

Influence of the fungicide strobilurin on forage rye production under different harvesting systems

Influência do uso de fungicida (estrobilurina) na produção de forragem de centeio sob diferentes sistemas de corte

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Highlights

Fungicide application promotes a reduction in NDF content.

Management with a cut at the vegetative stage increases the rye biomass yield.

Harvest management does not change the nutritional value of the forage.

Abstract

The goal of this study was to evaluate the effects of successive harvests, with or without application of foliar fungicide, on the morphometric characteristics, yield and nutritional value of rye forage harvested at the hard dough grain stage, aiming at silage production. The preventive control of foliar diseases was carried out with the fungicide Orkestra™ SC®, in a single application, at early flowering, for both harvesting systems. The first harvest was conducted at the full vegetative stage and the second, at the hard dough grain stage. The system with two harvests resulted in higher cumulative yield compared with the single-harvest system (40,680 and 9,029 kg ha⁻¹ fresh and dry biomass with two harvests, against 8,816 and 5,375 kg ha⁻¹ fresh and dry biomass). Fungicide application promoted a reduction in neutral detergent fiber content in both systems, with values of 753.9 against 790.6 g kg⁻¹ for the single-harvest system and 734.4 against 773.3 g kg⁻¹ for the two-harvest system, with and without fungicide, respectively. For lignin content, the application of the fungicide reduced values (97.2 against 110.3 g kg⁻¹) only in the two-harvest system. Rye management with a harvest at the vegetative stage increases the cumulative dry biomass yield without negatively affecting the harvest yield at the hard dough grain stage, and without drastic changes in the nutritional value of the plant. Even under adverse conditions, fungicide application positively interferes with plant cell wall components.

Key words: Carboxamide. Winter cereal. Biomass yield. *Secale cereale*. Silage.

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Resumo

O objetivo desse estudo foi avaliar os efeitos de sistemas de cortes sucessivos, com ou sem uso de fungicida foliar, sobre as características morfométricas, o rendimento e o valor nutricional da forragem de centeio colhida em estágio de grão farináceo, visando a confecção de silagem. O controle preventivo de doenças foliares foi feito com o fungicida Orkestra™ SC®, em aplicação única, em estágio inicial de florescimento, para ambos sistemas de corte. A sucessão de cortes teve o primeiro em estágio de pleno vegetativo e o segundo em estágio de grão farináceo. O sistema com dois cortes gerou maior rendimento acumulado em comparação ao sistema de corte único (40.680 e 9.029 kg ha⁻¹ de biomassa fresca e seca com dois cortes, contra 8.816 e 5.375 kg ha⁻¹ de biomassa fresca e seca). O uso de fungicida promoveu redução nos teores de fibra em detergente neutro em ambos os sistemas, com valores de 753,9 contra 790,6 g kg⁻¹ para o sistema de um corte e 734,4 contra 773,3 g kg⁻¹ para o sistema de dois cortes, com e sem fungicida respectivamente. Para teores de lignina, apenas no sistema de dois cortes o uso de fungicida promoveu redução de valores (97,2 contra 110,3 g kg⁻¹). O manejo do centeio com um corte no estágio vegetativo eleva o rendimento de biomassa seca acumulada sem prejudicar o rendimento para colheita em grão farináceo, e tampouco promove mudanças drásticas no valor nutricional da planta. Mesmo em condições adversas, o uso de fungicida interfere positivamente nos componentes da parede celular da planta.

Palavras-chave: Carboxamida. Cereais de inverno. Rendimento de biomassa. *Secale cereale*. Silagem.

Introduction

Ensiling is the most used forage preservation method in the world (Haselmann et al., 2020), since the launch of technologies has made the process less dependent on weather conditions and the advance in research has shown ways to reduce losses and increase production. Thus, hay has been constantly replaced with this type of preservation. The proportion of silages is also increasing over green forages in ruminant diets by promoting greater control of ingested nutrients, especially in total mixed ration (González et al., 2007). However, for feeding strategies to be adequate, silage has to be available throughout the year (Horst et al., 2017).

