Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2025v46n6p1807

Effect of pyraclostrobin on disease control and ruminal degradation of winter cereal forage harvested at different developmental stages

Efeito da piraclostrobina sobre o controle de doenças e degradação ruminal da forragem de cereais de inverno colhidos em diferentes fases de desenvolvimento

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Highlights

Pyraclostrobin improved ruminal DM degradation at stage 11.2.
The use of pyraclostrobin improved plant health at stage 5.
Pyraclostrobin concentrated the total nitrogen content at stage 5.

Abstract

The objective was to evaluate the incidence of diseases, ruminal degradation of dry matter and neutral detergent fiber, and possible alterations in the chlorophyll and nitrogen content of the forage of three winter crops: wheat (*Triticum aestivum* L.; cultivar BRS Umbu), white oat (*Avena sativa* L.; cultivar URS Guará), and black oat (*Avena strigosa* Schreb.; cultivar Embrapa 139), associated with the application or not of the fungicide pyraclostrobin, with the forage harvested at different developmental stages. Pyraclostrobin was sprayed at a rate of 0.6 L ha^{-1} (250 g L^{-1}) in two applications during stage 5, the first

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Received: May 07, 2025 Approved: Nov. 24, 2025

application at the V5 phenological stage (elongation), 54 days after sowing (DAS), and the second at 66 DAS. Evaluations were carried out at three successive and distinct stages during crop development, by manually harvesting the plants 8 cm above the ground, according to each treatment, at stages 5 and 7, following the Feekes scale (Large, 1954) for hay production, as well as sequentially at stage 11.2 for silage production. Disease control evaluations were carried out at 59, 81, and 127 DAS, showing that pyraclostrobin increased the percentage of symptom-free plants from 41.1% to 56.7% at stage 5 and from 0.3% to 46.7% at stage 7 compared to the control treatment, regardless of the forage species. The application of pyraclostrobin, regardless of the forage species, improved ruminal degradation at stage 11.2, from 58.1% to 68.4% compared to the control treatment; decreased chlorophyll a content from 37.7% to 36.4% during the vegetative phase and from 38.3% to 37.0% during the full vegetative phase, while increasing total nitrogen content from 3.64% to 4.10% at the vegetative phase and from 3.33% to 3.56% at the full vegetative phase, respectively. Using pyraclostrobin in winter cereals for forage production is recommended because it efficiently controls diseases, increases rumen degradability of neutral detergent fiber and dry matter, and promotes positive changes in plant nitrogen content.
Key words: Fungicide. Helminthosporiosis. Powdery mildew. Rust. Strobilurin.

Resumo

Objetivou-se avaliar a incidência de doenças, a degradação ruminal da matéria seca e da fibra em detergente neutro e possíveis alterações nas concentrações de clorofila e de nitrogênio na forragem de três culturas de inverno: trigo (*Triticum aestivum* L.; cultivar BRS Umbu), aveia branca (*Avena sativa* L.; cultivar URS Guará) e aveia preta (*Avena strigosa* Schreb.; cultivar Embrapa 139) associadas a aplicação ou não do fungicida piraclostrobina, colhida a forragem em diferentes fases de desenvolvimento. A pulverização do fungicida piraclostrobina foi realizada na dose de 0,6 L ha^{-1} (250 g/L^{-1}) em dois momentos durante o estádio 5, sendo a primeira aplicação no estádio fenológico V5 (alongamento) aos 54 dias após a semeadura (DAS), e a segunda aos 66 DAS. As avaliações foram realizadas em três momentos sucessivos e distintos ao desenvolvimento das culturas, por meio do corte das plantas a 8 cm de altura do solo, de forma manual de acordo com cada tratamento, no estádio 5 e estádio 7, seguindo a escala Feekes (Large, 1954), visando produção de feno, assim como sequencialmente em estádio 11.2, visando produção de silagem. As avaliações de controle de doenças se deram aos 59 DAS, 81 DAS e 127 DAS, mostrando que a piraclostrobina aumentou a porcentagem de plantas sem sintomas, de 41,1% para 56,7% no estádio 5 e de 0,3% para 46,7% no estádio 7 frente ao tratamento controle, independentemente da espécie forrageira. A aplicação de piraclostrobina, independente da espécie forrageira testada, melhorou a degradação ruminal, em fase de estádio 11.2, de 58,1 para 68,4% em relação ao tratamento controle; diminuiu as concentrações de clorofila a de 37,7% para 36,4% na fase vegetativa e de 38,3% para 37,0% na fase plena vegetativa, assim como aumentou as concentrações de nitrogênio total de 3,64% para 4,10% na fase vegetativa e de 3,33% para 3,56% na fase plena vegetativa, respectivamente. Recomenda-se o uso de piraclostrobina em cereais de inverno para produção de forragem por ser eficiente no controle de doenças e no incremento da degradabilidade ruminal da fibra em detergente neutro e da matéria seca, e ainda promover alterações positivas nas concentrações de nitrogênio na planta.
Palavras-chave: Estrobilurina. Ferrugem. Fungicida. Helmintosporiose. Oídio.

