http://dx.doi.org/10.1590/1678-4162-13106

***Arq. Bras. Med. Vet. Zootec.*, v.76, n.2, p.333-345, 2024**

Effectiveness of pyraclostrobin on the production, morphology and nutritional value of winter cereal forage in successive cuts

[Eficácia da piraclostrobina sobre a produção, a morfologia e o valor nutricional da forragem de cereais de inverno em cortes sucessivos]

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ABSTRACT

This study aimed to evaluate the forage yield, morphology, and nutritional value of three winter cereal wheat (*Triticum aestivum* cv. BRS Umbu), white oats (*Avena sativa* cv. URS Guará), and black oats (*Avena strigosa* cv. Embrapa 139), harvested in two successive cuts, at the vegetative and full vegetative stages, applied or not with fungicide pyraclostrobin. Pyraclostrobin was sprayed two times during the vegetative stage, the first application at the phenological stage V5, and the second, 12 days after the first cut of each forage species. In general, the application of pyraclostrobin resulted in an increase in the participation of leaves in the plant structure, from 72.2% to 86.9% in the second cut; and crude protein increased, and lignin decreased (from 22.75% and 9.89% to 25.60% and 6.30%, respectively) in the first cut and (from 20.82% and 11.73% to 22.28% and 9.20%, respectively) in the second cut. Black and white oats had a higher cumulative biomass production, 3,698 kg ha^{-1}, and 3,277kg ha^{-1}, respectively, and white oats had the lowest content of acid detergent fiber (27.90%) at the second cut.

Keywords: ruminal DM degradation, lignin, crude protein, DM production

RESUMO

O objetivo do trabalho foi avaliar a produtividade, a morfologia e o valor nutricional da forragem de três cereais de inverno: trigo (Triticum aestivum *cv. BRS Umbu), aveia-branca* (Avena sativa *cv. URS Guará) e aveia-preta* (Avena strigosa *cv. Embrapa 139), colhidos em dois cortes sucessivos, na fase vegetativa e plena vegetativa, submetidos ou não ao tratamento com o fungicida piraclostrobina. A pulverização da piraclostrobina foi realizada em dois momentos durante a fase vegetativa, sendo a primeira aplicação no estádio fenológico V5 e a segunda aos 12 dias após o primeiro corte de cada espécie forrageira. A aplicação da piraclostrobina proporcionou incremento na participação de folhas na estrutura das plantas de 72,2% para 86,9%, no segundo corte; e os teores de proteína bruta aumentaram e de lignina diminuíram (de 22,75% e 9,89% para 25,60% e 6,30%, respectivamente) no primeiro corte e (de 20,82% e 11,73% para 22,28% e 9,20%, respectivamente) no segundo corte. A aveia-preta e a aveia-branca tiveram maior produção de biomassa acumulada, de 3.698 kg ha^{-1} e 3.277 kg ha^{-1}, respectivamente, e a aveia-branca os menores teores de fibra em detergente ácido (27,90%) ao segundo corte.*

Palavras-chave: degradação ruminal da MS, lignina, proteína bruta, produção de MS

INTRODUCTION

Winter cereals are excellent forage for ruminant feeding, which are used in different ways, whether as grazing, successive cuts with the ready supply to the animals, or as haylage or hay. When winter cereals are used at the vegetative development stage, the forage obtained, in the general average, is nutritionally characterized by a high content of crude protein (>15%) and mineral matter (>8%), with greater digestibility

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Submitted: July 21, 2023. Accepted: October 6, 2023.

of dry matter (>75%), and low content of dry matter (<20%) and neutral detergent fiber (<45%) (Silva *et al.*, 2022).

An aggravating factor in the production of winter cereals is the high incidence of foliar diseases, especially at the end of the cycle, as a result, the use of fungicides has become more important to produce grains of these cereals (Reis, 2001).

Studies show that some strobilurin fungicides cause some changes in plant physiology (Fernandes and Pacinini, 1999), which result in increased grain productivity (Trojan, 2009), but there are no reports of changes in productivity and nutritional value of winter cereals at the vegetative phase aiming at the production of high-quality forage.

The evaluation of different winter cereals applied with pyraclostrobin must be carried out, because possibly each cereal will respond differently, in terms of physiological changes, generating physical changes, alterations in productivity, and nutritional value in the forage produced.