The accumulation of high forage biomass yields per year requires sequences

of successful summer and winter harvests, in order to maximize the photosynthetic rate of plants in different seasons (Yusoff et al., 2013). Our study focused on the evaluation of a winter forage, rye (*Secale cereale*), mainly because approximately 80% cattle feed worldwide is based on temperate grasses (McEvoy et al., 2011), but studies on this forage are still scarce in the literature. Compared to other winter forages, rye is more tolerant to low temperatures, soil types and fertility levels, has a higher plant height, and can be cultivated from sea level to 4,300 m altitude (Heker et al., 2020a). Despite this, rye is still not widespread, perhaps due to its susceptibility to end-of-cycle foliar diseases, as reported by Heker et al. (2020b). According to these same authors, fungicide application at specific times can increase the biomass yield and the nutritional value of the forage, placing rye at a higher prominence level.

Since their first record in 1997, strobilurin fungicides have shown the ability to extend leaf life span (Pepler et al., 2005), increase forage biomass yield (Haerr et al., 2016) and plant digestibility (Wise & Mueller, 2011). More recently, Kalebich and Cardoso (2017) observed improvements in yield and chemical quality of vegetative parts of the corn plant with the application of pyraclostrobin combined with fluxapyroxad. Therefore, the objective of this study was to evaluate the effects of successive harvests, with or without application of pyraclostrobin + fluxapyroxad, on the morphometric characteristics, yield and nutritional value of rye forage harvested at the hard dough grain stage, aiming at silage production

Material and Methods

The experiment was carried out at the Universidade Estadual do Centro-Oeste, located in the municipality of Guarapuava, state of Paraná, Brazil, located in the subtropical zone of the state at coordinates 25°23'02" S and 51°29'43" W and 1,026 m altitude. The climate of the region according to the Köppen classification is Cfb (mesothermal humid subtropical), with mild summers and moderate winters, with no defined dry season and severe frosts. Figure 1 shows the climate data, in decennial, during the experimental period in 2018.

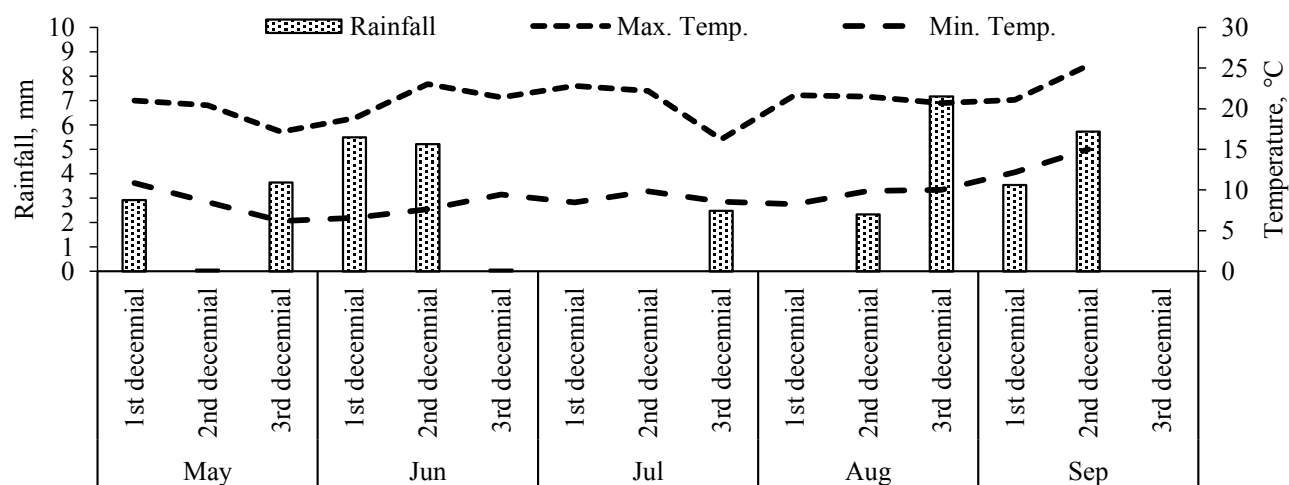


Figure 1. Data on rainfall (mm) and maximum and minimum temperature (°C) during the experimental period.

