number of ears per plant, which can justify the early application of fungicides.

Helminthsporiosis (*Exserohilum turcicum*) and white spot (*Pantonea annatis*) are among the main diseases of corn plants. When studying corn cultivation areas in India in 2003/04, Harlapur (2005) found that the average percentage of the incidence of the disease showed that it occurred in all corn crop areas in the state ranging from 20 to 86%, and 47% was the average incidence of the disease. The pathosystem of the white spot of corn (CWS) has acquired endemic character in several producing regions in the country, being able to reduce by up to 60% the grain yield (Brito *et al*., 2013). The etiological agent of CWS was initially described as the fungus *Phaeosphaeria maydis* by Rane *et al*. (1966). However, some studies have questioned the pathogenicity of *Phaeosphaeria maydis*, characterizing bacterium *Pantoea ananatis* as the CWS causative agent (Paccola-Meirelles *et al*., 2001; Lanza *et al*., 2013). A 10-20% CWS severity can reduce the net photosynthetic rate by 40% and yield losses can be as high as 60% depending on environmental conditions (Godoy *et al*., 2001).

The objective of this work was to evaluate the effect of application period of the fungicide (fluxapriroxade + epoxiconazole + piraclostrobin) on the control of helmontosporiosis and white spot diseases and on the reduction of mycotoxin levels in corn grains.

MATERIAL AND METHODS

This study was carried out in the CCGL Tecnologia experimental area, municipality of Cruz Alta/RS in Brazil, using P 1630 Dupont Pioneer corn hybrid with a super early cycle, in which one of the hybrids was more susceptible to leaf diseases.

The seeds were treated with the fungicide (Carboxin 200 gL^{-1} + Thiram 200 gL^{-1}) at a dose of 0.3 L per 100 kg of seed and with the insecticide (Imidacloprid 150 gL^{-1} + Thiodicarb 450 gL^{-1}), at a dose of 0.3 $L.ha^{-1}$. Sowing was carried out in December 2017, with a population of 75,000 plants ha^{-1}. Each plot consisted of six rows of 6 m, with row spacing of 0.47 m. The useful area of the harvested experimental plot was 5.4 m². The soil was previously fertilized with 300 kg.ha^{-1} of formula 10- 30-20 (NPK), performing the other topdressing fertilizations and crop treatments according to recommendations for corn crop.

The applications of the treatments were done using with a ground bar, provided with six tips (double flat deflector fan model - TTJ 60 110 02 / TEEJET), with CO_2 pressurized costal sprayer, walking speed of 1 ms^{-1}, pressure 30 lbs. pol^2 and spray volume of 150 L ha^{-1}. Dates, growth stages (treatments), and weather conditions at application periods of fluxapiroxade + piraclostrobin + epoxiconazole were identified (Table 1).

Table 1. Dates, growth stages and climatic conditions at application of fungicide Fluxapioroxade + Epoxiconazole + Piraclostrobina. INMET- Cruz Alta-RS. 2018.

Treatments	Date	DAE[1]	Stage	Initial hour / Final hour	Temp. (^{0}C) Maximum	Temp. (^{0}C) Minimum	R.H. (%)	Wind speed (km/h)
V4	03/01	18	V4	09:00	17	15	92	1.4
				09:20	17	15	92	1.4
V8	25/01	40	V8	09:00	22	21.8	82	9.3
				09:30	22	21.8	82	9.3
PP	06/02	52	PP	09:15	16.5	15.9	88	7.1
				09:55	16.5	15.9	88	7.1
DAA	22/02	68	R2	11:00	18.8	16.9	77	2.5
				11:25	18.8	16.9	77	2.5

[1]DAE Days after emergence.