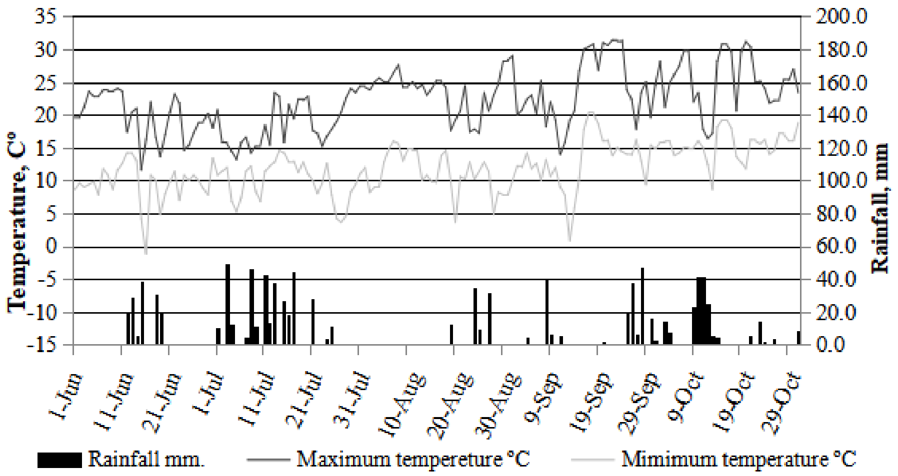
Thus, this study aimed to evaluate the productivity, physical composition, and nutritional value of the forage of three winter cereals, harvested in two successive cuts, at the vegetative and full vegetative stages, applied or not with fungicide pyraclostrobin.

MATERIAL AND METHODS

The experiment was carried out at the Animal Production Center (Nupran), Master's Program in Veterinary Sciences, in the area of animal health and production of the Agrarian and Environmental Sciences Sector, Midwest State University (Unicentro), in Guarapuava, state of Paraná, located in the subtropical zone of the state, at the geographical coordinates 25º23'02" South latitude and 51º29'43" West longitude and 1,026 m altitude.

According to the Köppen classification, the climate of the region is Cfb (subtropical humid mesothermal), with mild summers and moderate winters, without a defined dry season and with severe frosts. The average annual rainfall is 1,944 mm, the average annual minimum temperature is 12.7°C, the average annual maximum temperature is 23.5°C, and the relative humidity is 77.9%.

The soil in the experimental area was classified as Typical Bruno Latosol (Pott, 2007), and upon crop implementation, it showed the following chemical properties (profile from 0 to 20cm): pH $CaCl_2$ 0.01M: 4.7; P: 1.1 mg dm^{-3}; K^+: 0.2cmolc dm^{-3}; OM: 2.62g dm^{-3}; Al^{3+}: 0.0cmolc dm^{-3}; H^+ $+Al^{3+}$: 5.2cmolc dm^{-3}; Ca^{2+}: 5.0cmolc dm^{-3}; Mg^{2+}: 5.0cmolc dm^{-3} and base saturation (V%): 67.3%. Fig. 1 illustrates the average rainfall in mm, as well as the daily maximum and minimum temperatures, in ºC, during the experimental period.



Source: SIMEPAR/UNICENTRO experimental station, Guarapuava, state of Paraná, 2015.

Figure 1. Average rainfall (mm), maximum and minimum temperature (ºC) during the winter cereal growing period.

The experimental material consisted of wheat (*Triticum aestivum* cv. BRS Umbu), white oats (*Avena sativa* cv. URS Guará), and black oats (*Avena strigosa* cv. Embrapa 139), sown according to the agricultural zoning for the region of Guarapuava, state of Paraná, in a no-till system. Sowing was done in plots with a useful area of $5m^2$, using a Semina 1 seeder, with row spacing of 0.17 meters, a mean sowing depth of two centimeters, and a sowing density of 80kg ha^{-1}.

Upon sowing, basal fertilization was carried out with 250 kg ha^{-1} fertilizer 08-20-20 ($N-P_2O_5-K_2O$), respecting the recommendations of the Soil Fertility Commission of Santa Catarina and Rio Grande do Sul (Manual…, 2004). Topdressing nitrogen fertilization was carried out at once, 42 days after sowing with 150kg ha^{-1} urea (46-00-00).

Weeds were chemically controlled using a Glyphosate-based herbicide (Roundup WG® commercial product: 3.0kg ha^{-1}) for burndown of the experimental area, 15 days before sowing, and in crop management, 30 days after sowing, with the application of metsulfuron-methyl herbicide (commercial product Ally®: 6.6g ha^{-1}).

(Comet®, pyraclostrobin, BASF, Brasil) 250g L^{-1} of the active ingredient was sprayed at a dose of 0.6L ha^{-1} in two moments during the vegetative stage, the first application at the V5 phenological stage (elongation), and the second at 12 days after the first cut of each forage species. Sprayings were carried out using a motorized backpack sprayer, equipped with a bar containing four double flat fan nozzles, Twinjet TJ 60 110.02, spaced 0.50m apart, with a spray volume of 200 L ha^{-1} and sprinkler pressure of 2.0kgf cm^{-2}.

Plants were manually harvested at 8 cm above the ground according to each treatment. The cuts were made at the vegetative and full vegetative stages, when light interception reached 90 to 95% in the plot, following the recommendations of Fontaneli *et al*. (2009), where cutting should occur when the plants reach a height close to 30-35cm. Therefore, plants were harvested at the vegetative stage between 60 and 75 days and at the full vegetative stage, between 82 and 97 days after sowing, according to the behavior of the different evaluated forages. Light interception (LI) was estimated using photosynthetically active radiation (RAF) and measured by a digital linear ceptometer, AccuPAR LP-80, (Decagon, Devices).