The soil of the experimental area was classified as Latosol Bruno Típico, with the following chemical properties (0 - 20 cm layer): pH CaCl_2 0.01M: 4.8; P (Mehlich): 1.0 mg dm^{-3} ; K+: 0.2 cmol dm^{-3} ; OM: 2.62%; Al_3+ : 0.0 cmolc dm^{-3} ; H+ and Al_3+ : 5.2 cmolc dm^{-3} ; Ca_2+ : 5.2 cmol dm^{-3} ; Mg_2+ : 5.1 cmolc dm^{-3} and base saturation: 65.8%.

The total experimental area was 540 m^2 , divided into 36 plots of 9.45 m^2 each (3.15 × 3.00 m), which corresponded to 54 linear meters of sowing, with only 24 central linear meters used as a useful area, that is, 4.2 m^2 (2.10 × 2.00 m). No-till sowing was carried out in rows spaced 17.5 cm apart, at a sowing depth of 2.0 cm and average distribution of 220 seeds m^{-2} . Upon planting, basal fertilization was carried out with 280 kg ha^{-1} 08-30-20 (N-P₂O₅-K₂O) fertilizer, respecting the recommendations of the fertilization and liming manual for the state of Paraná (SBCS/NEPAR, 2017). Nitrogen topdressing was performed 51 days after sowing (DAS), in a single application of 444 kg urea ha^{-1} , ensuring the application of 200 kg N ha^{-1} .

The experimental material was rye (*Secale cereale*) cv. Temprano. The design was randomized blocks in a 2 × 2 + 1 factorial arrangement, with two harvests to evaluate the forage at the hard dough grain stage (one and two harvests); two foliar disease control managements (with and without application of fungicide); plus a harvest at the vegetative stage that is part of the two-harvest system. Each treatment contained six replicates (blocks). The preventive control of foliar diseases in treatments with fungicide application was carried out with a product based on Fluxapyroxad + Pyraclostrobin (Orkestra™ SC®: 0.35 L ha^{-1}),

in a single application, at early flowering, for both systems. Each harvesting system had a different time of application due to the delay in the treatment with harvest at the full vegetative stage. The first harvest was conducted at the full vegetative stage and the second, at the hard dough grain stage. Stages related to the first and second harvests are called points 5 and 11.2, respectively, on the scale of Feekes (1940), modified by Large (1954).

The forage was manually harvested using a serrated sickle at 7 cm above the ground, following recommendations described by Fontaneli et al. (2009). The harvested forage weight allowed the estimation of the fresh biomass yield, and with correction for the moisture content, the dry biomass yield was determined. Two homogeneous samples of 500 g plants from each plot were taken upon harvesting and sent to the laboratory, one for separation of the structural components stems, leaves and ears, and the second, for chemical analysis. Samples were pre-dried in a forced-air oven at 55 °C to constant weight (Association of Official Analytical Chemists [AOAC], 1995), and then ground in a Wiley mill with a 1 mm mesh sieve. The pre-dried and ground samples were subjected to analysis of total dry matter in an oven at 105 °C for 4 hours and the mineral matter (MM) obtained by incineration at 550 °C for 4 hours. Crude protein (CP) was determined by the micro-Kjedahl method according to AOAC (1995). Neutral detergent fiber (NDF) content was obtained without α -amylase and sodium sulfite. The acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were sequentially determined according to Goering and Van Soest (1970). Digestible energy (DE) was estimated according to NRC (2001).