Introduction

The cultivation of winter cereals for grain or forage production faces the main challenge of fungal disease occurrence. Considering that southern Brazil has humid winters and cloudy days, these conditions favor such diseases (Pott et al., 2007). Thus, the chemical control of such diseases by applying fungicides to plant shoots is one of the most important management measures for these crops (Reis et al., 2001).

Fungicides from the strobilurin chemical group interfere with the physiological and biochemical activities of the crops, causing a *green effect* in the plants. This increases the activity of the nitrate reductase enzyme, which maintains the active photosynthetic area of the plant. Consequently, there is better nitrogen assimilation and increased crude protein content (Fagan et al., 2010; B. J. Venancio et al., 2024).

Several fungicides are available on the market for the prevention and control of diseases, including strobilurins, which promote positive results for disease control and minimize productivity losses. Strobilurins inhibit quinone oxidase by acting on complex III of the mitochondrial electron transport chain and preventing ATP production. This active ingredient demonstrates effective preventive results and should be applied before or shortly after infection (Schoffel et al., 2023).

When plants are attacked by pathogens, they undergo stress conditions that increase the production of defense compounds, including lignin (B. J. Venancio et al., 2024). This increases the concentration of fiber in the plant. Ruminants cannot digest lignin; therefore, the lower the fungal load, the lower the production of defense compounds and the greater the digestibility of the material (Van Soest et al., 1991).

The application of fungicides to plant shoots can change leaf pigmentation and leave them free of pathogens (Martinazzo et al., 2016). In addition, the strobilurin chemical group can increase the activity of the nitrate reductase enzyme, making more nitrogen available for plant metabolism and chlorophyll synthesis (W. S. Venancio et al., 2004; Groff et al., 2020).

In this context, this study aimed to evaluate the effectiveness of pyraclostrobin in improving dry matter and plant fiber digestibility, controlling diseases, and altering chlorophyll and nitrogen content in forage from successive harvests of winter cereal plants.

Material and Methods

Study area

The Animal Research Ethics Committee (CEUA/UNICENTRO) reviewed and approved the experiments (Official Letter number 02/2018). After receiving approval, the experiments were conducted at the Animal Production Center (NUPRAN), which is affiliated with the Master’s Program in Veterinary Sciences, in the area of Sustainable Animal Health and Production. NUPRAN is part of the Agricultural and Environmental Sciences sector of the State University of the Midwest (UNICENTRO), which is located in the municipality of Guarapuava, state of Paraná. This area is in the subtropical zone, at the following geographical coordinates: 25°23’02” S, 51°29’43” W, and an altitude of 1,026 m.