At the time of harvesting on different dates, forages were weighed to determine the production of green biomass (kg ha^{-1}). Homogeneous samples of each material were sent to the laboratory for analysis of the structural physical composition of the plant by manual separation of the physical components stem and leaves (leaf blade + sheath), in addition to determining the dry matter content of the plant and its components, using a forced air oven at 55°C, to constant weight. The relationship between the weight of the material harvested in the plot, corrected to the area unit and the dry matter content of the plant, allowed for estimating the dry biomass production (kg ha^{-1}).

The pre-dried samples of the original material were ground in a Wiley mill, with a 1 mm sieve and sequentially the content of crude protein (CP) was determined by the micro Kjeldahl method, and the mineral matter (MM) by incineration at 550ºC (4 hours), as well as neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) contents, following methodologies described by Silva and Queiroz (2009).

The rate of ruminal dry matter disappearance was estimated by the in-situ technique.The ruminal disappearance rate of dry matter was estimated by the in-situ technique using nylon bags measuring 12cm x 8cm and pore sizes of 40 to 60μm, containing 5g each material, ground to 1 mm, for later incubation in the rumen (Nocek, 1988). The incubation times used were 0, 6, 12, 18, 24, 36, and 48 hours, where the time of 0 hours represented the dry matter soluble fraction. For this purpose, two steers, 48 months of age, mean body weight of 750kg, fitted with a ruminal cannula were used. All experimental procedures were previously submitted to the Ethical Animal Experimentation Committee (Ceua/Unicentro) and approved for execution under letter 02/2018 of March 2, 2018.

The experimental design used was a 3 x 2 factorial completely randomized blocks, with three forage species of winter cereals (wheat, white oats, and black oats) and the application or not of fungicide, with four replications for each treatment. The results obtained were tested by analysis of variance and the means values were compared by F-test and Tukey's test at 5% significance using the SAS statistical software (1993). Data referring to the rate of ruminal dry matter disappearance were subjected to regression analysis (proc reg) of SAS.

RESULTS

The analysis of variance evidenced no significant interaction between forage species and fungicide application for the productive and chemical parameters, both for the first cut and for the second cut, respectively.

The application of pyraclostrobin, regardless of the evaluated forage species, caused no changes (P>0.05) in the dry biomass production, both in the first cut (960 against 902kg ha^{-1}) and in the second cut (2,160 against 2,092kg ha^{-1}), nor on the mean cumulative values (3,120 against 2,994kg ha^{-1}) compared to the control treatment, respectively (Table 1).

Table 1. Dry biomass production and mean forage dry matter contents of different winter cereals, harvested at the vegetative and full vegetative stages, with or without treatment with pyraclostrobin

Pyraclostrobin	Forage	Harvest		Cumulative biomass
		Vegetative	Full vegetative	
		Dry biomass, kg ha^{-1}		
With	Wheat	489	1,794	2,283
	White oats	798	2,588	3,387
	Black oats	1,592	2,098	3,690
	Mean	960 A	2,160 A	3,120 A
Without	Wheat	526	1,583	2,109
	White oats	851	2,315	3,166
	Black oats	1,330	2,377	3,707
	Mean	902 A	2,092 A	2,994 A
	Wheat	507 c	1,689 b	2,196 b
	White oats	825 b	2,452 a	3,277 ab
	Black oats	1,461 a	2,237 ab	3,698 a
		Forage dry mass, %		
With	Wheat	16.58	17.33	
	White oats	14.18	18.25	
	Black oats	11.42	11.79	
	Mean	14.06 B	15.79 A	
Without	Wheat	17.58	18.38	
	White oats	14.91	14.64	
	Black oats	12.75	12.75	
	Mean	15.06 A	15.26 A	
	Wheat	17.08 a	17.85 a	
	White oats	14.54 b	16.44 b	
	Black oats	12.05 c	12.27 c	

ha = hectare.
Means, followed by different uppercase letters, in the same column, in the comparison between treatments with and without fungicide, are significantly different by F-test at 5%.
Means, followed by different lowercase letters, in the same column, in the comparison between forage species, are significantly different by Tukey's test at 5%.

In the overall mean, considering only the effect of the evaluated forage species, regardless of the application or not of pyraclostrobin, black oats reached the highest (P<0.05) production of dry biomass with 1,461kg ha^{-1} compared to white oats (825kg ha^{-1}) and wheat (507kg ha^{-1}), at the vegetative stage. At the full vegetative stage, the dry biomass production of black oats (2,237kg ha^{-1}) was similar to white oats (2,452kg ha^{-1}), both, however, higher (P<0.05) than the production of wheat (1,689kg ha^{-1}). Therefore, the cumulative dry biomass production in the two harvests showed the superiority (P<0.05) of black oats (3,698kg ha^{-1}) and white oats (3,277kg ha^{-1}) compared to wheat (2,196kg ha^{-1}) (Tab. 1).