Dry matter digestibility at 24 and 48 hours was estimated by the in-situ technique using nylon bags measuring 12 × 8 cm and 40 - 60 µm pores, containing 5 g of each material, ground to 1 mm, for subsequent incubation in the rumen (Nocek, 1988). Before incubation, bags were dipped in water and inserted into the rumen at different times for removal at once. After removal, bags were rinsed in running water under moderate handling until the water ran clear. A blank bag was inserted at each time for correction. For this, two eight-year-old steers, average body weight of 750 kg, with permanent ruminal fistula were used. This procedure was previously submitted for consideration and approval by the Animal Ethics Committee under official letter 035/2017-CEUA/UNICENTRO.

Data were checked for normality and homoscedasticity by Shapiro-Wilk and Bartlett tests, respectively. Once these assumptions were met, data were analyzed using the General linear models procedure (PROC GLM) and tested by analysis of variance (ANOVA) according to the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \alpha_i\beta_j + \varepsilon_{ijk}$$

where:

Y_{ijk} = Response variable related to factor i with factor j in block k ;

μ = Overall mean;

α_i = Effect of factor i ; harvesting system;

β_j = Effect of factor j ; with or without fungicide;

$\alpha_i\beta_j$ = Effect of the interaction between factors i and j

δ_k = Effect of block k ;

ε_{ijk} = Random error associated with each Y_{ijk} observation.

Subsequently, the F-test of multiple comparison of means at 5% of significance was applied. All analyses were run in the SAS software (v. 9.2; SAS Institute Inc., Cary, NC).

Results and Discussion

Table 1 presents the probability values of the analysis of variance of the variables analyzed in this study.

Table 1
Probability values of the analysis of variance (ANOVA)

Source of variation	P-value			
	B	S	F	S×F
Fresh biomass	0.078	0.001	0.770	0.861
Dry biomass	0.068	0.001	0.640	0.946
Plant height	0.768	0.001	0.358	0.453
Stem participation	0.469	0.915	0.101	0.390
Leaf participation	0.884	0.866	0.073	0.296
Ear participation	0.324	0.874	0.130	0.234
Plant DM	0.610	0.001	0.407	0.512
Leaf DM	0.155	0.563	0.582	0.697
Stem DM	0.650	0.449	0.425	0.773
Ear DM	0.034	0.808	0.156	0.280
Mineral matter	0.422	0.001	0.836	0.194
Crude protein	0.712	0.899	0.071	0.497
NDF	0.812	0.123	0.006	0.923
ADF	0.704	0.087	0.059	0.895
Lignin	0.268	0.855	0.745	0.203
DE	0.701	0.268	0.069	0.907
ISDMD-24h	0.970	0.046	0.933	0.066
ISDMD-48h	0.871	0.764	0.911	0.066

B: Block; S: Successive harvest system (1 or 2 harvests); F: Fungicide (with or without); S×F: Interaction S×F.

The cumulative fresh and dry forage biomass yields were not statistically affected by the application of foliar fungicide (Table 2). The two-harvest system resulted in higher cumulative yield compared to the single-harvest system ($P < 0.05$), with mean values of 40,680 and 9,029 kg ha⁻¹ fresh and dry

biomass with two harvests, against 8,816 and 5,375 kg ha⁻¹ fresh and dry biomass obtained in the single-harvest system. Regardless of the application of fungicide, the most expressive biomass yield was obtained at the vegetative stage, leading to superiority the system with harvest at this stage.

Table 2

Yield of fresh and dry forage biomass of rye cv. Temprano, subjected to a successive harvest system, with and without fungicide application

Variable	Harvests		Fungicide		SEM
	1	2	Without	With	
Fresh biomass (kg ha ⁻¹)					262
Full vegetative		31,919	31,919	31,919	
Hard dough grain	8,816	8,761	8,543	9,035	
Cumulative	8,816 b	40,680 a	40,462	40,954	
Dry biomass (kg ha ⁻¹)					304
Full vegetative		4,938	4,938	4,938	
Hard dough grain	5,375	4,091	4,694	4,773	
Cumulative	5,375 b	9,029 a	7,163	7,242	

^{a, b} Mean values followed by different letters, in the same row, between harvesting system or between fungicide application, are significantly different by F-test at 5%. SEM: standard error of the mean.