The region’s climate, according to the Köppen classification, is Cfb (humid subtropical mesothermal), with mild summers and moderate winters, no defined dry season, and 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 a Typical Brown Latosol (Michalovicz et al., 2018). After crop establishment, the soil presented the following chemical properties (profile from 0 to 20 cm): pH $CaCl_2$ 0.01M: 4.7; P: 1.1 mg dm^{-3}; K^+: 0.2 cmolc dm^{-3}; OM: 2.62 g dm^{-3}; Al^{3+}: 0.0 cmolc dm^{-3}; $H^+ + Al^{3+}$: 5.2 cmolc dm^{-3}; Ca^{2+}: 5.0 cmolc dm^{-3}; Mg^{2+}: 5.0 cmolc dm^{-3} and base saturation (V%): 67.3%.

Figure 1 shows the average rainfall in mm, as well as the daily maximum and minimum temperatures during the experimental period.

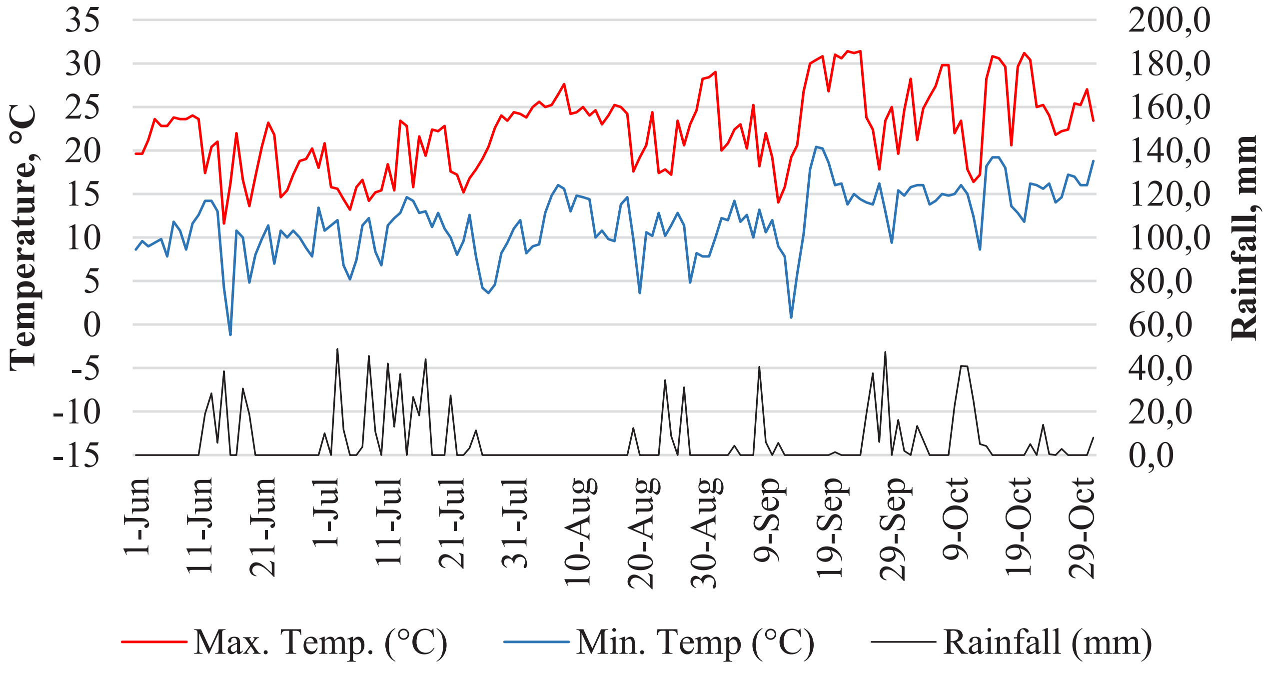

Figure 1. Average rainfall (mm), maximum and minimum temperatures (°C) during the growing season for different winter cereals.
Source: SIMEPAR/UNICENTRO experimental station, Guarapuava, state of Paraná, 2015.

Experimental design

A randomized block design was used in a 3 x 2 factorial arrangement with three winter cereal forage species: wheat (*Triticum aestivum* cv. BRS Umbu), white oat (*Avena sativa* cv. URS Guará), and black oat (*Avena strigosa* cv. Embrapa 139). These species were associated or not associated with the application of pyraclostrobin and were harvested at different developmental stages.