Regarding the mean forage dry matter (Table 1), the forage harvested at the vegetative stage, from the treatment with pyraclostrobin, presented a lower (P<0.05) value (14.1% against 15.1%) compared to the control treatment. At the full vegetative stage, forage dry matter contents were similar (P>0.05) with or without treatment with pyraclostrobin fungicide, with a mean value of 15.6%.

Also in Tab. 1, in the general average, in the comparison between the forage species, the dry matter contents of the forage, both at vegetative and full vegetative stages, were higher (P<0.05) in wheat (17.1% and 17.9 %, respectively) and lower (P<0.05) in black oats (12.1% and 12.3%, respectively), compared to intermediate values found in white oats (14.5% and 16.4%, respectively).

The use of pyraclostrobin, regardless of the evaluated forage species, did not change (P<0.05) the morphological composition of the plant at the vegetative stage. The opposite behavior was found at the full vegetative stage, where a difference (P<0.05) was observed in the morphology of the plant, between stem and leaves in terms of treatment with fungicide. In general, pyraclostrobin application resulted in forage with greater participation of leaves (86.9% against 72.2%) and lower participation of stems (13.1% against 27.8%) compared to the control treatment (Tab. 2).

The evaluation of forage species in Tab. 2 shows the morphological composition of the plant at the vegetative stage, where only black oats presented a mean value of 4.5% stem and 95.5% leaves (P<0.05), while wheat and white oats presented a structure with 100% leaves. In the second harvest, at the full vegetative stage, white oats and black oats had a higher proportion of stem (26.3% and 22.3%, respectively) and a lower proportion of leaves (72.7% and 77.7%, respectively), differing (P<0.05) from wheat, which had 12.8% stem and 87.2% leaves.

As for the stem at the vegetative stage, dry matter content (Table 2) did not differ (P>0.05%) regarding the effect of the treatment with pyraclostrobin, with a mean value of 7% dry matter for black oats since the other species did not present a stem at the vegetative stage. For the leaves, the fungicide application maintained lower dry matter contents (14.4% against 15.5%; P<0.05) compared to the control treatment.

At the full vegetative stage, the dry matter content of the stem differed (P<0.05%) regarding the effect of the treatment with pyraclostrobin. The application of the fungicide resulted in lower dry matter contents (10.5% against 12.1%) compared to the control treatment; while the dry matter contents of the leaves remained stable (P>0.05) compared to the control treatment (Table 2).

As for the dry matter content of the stem, at the vegetative stage, which was present only in black oats, presented a mean value of 7.0%, regardless of the treatment with pyraclostrobin. At the full vegetative stage, wheat presented the highest (P<0.05) values of stem dry matter with 13.1% compared to white oats (9.9%) and black oats (10.9%) (Tab. 2).

For the dry matter content of leaves, regardless of the application of pyraclostrobin, at the vegetative stage, wheat presented the highest (P<0.05) value with 16.7%, followed by white oats and black oats, with 14.9% % and 13.2% dry matter, respectively. Similar behavior in leaf dry matter content was observed at the full vegetative stage, where white oats (16.2%) and black oats (15.6%) did not differ (P>0.05) from each other, but with lower values (P<0.05) compared to wheat (23.4%) (Table 2).

Table 2. Participation of stem and leaves, on a dry matter basis, and dry matter content of stem and leaves in the forage of different winter cereals, harvested at the vegetative and full vegetative stages, with or without treatment with pyraclostrobin

Pyraclostrobin	Forage	Harvests			
		Vegetative		Full vegetative	
		Composition (%)	DM (%)	Composition (%)	DM (%)
		Stem			
With	Wheat	0	-	7.1	12.48
	White oats	0	-	11.4	8.66
	Black oats	3.4	6.36	20.8	10.43
	Mean	1.1 A	6.36 A	13.1 B	10.52 B
Without	Wheat	0	-	18.5	13.76
	White oats	0	-	41.1	11.11
	Black oats	5.6	7.64	23.8	11.40
	Mean	1.87 A	7.64 A	27.8 A	12.09 A
	Wheat	0.0 b	-	12.8 b	13.12 a
	White oats	0.0 b	-	26.3 a	9.88 b
	Black oats	4.5 a	7.00	22.3 a	10.91 b
		Leaves			
With	Wheat	100.0	16.33	92.9	25.10
	White oats	100.0	14.30	88.6	15.79
	Black oats	96.6	12.64	79.2	16.31
	Mean	98.9 A	14.42 B	86.9 A	19.07 A
Without	Wheat	100.0	17.09	81.5	21.80
	White oats	100.0	15.47	58.9	16.55
	Black oats	94.4	13.86	76.2	14.89
	Mean	98.1 A	15.47 A	72.2 B	17.75 A
	Wheat	100.0 a	16.71 a	87.2 a	23.45 a
	White oats	100.0 a	14.88 b	73.7 b	16.17 b
	Black oats	95.5 b	13.25 c	77.7 b	15.60 b

DM = dry matter.
Means, followed by different uppercase letters, in the same column, in the comparison between treatments with and without fungicide, are significantly different by F-test at 5%.
Means, followed by different lowercase letters, in the same column, in the comparison between forage species, are significantly different by Tukey's test at 5%.