Plant height was significantly higher at the hard dough grain stage compared with the vegetative stage (Table 3). Between systems, when harvested only at the hard dough grain

stage, rye presented a height of 119 cm, higher ($P < 0.05$) than in the two-harvest system, 107 cm when fungicide was applied and 102 cm when not applied.

Table 3

Yield of fresh and dry forage biomass of rye cv. Temprano, subjected to a successive harvest system, with and without fungicide application

Variable	Full vegetative	Hard dough grain				SEM
		Harvests		Fungicide		
		1	2	Without	With	
Plant height (cm)	29.1	119.1 a	105.0 b	111.6	113.3	0.1
Leaves (% plant DM)	78.33	2.31	2.33	1.73	2.90	0.58
Stem (% plant DM)	21.67	70.18	70.48	73.11	67.45	3.12
Ear (% plant DM)	0.00	27.70	27.26	25.20	29.75	2.71
DM (g kg FM ⁻¹)	155.4	610.7 a	467.0 b	550.7	526.9	0.8
Leaf DM (g kg DM ⁻¹)	168.8	633.9	679.6	635.0	678.5	7.6
Stem DM (g kg DM ⁻¹)	133.0	545.4	514.7	546.2	513.8	3.9
Ear DM (g kg DM ⁻¹)	0.0	603.2	590.8	635.3	558.6	5.0

^{a, b} Mean values followed by different letters, in the same row, between harvesting system or between fungicide application, are significantly different by F-test at 5%; DM: dry matter; FM: fresh matter; SEM: standard error of the mean.

The morphological composition of the plant differed ($P < 0.05$) for all structural components between the vegetative and hard dough grain stages. At the vegetative stage, the plant consisted of 78.33% leaves and 21.67% stems. At the hard dough grain stage, there was no difference between the one- and two-harvest systems, but the application of fungicide maintained the participation of leaves higher in the single-harvest system (3.16 against 1.34%), while the participation of stem was lower (68.66 against 71.53%), with no difference in ear percentage in this system. As for the two-harvest system, there was a greater participation of ear for the fungicide treatment (31.20 against 23.23%), offset by the lower participation of stem (66.19 against 74.69%). There was no difference between harvesting systems for any of the components.

The significant difference observed in the yield of fresh and dry biomass between the single- and two-harvest systems may have been influenced by climatic conditions during the experimental period (Figure 1). The low rainfall may have contributed to the

acceleration of the plant cycle, more evident after flowering (Pepler et al., 2005), and thus, the harvest at the vegetative stage in the two-harvest system, while the plant was healthy, was essential for the greater cumulative biomass yield in this system. In a similar study, Heker (2020c) obtained a similar biomass yield at the vegetative stage, however, the author described an average yield of 8,934 kg ha⁻¹ at the pasty to hard dough grain stage after regrowth of the harvest at the vegetative stage, without any poor environmental condition during the growing season.

According to Heker et al. (2020a) and Meinerz et al. (2011), more than two harvests seem not to be indicated for rye, because its rapid stem elongation and reduction in the proportion of leaves turn its photosynthetic rate less effective (Horst et al., 2018). On the other hand, this pronounced stem height is a major responsible for the biomass yield (Horst et al., 2017). The participation of stem in plants at the hard dough grain stage ranged from 66 to 74%, while the participation of leaves was limited to 3% (Table 4).