The forage species were sown according to the agricultural zoning for the Guarapuava region, in a no-till system. Sowing was carried out in 5 m^2 plots, using a Semina 1 model seeder, with a row spacing of 0.17 m, an average sowing depth of 0.2 m, and a sowing density of 80 kg ha^{-1}.

Upon sowing, a base fertilization of 250 kg ha^{-1} of 08-20-20 fertilizer (N-P_2O_5-K_2O) was used, respecting the recommendations of the soil fertility commission of Santa Catarina and Rio Grande do Sul (Comissão de Química e Fertilidade do Solo [CQFS RS/SC], 2004). A single application of nitrogen topdressing was carried out 42 days after sowing, with 150 kg ha^{-1} of urea (46-00-00).

Weeds were chemically controlled using a glyphosate-based herbicide (commercial product Roundup WG®: 3.0 kg ha^{-1}) during the desiccation of the experimental area, 15 days before sowing, and during crop management, 30 days after sowing. A metsulfuron-methyl-based herbicide (commercial product Ally®: 6.6 g ha^{-1}) was also applied.

The fungicide pyraclostrobin (trade name Comet®, BASF, 250 g L^{-1} of active ingredient) was sprayed at a rate of 0.6 L ha^{-1} in two applications during stage 5, following the Feekes scale (Large, 1954), 54 days after sowing (DAS), and the second at 66 DAS. Spraying was carried out using a motorized backpack sprayer equipped with a boom containing four double flat jet nozzles, Twinjet TJ 60 110.02, spaced 0.50 m apart. The spray volume was 200 L ha^{-1}, and the spray pressure was 2.0 kgf cm^{-2}.

Evaluation methodology

The plants were harvested to evaluate plant disease incidence, ruminal degradation, total nitrogen (TN), and fiber analysis. They were manually cut 8 cm above the ground at stages 5 and 7, when light interception (LI) averaged 90-95% in the plot. This adjustment followed the recommendations of Fontaneli et al. (2009) for hay production. The plants were also harvested sequentially at stage 11.2 for silage production. LI was estimated using photosynthetically active radiation (PAR) with an AccuPAR LP-80 digital linear ceptometer (Decagon Devices).

Disease incidence was assessed by directly counting 30 plants per plot, one day before each harvest. Incidence of helminthosporium leaf spot (*Drechslera avenae*), rust (*Puccinia coronata* f. sp. avenae), and powdery mildew (*Blumeria graminis* f. sp. avenae) was verified in white and black oats, while incidence of helminthosporium leaf spot (*Drechslera tritici-repentis*), rust (*Puccinia triticina*), and powdery mildew (*Blumeria graminis* f. sp. *tritici*) was verified in wheat. Symptomatic plants were assessed in the three cuts, established by determining the percentage incidence of asymptomatic plants using the following formula: Incidence (%) = (number of infected plants/30)*100. The same formula was used for asymptomatic plants. For chlorophyll analysis, a portable chlorophyll meter, Clorofilog®, was used on the flag leaf of ten plants from each plot. This analysis was carried out on the three forages.

Samples of plant segments obtained from each harvest were sent to the laboratory to determine the dry matter content of the plants. Samples were dried in a forced-air oven at 55 °C until a constant weight was obtained. The pre-dried samples of the original material were ground in a Wiley mill, with a 1 mm sieve, and subsequently the total dry matter (DM) content was determined in an oven at 105 °C for 4 hours. The total nitrogen (TN) content was determined using the micro-Kjeldahl method. The neutral detergent fiber (NDF) content was determined using the methodologies described by D. J. Silva and Queiroz (2009).