The experiment was carried out in a randomized block design with eight treatments (growth stages): Control, V8 (eight fully open leaves), PT (pre-tasseling), V4 (four completely open leaves) + V8, V4+V8+PT, V8+PT, V8+PT+15DAA (days after previous application), PT+15DAA (days after previous application), PT + 15DAA and three repetitions. Fluxapiroxade 50g.L^{-1} + piraclostrobin 81 g.L^{-1} + epoxiconazole 50 g.L^{-1} fungicide were used in all applications at a dose of 1.0 L.ha^{-1}).

The diseases occurring over the crop cycle were helminthsporiosis and white spot. Seven severity evaluations of leaf diseases that occurred throughout the crop cycle were carried out with the aid of a diagrammatic scale for white spot according to (Azevedo, 1998) and for helmintosporiosis, a scale proposed by Utfpr, (2012) was used, giving a visual plot score. With these results, it was calculated the area below the disease progress curve (ABDPC), which takes into account the intensity of the disease and its evolution over time (Shaner & Finney, 1977). In the growth phase R4 (kernel dough stage), the final severity of the diseases was evaluated. Control efficiency (EC%) was calculated in relation to the area of the disease progress curve.

Harvest was done manually on April 25, 2018, in a useful area of 5.4 m², and later threshed in a stationary machine. The grains were cleaned in a specific stationary air machine, and then the grain mass (kg. ha^{-1}) and weight of one thousand seeds (g) were determined, by adjusting moisture to 13% of the samples collected for the productivity calculation.

For the determination of mycotoxin levels in the grain, 1 kg of the productivity samples were removed. All samples were ground in a blender for three minutes, and the blender was cleaned whenever the sample was changed, using paper towels and Vacuum cleaner, so that there was no contamination between samples. After grinding, they were stored in paper bags in a freezer until analysis.

Mycotoxin was analyzed using the standard procedure recommended by the Neogen Veratox® Kit method, based on Enzyme Linked Immuno Sorbent Assay (ELISA), was followed for aflatoxin, ocratoxin, fumonisin and zearalenone mycotoxins. The reading was performed on the specific reader (stall fax 4700) using the 650-nm filter. Results were given in parts per billion (ppb), (1 ppb is equivalent to 1 µg kg^{-1}).

The data were submitted to analysis of variance and compared using Scott-Knott test at 5% probability of error. Analyses were performed using SASM-Agri software (Canteri *et al*. 2001).

RESULTS AND DISCUSSION

The high rainfall observed in December (102.8mm) and January (361.2mm) resulted in a favorable environment for the occurrence of diseases in corn (Table 2), as well as that observed in February and March, which provided a favorable environment for the development of the diseases, in which the most common were helminths and white spot.

Table 2. Tem-day period for maximum and minimum temperatures (ºC), rainfall (mm) and average air relative humidity (%), during corn crop growth. Cruz Alta-RS. 2017/2018

		Dec	**Jan**	**Feb**	**Mar**	**Apr**
Maximum temperature * (ºC)	1st ten-day period	30.7	28.6	29.9	24.8	28.1
	2nd ten-day period	31.0	28.5	26.6	28.0	27.1
	3rd ten-day period	29.1	25.8	27.6	25.6	29.2
Monthly average		**30.3**	**27.6**	**28.1**	**27.3**	**28.1**
Minimum temperature * (ºC)	1st ten-day period	17.4	17.0	17.1	16.6	15.7
	2nd ten-day period	16.5	19.0	16.6	16.1	15.9
	3rd ten-day period	18.0	17.6	15.6	14.9	17.5
Monthly average		**17.3**	**17.8**	**16.5**	**15.8**	**16.4**
Rainfall (mm)	1st ten-day period	25.0	22.2	23.0	15.4	29.2
	2nd ten-day period	33.4	77.8	46.8	5.7	18.2
	3rd ten-day period	44.4	261.2	0.4	168.0	12.6
Total (mm)		**102.8**	**361.2**	**70.2**	**240.4**	**60**
Average RH (%)		**62.5**	**74.5**	**72.3**	**71.4**	**68.2**

* INMET, CCGL weather station - Cruz Alta-RS.