Table 4
Chemical composition of rye cv. Temprano subjected to harvesting systems (S) with or without fungicide application (F)

Variable	Full vegetative	Hard dough grain				SEM
		Harvests		Fungicide		
		1	2	Without	With	
Mineral matter (g kg ⁻¹)	67.6	22.8 b	29.8 a	26.4	26.2	0.1
Crude protein (g kg ⁻¹)	214.2	76.2	79.5	71.5	84.3	0.3
NDF (g kg ⁻¹)	446.6	772.3	753.9	782.0 a	744.2 b	1.1
ADF (g kg ⁻¹)	236.9	478.0	453.7	479.5 a	452.1 b	1.0
Lignin (g kg ⁻¹)	36.7	105.2	103.8	105.8	103.2	0.7
DE (MJ kg DM ⁻¹)	13.15	10.0	10.4	10.0 a	10.4 b	0.02

^{a,b} Mean values followed by different letters, in the same row, between harvesting system or between fungicide application, are significantly different by F-test at 5%; NDF: neutral detergent fiber; ADF: acid detergent fiber; DE: digestible energy; SEM: standard error of the mean.

The DM content of the plant and its components differed ($P < 0.05$) between the vegetative and hard dough grain stages, and the DM content at the vegetative stage was 155.4 g kg^{-1} for plant, 168.8 g kg^{-1} for leaves and 133.0 g kg^{-1} for stem. Regardless of fungicide application, in the two-harvest system, no difference was detected in the DM contents of the plant, leaves and stem at the hard dough grain stage. As for the single harvest system, fungicide application caused a difference ($P < 0.05$) for all of them, with values of 589.4 against 631.9 g kg^{-1} for plant; 670.9 against 596.8 g kg^{-1} for leaves; 523.4 against 567.3 g kg^{-1} for stem and 536.3 against 670.0 g kg^{-1} for ear, with and without fungicide, respectively. In the system with two harvests, the plant and the ear had lower ($P < 0.05$) DM content compared to the single-harvest system, with averages of 466.9 against 610.6 g kg^{-1} for plant, and 590.7 against 603.1 g kg^{-1} for ear.

Under the conditions found in this study, a single fungicide application was not able to effectively control the development of fungal diseases from the stage of grain development, complicating conclusions about its interference with biomass yield. Clear evidence of this is the high DM content of stem, leaves and ear. On the other hand, the high DM content of the grains combined with the greater participation of this fraction in the plant gives evidence that at this stage we can obtain a forage with high starch content, increasing the energy density of the food and reducing the need to use concentrates in the diet (Harper et al., 2017). In agreement with Harper et al. (2017), it is entirely possible to replace corn silage with winter forage silage in the diet for dairy cows without affecting milk yield, but for this purpose, rye harvesting must

be done with as much starch as possible, that is, at the hard dough grain stage. However, in order to reach this stage, the sowing of summer corn must often be delayed, which can reduce the biomass yield between 10 and 20%, raising questions about the feasibility of this management. With the advancement of maturity, there is a reduction in the quality of the fiber fraction, either by the loss in the participation of leaves or by the greater thickening and lignification of the cell wall (Leão et al., 2016).

The mineral matter content at the vegetative stage was 67.6 g kg^{-1} , significantly higher ($P < 0.05$) than observed at the hard dough grain stage, regardless of fungicide application (Table 3). At the hard dough grain stage, fungicide application did not affect this variable, unlike the harvesting system, where the single-harvest system showed a lower average mineral matter ($P < 0.05$) than the two-harvest system (22.7 against 29.8 g kg^{-1}). The CP content, with 214.2 g kg^{-1} at the vegetative stage, was not affected by the harvesting system at the hard dough grain stage. However, fungicide application resulted in higher content of CP ($P < 0.05$) in both systems, 80.2 against 72.2 g kg^{-1} for the single-harvest system, and 88.3 against 70.7 g kg^{-1} for the two-harvest system.

The NDF, ADF and lignin contents were not affected by the harvesting system. At the vegetative stage, they had a participation of 446.6 ; 236.9 and 36.7 g kg^{-1} in the nutritional composition of the plant. Fungicide application also did not change the ADF content, but promoted a reduction ($P < 0.05$) in NDF in both systems, with values of 753.9 against 790.6 g kg^{-1} for the single-harvest system and 734.4 against 773.3 g kg^{-1} for the two-harvest system, with and without

fungicide, respectively. For lignin, only in the two-harvest system, fungicide application promoted a reduction ($P < 0.05$) in the observed content (97.2 against 110.3 g kg⁻¹).