The application of pyraclostrobin, regardless of the evaluated forage species, resulted in significant changes ($P<0.05$) in the chemical composition of the forage, both at the vegetative stage and at the full vegetative stage, where on average the crude protein contents were higher ($P <0.05$) in the fungicide treatment (25.60% and 22.28%, respectively) compared to the control treatment (22.75% and 20.82%, respectively), while the mineral matter contents remained stable ($P>0.05$) (Table 3).

Table 3. Mean contents of crude protein and mineral matter in the forage of different winter cereals, harvested at the vegetative and full vegetative stages, with or without treatment with pyraclostrobin

Pyraclostrobin	Forage	Harvests	
		Vegetative	Full vegetative
		Crude protein, %	
With	Wheat	28.30	26.49
	White oats	26.23	19.86
	Black oats	22.25	20.48
	Mean	25.60 A	22.28 A
Without	Wheat	27.50	22.15
	White oats	17.65	16.64
	Black oats	23.11	20.27
	Mean	22.75 B	20.82 B
	Wheat	27.91 a	24.31 a
	White oats	21.95 b	18.25 b
	Black oats	22.68 b	20.35 b
		Mineral matter, % DM	
With	Wheat	7.40	7.33
	White oats	9.05	7.01
	Black oats	8.91	8.66
	Mean	8.45 A	7.67 A
Without	Wheat	7.00	6.36
	White oats	8.39	7.27
	Black oats	9.25	8.41
	Mean	8.21 A	8.10 A
	Wheat	7.22 b	6.86 b
	White oats	8.72 a	7.14 b
	Black oats	9.07 a	8.50 a

DM = dry matter.
Means, followed by different uppercase letters, in the same column, in the comparison between treatments with and without fungicide, are significantly different by F-test at 5%.
Means, followed by different lowercase letters, in the same column, in the comparison between forage species, are significantly different by Tukey's test at 5%.

In the general average, in the comparison between forage species, in Tab. 3, the forage crude protein contents, both at vegetative and full vegetative stages, were higher ($P<0.05$) in wheat (27.91% and 24.31%, respectively) and lower ($P<0.05$) in black oats (22.68% and 20.35%, respectively) and white oats (21.95% and 18.25%, respectively), regardless of the use of pyraclostrobin. Mineral matter contents, both at vegetative and full vegetative stages, were higher ($P<0.05$) in black oats (9.07% and 8.50%, respectively) and lower ($P<0.05$) in white oats (8.72% and 7.14%, respectively) and wheat (7.22% and 6.86%, respectively).

In Tab. 4, the forage neutral detergent fiber contents, regarding the use of pyraclostrobin fungicide, regardless of the evaluated forage species, were similar ($P>0.05$) both at the vegetative and full vegetative stages.

In the evaluation of the forage species, at the vegetative stage, black oats presented the highest values of neutral detergent fiber (54.72%) differing ($P<0.05$) from white oats with the lowest value (48.51%), and intermediate value in wheat (51.52%). At the full vegetative stage, there were no differences ($P>0.05$) for neutral detergent fiber contents between the different species (Table 4).

Table 4. Mean contents of neutral detergent fiber and acid detergent fiber in the forage of different winter cereals, harvested at the vegetative and full vegetative stages, with or without treatment with pyraclostrobin

Pyraclostrobin	Forage	Harvests	
		Vegetative	Full vegetative
		Neutral detergent fiber, % DM	
With	Wheat	53.53	59.07
	White oats	50.50	54.62
	Black oats	54.24	57.55
	Mean	52.75 A	57.08 A
Without	Wheat	49.53	58.33
	White oats	46.55	57.97
	Black oats	55.17	57.68
	Mean	50.41 A	55.97 A
	Wheat	51.52 b	58.70 a
	White oats	48.51 c	56.30 a
	Black oats	54.72 a	57.61 a
		Acid detergent fiber, % DM	
With	Wheat	24.87	30.88
	White oats	24.03	26.81
	Black oats	25.85	31.02
	Mean	24.92 A	29.57 A
Without	Wheat	25.04	31.09
	White oats	22.86	29.04
	Black oats	25.06	31.88
	Mean	24.32 A	28.65 A
	Wheat	24.96 a	30.97 a
	White oats	23.44 a	27.90 b
	Black oats	25.43 a	31.44 a

DM = dry matter.
Means, followed by different uppercase letters, in the same column, in the comparison between treatments with and without fungicide, are significantly different by F-test at 5%.
Means, followed by different lowercase letters, in the same column, in the comparison between forage species, are significantly different by Tukey's test at 5%.