All treatments reduced the final severity of helminthesporiosis when compared to the control with no treatment. Final severity was 45%. Also, the best controls were observed when the application was performed preventively in V4. In the application performed in V8, early symptoms of the disease had already been observed.

The smallest severities caused by helminthsporiosis were obtained for the treatment where fungicide applications were performed on V4 + V8 and V4 + V8 + PT, followed by V8 + PT and V8 + PT + 15DAA (Table 3). According to Boller *et al*. (2007) the effectiveness of control depends on the age of the infection. A fungicide application on newly established infections results in the death of the pathogen.

Table 3. Final severity (%), area below disease progress curve (ABDPC), control percentage (%C) of helminthsporiosis in corn, Cruz Alta-RS. 2017/2018.

Treatments	Final severity (%)	ABDPC	%C[1]
V4+V8+PT	12.0 a*	247.8 a	81.3
V4+V8	12.0 a	299.7 b	77.4
V8+PT	16.6 b	336.3 b	74.7
V8+PT+15DAA	19.3 b	372.3 c	72.0
V8	24.0 c	570.8 e	57.1
PT+15DAA	24.6 c	453.8 d	65.9
PT	29.0 d	685.8 f	48.9
Control	45.0 e	1331.5 g	0.0
C.V%	6.7	5.6	

*Means followed by the same letter in the column are not different from each other by the test of Scott-Knott at 5% probability. [1] Calculated on the basis of ABDPC.

Azevedo (2007) reports the most pronounced curative/eradicating effect of fungicides up to 48 to 72 hours after pathogen infection. In older infections, the energy no longer used for growth is reallocated for reproduction, causing lesions and forming viable or not viable spores. The cause, however, is the high number of non-visible infections at the incubation stage, which reinforces the need to apply treatments early, preemptively or at the onset of symptoms (Boller *et al*., 2007).

The ABDPC considers the evolution of the disease over time, in which the best control was observed in treatment (V4 + V8 + PT). In relation to that treatment, application started preventively (V4), in which three applications were made, therefore providing more robustness and residual necessary to protect the active photosynthetic leaf area of the crop. Data on severity, ABDPC, and percentage control of helminthsporiosis are shown in (Table 3). Cota *et al*. (2010) observed that the application of Epoxiconazole + Piraclostrobin was efficient in the control of sorghum helminthsporiosis. Moreover, fungicide was applied 45 days after sowing, which is the key point for the success of sorghum helminthsporiosis chemical control because late applications may have no effect on disease control.

Similarly, all fungicides tested in the study reduced the final severity of white spot. The best controls were observed in preventive-initiated treatments (V4 and V8), as the disease was observed in the field seven days after application in V8. When analyzing the ABDPC, it was found that the treatment (V4 + V8 + PP) had the best controls (Table 4).

Table 4. Final severity, area below disease progress curve (ABDPC), white spot control percentage (%C), Cruz Alta-RS. 2017/2018.

Treatments	% Final severity	ABDPC	% C[1]
V4+V8+PT	20.6 a	332.7 a	79.3
V4+V8	25 b	428.7 b	73.3
V8+PT	26 b	423.3 b	73.6
V8+PT+15DAA	27.6 c	466.5 b	70.9
V8	30 d	500.6 b	68.8
PT+15DAA	32.3 d	825.6 c	48.6
PT	50 e	1094.6 d	31.8
CONTROL	56.6 f*	1605.3 e	0
C.V	5.12	5.7	

*Means followed by the same letter in the column are not different from each other by the test of Scott-Knott at 5% probability. [1] Calculated on basis of ABDPC.

Manerba *et al*. (2013) observed that at stage V8 there was a significant difference for all treatments in relation to the control up to 98 days after sowing. Also, the piraclostrobin + epoxyconazole mixture was higher at 82 DAS, probably due to a systemic residual of 20 to 30 days after application, which is a characteristic of the mixture of the strobilurin and triazole chemical groups.