Ruminal degradation of dry matter (DM) in the total digestive tract was performed at stage 11.2 and estimated using the in situ technique with 12 cm × 8 cm nylon bags and 40-60 μm pores, containing 5 g of material ground to 1 mm. The bags were incubated in the rumen for 240 hours. Ruminal degradation of NDF in the total digestive tract was measured by determining the NDF content before and after incubation according to Goeser and Combs (2009). Two 72-month-old cattle, each with an average body weight of 650 kg and a rumen cannula, were used for this purpose. This evaluation was performed only on forage samples at stage 11.2.

Statistical analysis

The experimental design was a 3 × 2 factorial randomized block design, with three winter cereal forage species (wheat, white oats, and black oats), either with or without the application of pyraclostrobin, and four repetitions. The data were analyzed using ANOVA, and the means were compared using an F-test and a Tukey test at a 5% significance level using the SAS statistical program (Statistical Analysis System Institute [SAS Institute], 1993).

Results and Discussion

The incidence of all evaluated diseases increased as the crop cycle progressed, in both plots treated with pyraclostrobin and control plots. However, powdery mildew was not observed in plots treated with pyraclostrobin.

There were no differences ($P > 0.05$) in the incidence of helminthosporium leaf spot at stage 5 with or without the application of pyraclostrobin, with a mean value of 42.4% infected plants. At stage 7, the fungicide application decreased ($P<0.05$) the incidence of helminthosporium leaf spot from 90.8% in the control treatment to 51.9% in the pyraclostrobin treatment ($P < 0.05$). At stage 11.2, 100% of the plants were infected in both treatments (Table 1).

These data highlight the need for fungicide use as a control or prevention measure for foliar diseases, as these diseases can compromise the plant's production cycle (Cardoso, 2020; Neumann et al., 2024).

In all evaluation phases, there was a significant difference ($P < 0.05$) in rust incidence between treatments. In stage 5, the incidence was reduced from 16.4% to 0.3% in plants treated with pyraclostrobin. At stage 7, the reduction was from 36.4% to 1.4%. For stage 11.2, control plants showed a 100% incidence, whereas the incidence was 63.1% with the pyraclostrobin treatment (Table 1).

Tormen et al. (2013) evaluated 10 wheat cultivars and found that the application of the fungicide pyraclostrobin + metaconazole resulted in a lower percentage of leaf area infected with rust (7.2% versus 95.4%, respectively) compared to the control treatment. Fochesatto et al. (2020) studied wheat plants and found that treatments with pyraclostrobin resulted in less rust progression compared to the control treatment, ensuring a better plant health profile.

Table 1
Incidence of helminthosporium leaf spot and rust in wheat, white oat, and black oat forage at stages 5, 7, and 11.2, applied with pyraclostrobin

Pyraclostrobin	Forage	Evaluation time		
		Stage 5	Stage 7	Stage 11.2
		Incidence of helminthosporium leaf spot, %		
With	Wheat	16.7	47.5	100.0
	White Oat	59.2	56.7	100.0
	Black Oat	38.3	51.7	100.0
	Mean	38.1 A	51.9 B	100.0 A
Without	Wheat	15.8	91.7	100.0
	White Oat	63.3	89.2	100.0
	Black Oat	60.8	91.7	100.0
	Mean	46.7 A	90.8 A	100.0 A
	Wheat	16.3 b	69.6 a	100.0 a
	White Oat	61.3 a	72.9 a	100.0 a
	Black Oat	49.6 a	71.7 a	100.0 a
		Incidence of rust, %		
With	Wheat	0.8	4.2	100.0
	White Oat	0.0	0.0	50.8
	Black Oat	0.0	0.0	38.3
	Mean	0.3 B	1.4 B	63.1 B
Without	Wheat	14.2	17.5	100.0
	White Oat	33.3	86.7	100.0
	Black Oat	1.7	5.0	100.0
	Mean	16.4 A	36.4 A	100.0 A
	Wheat	7.5 ab	10.8 b	100.0 a
	White Oat	17.7 a	43.3 a	75.4 b
	Black Oat	0.8 b	2.5 b	69.2 b

Means, followed by different uppercase letters, in the same column, when comparing treatments with and without fungicide, differ from each other by the F-test at 5%.
Means, followed by different lowercase letters, in the same column, when comparing forage species, differ from each other by the Tukey test at 5%.