The content of acid detergent fiber and the nutritional value of the forage, regarding the use of the fungicide pyraclostrobin, regardless of the evaluated forage species, did not change significantly (P>0.05), both at the vegetative and full vegetative stages (Table 4). Also, regardless of the application of pyraclostrobin, at the vegetative stage, there was no difference (P>0.05) for acid detergent fiber contents in the comparison of different forage species; in turn, at the full vegetative stage, white oats with 27.90% acid detergent fiber stood out (P<0.05) compared to the values found in black oats (31.44%) and wheat (31.97%).

In Tab. 4, the use of the pyraclostrobin fungicide, regardless of the evaluated forage species, promoted significant changes (P<0.05) in the content of lignin in the fiber fraction of the forage, both at the vegetative and full vegetative stages, where the mean and lignin contents were lower (6.30% and 9.20% against 9.89% and 11.73%, respectively) in the fungicide treatment compared to the control treatment.

Comparing the different forage species, regardless of the application of pyraclostrobin, at the vegetative stage, higher content (P<0.05) of lignin was observed in white oats (9.39%) compared to black oats (7.80%) and wheat (7.11%), with similar values. At the full vegetative stage, the lignin content was higher in wheat (14.34%) compared to white oats (10.00%) and black oats (10.50%).
In Tab. 5, at the vegetative stage, regardless of the evaluated species, both with 24 hours and 48 hours of incubation, there were no differences (P>0.05) for in situ dry matter digestibility,

between treatments with and without pyraclostrobin, showing a mean value of 82.6% degradation in 24 hours and 87.0% in 48 hours of rumen incubation.

Table 5. Mean lignin content and relative forage value of different winter cereals, harvested at the vegetative and full vegetative stages, with or without treatment with pyraclostrobin

Pyraclostrobin	Forage	Harvests	
		Vegetative	Full vegetative
		Lignin, % DM	
With	Wheat	6.78	13.58
	White oats	6.97	7.06
	Black oats	5.16	6.97
	Mean	6.30 B	9.20 B
Without	Wheat	7.47	15.09
	White oats	11.81	12.93
	Black oats	10.39	14.02
	Mean	9.89 A	11.73 A
	Wheat	7.11 b	14.34 a
	White oats	9.39 a	10.00 b
	Black oats	7.80 b	10.50 b

DM = dry matter.
Means, followed by different uppercase letters, in the same column, in the comparison between treatments with and without fungicide, are significantly different by F-test at 5%.
Means, followed by different lowercase letters, in the same column, in the comparison between forage species, are significantly different by Tukey's test at 5%.

In the evaluation of the winter cereal species, regardless of the application of pyraclostrobin, with 24 hours of ruminal incubation, there were no differences (P>0.05) for in situ dry matter digestibility. For 48-hour incubation, wheat showed the highest digestibility, compared to black oats and white oats, with values of 88.7% for wheat; 86.4% for white oats, and 85.7% for black oats.

For the full vegetative stage, the application of pyraclostrobin had an effect (P<0.05), increasing the dry matter digestibility of the material, both in 24 and 48 hours of rumen incubation, with values of 80.6% against 78.9 %, at 24 hours of incubation, and 86.4% against 83.2% at 48 hours of ruminal incubation, for treatments with pyraclostrobin and control, respectively (Table 5).

In the evaluation of species, regardless of the application of pyraclostrobin, at the full vegetative stage, there were no differences (P>0.05) in dry matter digestibility, with a mean value of 79.8% with 24 hours of rumen incubation and 84.3 % with 48 hours of incubation (Table 5).

Fig. 2 shows the in situ ruminal dry matter disappearance rate curves in the first cut, in which the control treatment had a higher intercept point compared to that with pyraclostrobin, for all evaluated species, with intercept mean values of 43.54% for the control treatment and 40.30% for the treatment with pyraclostrobin, which may represent the readily soluble nutrients, however, the application of pyraclostrobin increased the rate of ruminal disappearance of DM per hour of ruminal incubation, for all species, with a mean value of 1.13% of the control treatment against 1.19% of the treatment with pyraclostrobin, resulting in a similar degradability at the end of 48 hours between the two treatments.