The use of practices such as susceptible cultivars, non-rotation of crops, associated with favorable conditions and the occurrence of epidemics, contribute to the increase in the importance of diseases in corn and consequently the use of fungicides (Oliveira, 1997; Julliati *et al*., 2004). Thus, disease management becomes a determining factor for the maintenance of crops with high productive potential (Fancelli, 2013). In addition to the control provided by the fungicide, some of them contain in their formulation molecules belonging to the strobilurin group, which may offer additional physiological effects, which positively contribute to the productivity of some cereals (Hoehle *et al*., 2002).

One of the factors involved for the complete expression of the productive potential of an hybrid is the ability to perform photosynthesis, which is related, among other factors, to its ability to capture sunlight through its leaves. This absorption will be maximum only if leaves are green and healthy and free of pathogens that can decrease this ability to capture light through necrosis. Economic benefit and response of fungicide use in corn will be achieved according to the susceptibility of the hybrid and the expected yield potential of the crop. More susceptible hybrids in crops with higher disease occurrence are likely to respond more to the number of fungicide applications and to the early entries (Wesp-Guterres *et al*., 2017). Therefore, when analyzing the productive data achieved in this experiment, it is observed that the applications started in V4 and V8 resulted in higher yields, which corroborates the low levels of diseases observed. Whenever disease control was started preventively, lower disease levels were observed, which was reflected in higher yields.

Brito *et al*. (2013) observed that leaf diseases *Cercosporiose* and White Spot reduced corn grain yield and this reduction was greater when diseases occurred early. In this work, it was found that the treatments (V4 + V8 + PT), (V8), (V4 + V8), (V8 + PT) and (V8 + PT + 15DAA) resulted in an average increment of 46.8 bags per hectare. Brito et al. (2013) analyzed 12 distinct corn hybrids at 3 different sites, and achieved 12% higher yield response on average compared to the use of two fungicide applications (azoxystrobin + cyproconazole) at V10 + PT, and the non-use. In treatments (PT) and (PT + 15DAA) in this work, where a disease level had been already observed at fungicide application, no significant difference of the control in relation to yield was observed. According to Fancelli and Dourado-Neto (2004), the beginning of the definition of the productive potential occurs during the vegetative phase, defining the number of rows of grains in the ear, size of ear and number of ears per plant, which can in some cases justify the early application of fungicides. For the mass of one thousand seeds, no statistical differences were obtained, as it can be observed in Table 5.

Table 5. Production (kg ha^{-1}), production in bags per ha (bags/ha), difference in relation to the non-treated control in bags per hectare (≠ for the control) and mass of one thousand seeds in grams (MTS), Cruz Alta-RS. 2017/2018.

Treatments	Production kg ha^{-1}		bags ha^{-1}	≠for control	MTS (g)	
V4+V8+PT	10862	a*	181.0	67.5	307.7	a
V8	9825	a	163.7	50.2	275.7	a
V4+V8	9810	a	163.5	50.0	291.3	a
V8+PT	8932	a	148.9	35.3	308.7	a
V8+PT+15DAA	8676	a	144.6	31.1	295.3	a
PT	7591	b	126.5	13.0	286.7	a
PT+15DAA	7436	b	123.9	10.4	293.3	a
Control	6812	b	113.5	0.0	257.0	a
CV	12.29%				5.51%	

*Means followed by the same letter in the column are not different from each other by the test of Scott-Knott at 5% probability.

The fungicides registered for corn crop are mainly to maintain plant health, reducing colonization of pathogens that cause burned grains (Duarte *et al*., 2009). This work analyzed the presence of four mycotoxins associated with corn grains in the post-harvest, and the time when fungicide was applied had no significant effect for ochratoxin and zearalenone, with a lower accumulation of aflatoxin and fumonisin (Table 6).

Table 6. Levels of Mycotoxin (μg kg^{-1}) found in the different treatments used in the P1630 corn hybrid. Cruz Alta, RS. 2017/2018.