The estimated digestible energy was higher ($P < 0.05$) at the vegetative stage compared to forage at the hard dough grain stage, 13.15 MJ kg DM⁻¹ for the first and an average of 10.19 MJ kg DM⁻¹ for the second. The forage of the two-harvest system under fungicide application showed the highest participation of ear in the plant and consequently the highest energy density (31.20% and 10.54 MJ kg DM⁻¹ of DE, respectively).

At the vegetative stage, the forage presented ISDMD of 663.6 and 844.6 g kg⁻¹ in 24 and 48 hours of ruminal incubation, respectively, higher values ($P < 0.05$) than obtained at the hard dough grain stage (Table 5). Fungicide application did not affect ISDMD in plants subjected to two harvests, however it resulted in higher ($P < 0.05$) digestibility in the single-harvest system, with 341.6 and 431.3 g kg⁻¹ against 268.8 and 344.9 g kg⁻¹ for 24 and 48 hours, respectively. After 24 hours of incubation, an effect of the harvesting system was found, where the plant subjected to two harvests had higher ($P < 0.05$) digestibility, with mean values of 334.9 against 305.2 g kg⁻¹ for the two-harvest and single-harvest systems, respectively.

Table 5

In situ dry matter digestibility (ISDMD) in 24 and 48 hours of incubation of rye plant cv. Temprano subjected to different harvesting systems (S) with or without fungicide application (F)

Variable	Full vegetative	Hard dough grain				SEM
		Harvests		Fungicide		
		1	2	Without	With	
ISDMD-24h (g kg ⁻¹)	663.6	305.2 b	335.0 a	297.2	343.0	0.9
ISDMD-48h (g kg ⁻¹)	844.6	388.1	396.7	366.8	418.1	1.0

^{a, b} Mean values followed by different letters, in the same row, between harvesting system or between fungicide application, are significantly different by F-test at 5%. SEM: standard error of the mean.

Fungicide application significantly reduced the lignin content in the two-harvest system, corroborating Haerr et al. (2016), who stated that fungi compete for nutrients with the plant, which will use defense mechanisms to try to control the development of the fungus, such as lignification and leaf fall. This can reduce dry matter digestibility (Weinberg & Chen, 2013), as statistically evidenced in the single-harvest system, with a reduction

from 344.3 to 325.6 g kg⁻¹ and from 404.8 to 388.6 g kg⁻¹, with 24 and 48 hours of incubation, respectively (Table 5). The higher ISDMD of forage applied with fungicide may also be related to the higher CP content in these plants, as strobilurins seem to promote greater assimilation of nitrate by the plant and consequently higher content of CP. Similar results were reported by Heker et al. (2020a) with the same cultivar.

Except for mineral matter, the harvesting system did not affect the chemical composition of the forage, proving the feasibility of the single-harvest at the vegetative stage for later harvest at the hard dough grain stage without nutritional losses. In a similar study, but with up to three harvests, Heker et al. (2020a) obtained the best nutritive result and in situ rumen disappearance in the two-harvest system, that is, one at the vegetative and subsequent harvest for silage production at the pasty to hard dough grain stage. Leão et al. (2019) also described higher content of TDN for wheat and triticale in a two-harvest system compared to one and three harvests.

Conclusion

The management of rye with a cut at the vegetative stage before harvesting at the hard dough grain stage is viable for preservation, because in addition to increasing the cumulative dry biomass yield per area, it caused no changes in the nutritional value of the plant. Fungicide application promoted no increase in forage biomass yield, but rather resulted in improvements in its nutritional value, especially in the decrease of cell wall components and increase in plant dry matter digestibility.

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