Regardless of pyraclostrobin application, helminthosporium leaf spot was less prevalent (P < 0.05) in wheat at stage 5, with an incidence of disease in 16.3% of plants compared to 49.6% and 61.3% for black and white oats, respectively. Other evaluations revealed no differences between species (P > 0.05) (Table 1). As for rust, white oats were more susceptible at stages 5 and 7, with incidences of 17.7% and 43.3%, respectively. Wheat showed the highest infestation at stage 11.2, with 100% of plants infested (Table 1).

The fungicide application resulted in 100% control of powdery mildew at stages 5 and 7, while at stage 11.2, only 1.1% of the plants were infected compared to 7.8%, 91.4%, and 100% for the control treatment (Table 2). Correa et al. (2013) evaluated the efficacy of pyraclostrobin and epixiconazole against powdery mildew in wheat and found an efficiency of 89.3% control of powdery mildew.

The number of symptom-free plants decreased as the cycle progressed for both treatments. Overall, at stage 5, pyraclostrobin application improved plant health (P < 0.05), increasing the percentage of plants without disease by 38.0%. At stage 7, the difference in plant health was even greater (P < 0.05), with the control treatment showing only 0.3% of plants without disease and the pyraclostrobin treatment showing 46.7%. By stage 11.2, no plants were disease-free for either the control or fungicide treatment (P > 0.05) (Table 2).

Poor plant health is characterized by a reduction in leaf area, which interferes with photosynthesis and, depending on the productive stage, compromises grain filling and the consumption of plant reserve nutrients (R. S. Silva et al., 2020).

The evaluation of powdery mildew among forage species, regardless of pyraclostrobin application, revealed no significant differences (P > 0.05). The average incidence was 3.9% at stage 5, 45.7% at stage 7, and 50.6% at stage 11.2 (Table 2).

Table 2

Incidence of powdery mildew and asymptomatic plants in wheat, white oat, and black oat forage at stages 5, 7, and 11.2, applied with pyraclostrobin

Pyraclostrobin	Forage	Evaluation time		
		Stage 5	Stage 7	Stage 11.2
		Incidence of powdery mildew, %		
With	Wheat	0.0	0.0	3.3
	White Oat	0.0	0.0	0.0
	Black Oat	0.0	0.0	0.0
	Mean	0.0 B	0.0 B	1.1 B
Without	Wheat	0.0	78.3	100.0
	White Oat	8.3	96.7	100.0
	Black Oat	15.0	99.2	100.0
	Mean	7.8 A	91.4 A	100.0 A
	Wheat	0.0 a	39.2 a	51.7 a
	White Oat	4.2 a	48.3 a	50.0 a
	Black Oat	7.5 a	49.6 a	50.0 a
		Incidence of asymptomatic plants, %		
With	Wheat	68.3	48.3	0.0
	White Oat	40.8	43.3	0.0
	Black Oat	60.8	48.3	0.0
	Mean	56.7 A	46.7 A	0.0 A
Without	Wheat	63.3	0.8	0.0
	White Oat	28.3	0.0	0.0
	Black Oat	31.7	0.0	0.0
	Mean	41.1 B	0.3 B	0.0 A
	Wheat	65.8 a	24.6 a	0.0 a
	White Oat	34.6 b	21.7 a	0.0 a
	Black Oat	46.3 b	24.2 a	0.0 a

Means, followed by different uppercase letters, in the same column, when comparing treatments with and without fungicide, differ from each other by the F-test at 5%.
Means, followed by different lowercase letters, in the same column, when comparing forage species, differ from each other by the Tukey test at 5%.

When evaluating symptom-free plants, regardless of pyraclostrobin application, there were differences ($P < 0.05$) between species only at stage 5, where wheat exhibited better health, with 65.8% of plants free of diseases. At stages 7 and 11.2, there were no differences ($P > 0.05$) between species, with a mean of 23.5% and no plants completely without symptoms, respectively (Table 2).