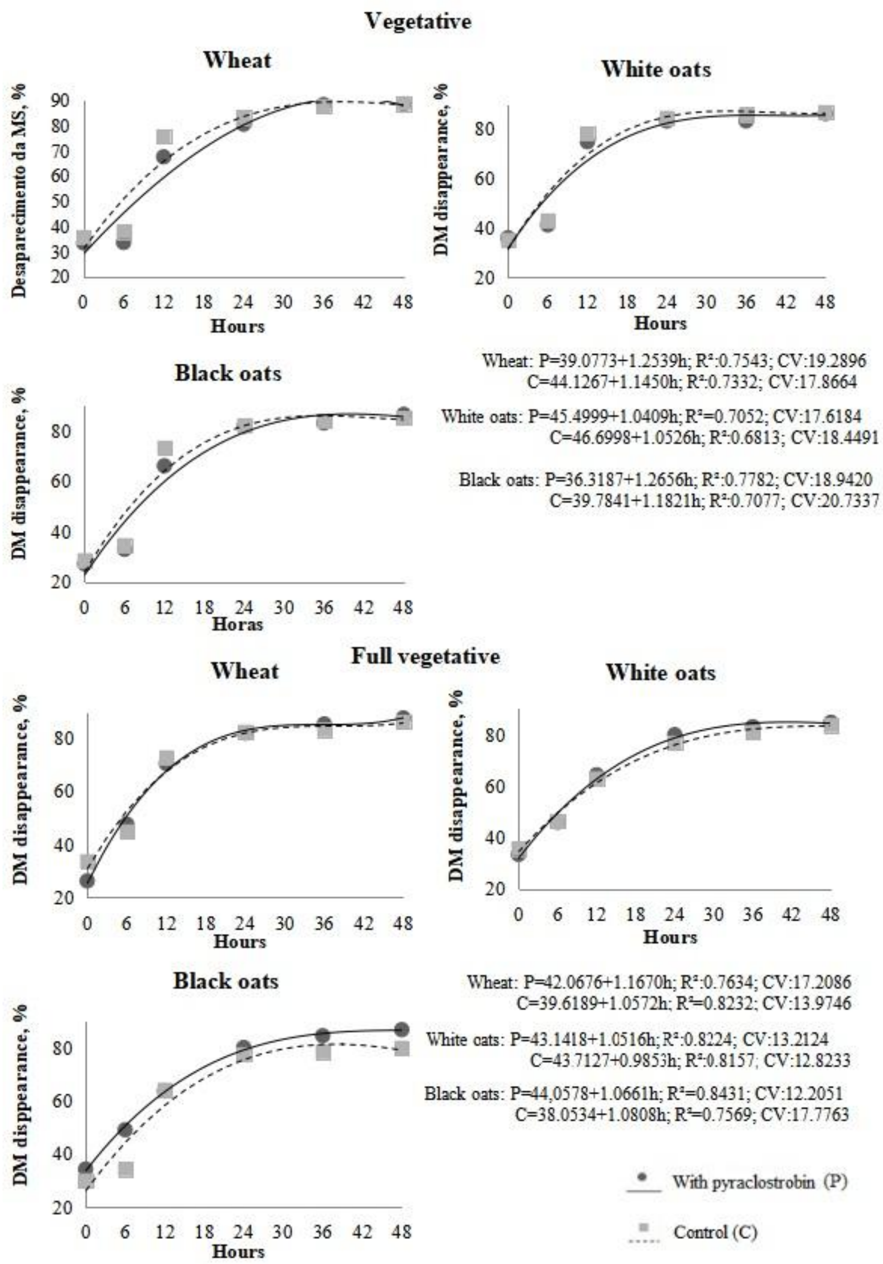


Figure 2. Ruminal disappearance rate (%DM) of forage at vegetative and full vegetative stages of wheat, white oats, and black oats, in successive harvests, applied with pyraclostrobin.

At the full vegetative stage, the treatment with pyraclostrobin reached higher DM digestibility than the control treatment, from the intercept with a mean value between species of 43.08% for the treatment with pyraclostrobin against 40.46% for the control treatment, and during incubation, with a rate of DM disappearance per hour of rumen incubation of 1.09% against 1.04% of the control treatment, resulting in the treatment with pyraclostrobin a greater digestibility at all incubation times (Fig. 2).

DISCUSSION

The application of pyraclostrobin in the wheat crop increased grain production by 3% and 6% in one and two applications of the fungicide (Trojan, 2009). In the soybean crop, an increase of 7% in grain mass was possible, and an increase in productivity of 1,080 kg compared to the control group, where such an increase was justified by the increase in the photosynthetic rate, decrease in respiration and increased activity of the nitrate reductase enzyme (Fagan *et al*., 2010). The same authors showed that for forage production the application of pyraclostrobin did not cause the same effect.

A study carried out by Leão *et al*. (2016), for an average of two harvests of winter cereal, found a forage productivity of 1,437kg ha^{-1} dry matter. On the other hand, Meinerz *et al.* (2012) obtained a mean production of 2,200kg ha^{-1} per harvest, evaluating several genotypes of winter cereals.

In the second harvest, the percentage of stem increased compared to that of leaves, corroborating Meinerz *et al*. (2012), who state that the leaf: stem ratio tends to decrease with the increase and/or advancement of harvests. With the application of pyraclostrobin, the increase in the proportion of leaves corroborates the data found by Silva *et al*. (2022), which showed an increase in leaf area, when pyraclostrobin was applied to rye crops. Fungicide application increased the number of viable leaves (Kalebich *et al*., 2017), thus improving the leaf: stem ratio.

It is probable that the low proportion of stems in the first harvest did not allow the identification of differences in the dry matter of the stems in the first harvest. Nevertheless, the high concentration of leaves that indicated differences regarding the application or not of pyraclostrobin influenced the differences in the dry matter of the entire plant.