Treatments	Fumonisin μg kg^{-1}	Aflatoxin μg kg^{-1}	Zearalenona μg kg^{-1}	Ocratoxin μg kg^{-1}
V8+PP+15DAA	20.1 b	5.5 a	13.8 a	0.1 a
V4+V8+PT	17.2 a	5.5 a	13.4 a	0.0 a
V4+V8	17.9 a	5.5 a	7.9 a	0.0 a
V8	18.9 a	6.6 a	14.8 a	0.0 a
PT+15DAA	20.7 b	6.9 a	16.5 a	0.2 a
PT	21.7 b	7.1 a	12.5 a	0.0 a
PT+15DAA	20.4 b*	7.8 b	25.9 a	0.1 a
Control	22.4 b*	9.6 b	19.9 a	0.3 a
CV	4.38%	8.55%	23.30%	112.10%

*Means followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% probability.

Ochratoxin, which is formed by *Penicilium* and *Aspergilus* storage fungi, was detected in this study in very low amounts, so it was not possible to verify whether or not its occurrence was reduced by the application of fungicides. The low occurrence of ochratoxin was also mentioned by Feddern *et al*. (2018), when they verified in only three samples of corn plots in 2016. Also, in 2017, they found that 95.9% of the samples did not accuse the presence and samples with presence were below the tolerable threshold.

No significant presence of zearalenone was observed in corn grain, possibly due to environmental conditions during cultivation, since the average minimum temperatures were around 15.8 ºC and the average maximum values were higher than 25ºC. Although Fusarium growh is favored by these temperatures, colder temperatures (8 to 14 ºC) are required for significant levels of zearalenone. These results corroborate those of Feddern *et al*. (2018) who found Zearalenone levels within the tolerable upper threshold in 99% of corn samples analyzed in 2016 and 2017.

Aflatoxin is caused by fungi of the *Aspergilus* genus and it has been found at levels below the maximum tolerable threshold. Even so, most of the treatments tested were able to decrease their occurrence in grains when compared to the control. All treatments with the exception of treatment (V8 + PT) decreased by 26.04% to 42.7% the amount of aflatoxins compared to treatment without fungicide.

Fumonisins are produced by secondary metabolism of toxigenic fungi of the genus *Fusarium* and *Alternaria*, in which *Fusarium moniliform* strains are the most productive (Lamic, 2018). As observed in this study, there was a lower accumulation of fumonisin in the treatments (V8), (V4 + V8) and (V4 + V8 + PT); however, in the others treatments, no significant interference with mycotoxin levels was observed. Similar to the other mycotoxins, the values found in the study are below the maximum acceptable threshold. Feddern *et al*. (2018), observed that although 76.1% of corn samples were contaminated with fumonisin, only 8.3% of the samples were above the maximum acceptable threshold. Juliatti *et al*. (2007) observed a significant difference in the incidence of *Fusarium moniliform* as a function of foliar application of fungicides, with a reduction in the infection of up to 33%. Yoshida *et al*. (2008) evaluated the period of methyl thiophanate applications in barley and concluded that applications reduce the mycotoxin content in harvested grains.

CONCLUSIONS

In relation to the diseases caused by helminthsporiosis and white spot, the best control efficiency of fluxapiroxade + piraclostrobin + epoxiconazole fungicide was obtained when applied in the V4 + V8 + PT growth stages, directly influencing the crop yield.

The highest yields of corn crop were achieved through preventive applications initiated at V4 and V8 growth stages.

Application on the levels of zearalenone and ochratoxin mycotoxins produced no effect, but a lower accumulation was observed for fumonisin and aflatoxin, but within the maximum accepted by the corn grain legislation.

AKNOWLEDGEMENTS, FINANCIAL SUPPORT and FULL DISCLOSURE

To Cooperativa Central Gaúcha Ltda (CCGL) and Unicruz. The authors declare that there is no conflict of interest in the publication of this paper.

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