At stage 11.2, pyraclostrobin application improved (P<0.05) dry matter degradability from 58.12% in the control treatment to 68.42% in the pyraclostrobin treatment. Pyraclostrobin application also improved (P < 0.05) neutral detergent fiber degradability, with a mean value of 61.63% compared to 53.81% in the control treatment (Table 3).

Table 3
Ruminal degradation of dry matter and neutral detergent fiber (240 hours of incubation) of wheat, white oat, and black oat forage at stage 11.2 applied with pyraclostrobin

Pyraclostrobin	Forage	Degradação ruminal	
		DMD, %	NDFD, %
With	Wheat	70.5	59.5
	White Oat	74.8	68.8
	Black Oat	60.0	56.6
	Mean	68.4 A	61.6 A
Without	Wheat	69.4	60.8
	White Oat	63.2	58.1
	Black Oat	41.5	42.5
	Mean	58.1 B	53.8 B
	Wheat	69.9 a	60.1 a
	White Oat	69.0 a	63.5 a
	Black Oat	50.9 b	49.5 b

Means, followed by different uppercase letters, in the same column, when comparing treatments with and without fungicide, differ from each other by the F-test at 5%.
Means, followed by different lowercase letters, in the same column, when comparing forage species, differ from each other by the Tukey test at 5%.

Due to the higher incidence of diseases, plants produce more lignin in response to infection (B. J. Venancio et al., 2024). Due to the lower disease burden in plants treated with pyraclostrobin, better degradability was obtained, likely because of the lower lignin content. Lignin is an indigestible plant component for ruminants (Van Soest et al., 1991).

The application of pyraclostrobin has also been shown to increase the activity of nitrate reductase, thereby increasing nitrogen availability to the plant (Groff et al., 2020; B. J. Venancio et al., 2024). Since nitrogen is a component of chlorophyll, an increase in chlorophyll levels was expected. However, this did not occur as the fungicide application decreased the concentration of chlorophyll a by 1% in both stage 5 and stage 7 (P < 0.05). Stage 11.2 showed no differences (P > 0.05) in chlorophyll a content, with a mean value of 33.2% (Table 4).

Chlorophyll b decreased with the application of pyraclostrobin at stage 5, with a reduction of 8.1%, compared to the control treatment. At stages 7 and 11.2, there were no differences in chlorophyll b concentrations, with mean values of 15.9% and 13.6%, respectively (Table 4).

According to data presented by Schumacher et al. (2017), using pyraclostrobin in corn (Zea mays L.) crops at various stages with different application strategies did not affect chlorophyll concentrations.

Table 4
Chlorophyll a and chlorophyll b in wheat, white oat, and black oat forage at stages 5, 7, and 11.2, applied with pyraclostrobin

Pyraclostrobin	Forage	Evaluation time		
		Stage 5	Stage 7	Stage 11.2
		Chlorophyll a, %		
With	Wheat	36.6	36.3	33.7
	White Oat	40.2	39.1	35.2
	Black Oat	32.6	3505	32.6
	Mean	36.4 B	37.0 B	33.8 A
Without	Wheat	35.8	36.8	32.4
	White Oat	40.7	41.6	33.5
	Black Oat	36.6	36.5	32.0
	Mean	37.7 A	38.3 A	32.6 A
	Wheat	36.2 b	36.5 b	33.0 a
	White Oat	40.4 a	40.3 a	34.3 a
	Black Oat	34.6 c	36.0 b	32.2 a
		Chlorophyll b, %		
With	Wheat	12.4	12.6	15.5
	White Oat	20.3	20.9	17.0
	Black Oat	11.8	13.9	11.8
	Mean	14.8 B	15.8 A	13.9 A
Without	Wheat	13.1	13.4	15.1
	White Oat	21.8	21.7	15.3
	Black Oat	13.3	13.0	12.1
	Mean	16.1 A	16.0 A	13.3 A
	Wheat	12.7 b	13.0 b	15.3 ab
	White Oat	21.1 a	21.3 a	16.1 a
	Black Oat	12.5 b	13.5 b	12.0 b

Means, followed by different uppercase letters, in the same column, when comparing treatments with and without fungicide, differ from each other by the F-test at 5%.
Means, followed by different lowercase letters, in the column, when comparing forage species, differ from each other by the Tukey test at 5%.