Dry matter values were lower in the treatment with pyraclostrobin, probably because it has characteristics that increase water retention within the plant due to reduced stomatal opening (Grossmann *et al*., 1999). However, Heker *et al*. (2020) and Silva *et al*. (2022) report that the application of pyraclostrobin did not change the dry matter values of plants harvested at the vegetative stage.

Dry matter content in the first harvest was found to be 15.7%; 17.1% and 16.7% dry matter for the stem, leaves, and whole plant, respectively, according to Carletto *et al*. (2017), values that were close to those found herein, except for the stem. The dry matter content of the leaves in the second harvest remained close to the values found by the aforementioned authors, who worked with wheat in successive cutting systems.

On the other hand, in the present study, the dry matter contents of the stem and whole plant showed lower values, when considering the mean value of the forages, however, when considering the values of wheat, these are similar to those found by the aforementioned authors.

Importantly, pyraclostrobin acts on plant metabolism, activating the nitrate reductase enzyme, generating greater assimilation of nitrogen, and justifying the increase in crude protein (Venancio *et al*., 2004). The activity of the nitrate reductase enzyme increases after the application of pyraclostrobin, by 87% and 72% when evaluated one and three days after application, respectively, in the soybean crop (Fagan *et al*., 2010).

Increased crude protein values were also found in rye silage treated with pyraclostrobin (Silva *et al*., 2022) and in wheat and black oat silage (Venancio *et al*., 2004).

Pyraclostrobin inhibits the transport of electrons in the mitochondria, which leads to a decrease in the cytosolic pH and an increase in the activity of the nitrate reductase enzyme (Barbosa *et al*., 2011), resulting in greater assimilation of nitrogen, consequently increasing the crude protein content in the plant.

In turn, Fontaneli *et al*. (2009) found differences in crude protein when evaluating different winter forages at the vegetative stage, where black oats had the highest values, followed by wheat and white oats, with mean values of 24.5%; 23.5%, and 22.5%, respectively, showing that differences in crude protein content between different species at the vegetative stage.

The use of strobilurin-based fungicides leads to an increase in neutral detergent fiber (Mendes *et al*., 2015), probably due to the greater plant height, which demands a greater amount of fiber for support. This increase in the content of neutral detergent fiber and acid detergent fiber was not observed in the present study, probably a response to the harvest performed for all treatments with similar management.

The differences between species for neutral detergent fiber content were verified, but this may be due to the characteristics of each species. Black oats had a higher neutral detergent fiber content in the first harvest, which has already been found in other experiments comparing winter cereal species (Horst *et al*., 2017; Leão *et al*., 2017).

The highest content of fiber in neutral detergent is not interesting, as this limits intake, usually by ruminal filling. This also has lower ruminal degradability; however, it depends on its composition since the relationship between hemicellulose, cellulose, and lignin present different values of degradability (Van Soest *et al*., 1991).

In an evaluation of different species Meinerz *et al.* (2011) found differences in acid detergent fiber, with white oats showing acid detergent fiber values in the first harvest of 24.1% and 23.5%; wheat with 21.3% and 30.1%; and black oats with 21.1% and 26.2%, respectively for the first and second harvests.

Lignin is basically indigestible by ruminants, so a higher value leads to a lower use of dry matter. Plants at a more advanced stage or that undergo stress tend to contain higher lignin contents. With the use of pyraclostrobin, lignin was reduced because the plant was possibly more "well-nourished" and/or without stress as pyraclostrobin acts by preventing the synthesis of ethylene, which is produced by the plant under stress conditions (Venancio *et al*., 2004).

Lignin concentration also changes according to the advance of the cycle and between crops, which was evidenced by Moreira *et al.* (2007), where they found differences when evaluating black oats and triticale, in two cuts (grazing), where the first cut presented a mean value of forages of 3.76%, and the second cut, 5.05%, and between the species with the black coat with a mean value between cuts of 4.03% and triticale with a mean value between cuts of 4.78% lignin.

Winter cereal forages have some variations in degradability kinetics. Horst *et al.* (2017) evaluated winter forages at the pre-flowering stage and reported different intercept values, which can be referred to as readily soluble nutrients, 28.63% for white oats; 26.06% for wheat, and 25.77% for black oats, and a ruminal disappearance rate per hour ranging from 0.66% for wheat to 0.82% for black oats, and white oats with intermediate values, however resulting in similar ruminal DM digestibility at the end of 48 hours between the evaluated forages.

Analyzing the treatment with pyraclostrobin on the ruminal disappearance rate of rye, at the pre-flowering stage, Heker *et al.* (2020) observed an increase in values of soluble nutrients, from 26.8% to 28.7%, and maintenance of this difference at approximately 2 percentage points during the evaluation period of 0-48 hours of ruminal incubation.

CONCLUSION

The application of the pyraclostrobin fungicide in winter cereal forage proved to be efficient to generate positive changes in the morphology and chemical characteristics of the plant managed for forage production in successive cuts. White oats expressed a greater balance between productivity and chemical characteristics for quality forage production compared to wheat and black oats.

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