As for concentration of chlorophyll a, in the comparison of the different forage species, regardless of the application of pyraclostrobin, white oats presented the highest content ($P < 0.05$) of chlorophyll a in stage 5 (40.4%) and stage 7 (40.3%), compared to wheat (36.2% and 36.5%) and black oats (34.6% and 36.0%). In stage 11.2, there was no difference between the forage species ($P > 0.05$) analyzed.

For the evaluation of chlorophyll b, white oats had the highest concentration ($P < 0.05$) at all three evaluation times (21.1%, 21.3% and 16.1%) compared to black oats (12.5%, 13.5% and 12.0%) and wheat (12.7%, 13.0% and 15.3%), which had similar values.

On average, regardless of the forage species evaluated, the application of pyraclostrobin increased ($P < 0.05$) the total nitrogen content in the plant, in stages 5 and 7. During the vegetative phase, the nitrogen content was 3.64% for the control treatment and 4.10% for the treatment with pyraclostrobin. During the full vegetative phase, the nitrogen content was 3.33% for the control treatment and 3.56% for the pyraclostrobin treatment. In stage 11.2, there were no differences ($P > 0.05$) in nitrogen concentrations between the control and pyraclostrobin treatments (Table 5).

Table 5
Mean nitrogen content of different winter cereals at stages 5, 7, and 11.2, applied with pyraclostrobin

Pyraclostrobin	Forage	Evaluation time		
		Stage 5	Stage 7	Stage 11.2
With	Wheat	4.53	4.24	1.50
	White Oat	4.20	3.18	1.44
	Black Oat	3.56	3.28	1.26
	Mean	4.10 A	3.56 A	1.40 A
Without	Wheat	4.40	3.54	1.52
	White Oat	2.82	2.66	1.12
	Black Oat	3.70	3.24	1.42
	Mean	3.64 B	3.33 B	1.35 A
	Wheat	4.47 a	3.89 a	1.51 a
	White Oat	3.51 b	2.92 b	1.28 b
	Black Oat	3.63 b	3.26 b	1.34 b

Means, followed by different uppercase letters, in the same column, when comparing treatments with and without fungicide, differ from each other by the F-test at 5%.
Means, followed by different lowercase letters, in the column, when comparing forage species, differ from each other by the Tukey test at 5%.

Research suggests that the increased nitrogen content in plants treated with pyraclostrobin may be due to the fungicide's ability to promote changes in leaf pigmentation, leading to a larger pathogen-free area (Martinazzo et al., 2016) and consequently the plant's photosynthetic activity. As a result, there is an increase in the activity of the nitrate reductase enzyme by improving nitrogen uptake and assimilation as well as chlorophyll synthesis (W. S. Venancio et al., 2004).

The application of pyraclostrobin increased nitrogen content and decreased chlorophyll in stage 5 and stage 7 crops, showing that the assimilated nitrogen was available to plants in other compounds, such as enzymes and other plant metabolites. Bourne and Danielli (1984) state that chlorophyll is indigestible; therefore, it is notable that there was more nitrogen available for animal nutrition.

In general, wheat showed higher nitrogen content values ($P < 0.05$) at the three evaluation times (4.47%, 3.89%, and 1.51%, respectively), regardless of pyraclostrobin application. White and black oats did not differ from each other at the evaluation times.

Conclusion

The use of pyraclostrobin effectively increased the digestibility of neutral detergent fiber and dry matter, helped control diseases, and promoted positive changes in the nitrogen content of winter cereal plants meant for hay or silage production.

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