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Effects on yield and nutritional value of corn silage from corn treated with foliar fungicide and microbial inoculant on ensiling

[*Efeitos na produtividade e no valor nutricional da silagem de milho tratada com fungicida foliar e inoculante microbiano na ensilagem*]

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ABSTRACT

The aim of this study was to evaluate the yield, the morphometric characteristics, and the nutritional value of corn with use of foliar fungicide associated with inoculant on ensiling on the chemical-fermentative characteristics of silage. The applications of fungicide pyraclostrobin + fluxpyroxade were carried out in vegetative-eight and tasseling stage, and the evaluations of plants occurred simultaneously at harvest (dough grain stage), during which application was made with inoculant consisting of strains of *Lactobacillus buchneri* and *L. casei* for silage production. The use of fungicide decreased the percentage of leaf area affected by *Diplodia macrospora* Earle, *Cercospora zeae-maydis* and *Phaeosphaeria maydis*. At harvest, corn showed fewer dry leaves and higher yields of fresh (66,368vs. 62,015kg·ha^{-1}) and dry biomass (20,964 vs. 19,485kg·ha^{-1}) with fungicide. The fungicide also reduced the LDA content from 5.99% to 5.16%, which generated greater ISDMD for whole plant (43.14 and 62.57%, for 24 and 48 hours, respectively). The association of fungicide with inoculant promoted higher concentration of acetic acid than when each was used alone, and the dry matter losses of silage with inoculant were higher than control silage (8.88vs. 12.78%, respectively). Fungicide and inoculant used in combination provided silages with lower fibrous content.

Keywords: digestibility, lactic acid bacteria (LAB), leaf diseases, pyraclostrobin, *zea mays* L.

RESUMO

O objetivo deste estudo foi avaliar a produtividade, as características morfométricas e o valor nutricional do milho, com uso de fungicida foliar associado a inoculante na ensilagem, sobre as características químico-fermentativas da silagem. As aplicações do fungicida piraclostrobina + fluxpiroxade foram realizadas nos estádios vegetativo-oito e pendoamento, e as avaliações das plantas ocorreram simultaneamente na colheita (fase de grãos pastosos), quando foi aplicado o inoculante composto por cepas de Lactobacillus buchneri *e* L. casei *para a produção de silagem. O uso de fungicida diminuiu a porcentagem de área foliar afetada por* Diplodia macrospora Earle, Cercospora zeae-maydis *e* Phaeosphaeria maydis. *Na colheita, o milho apresentou menor número de folhas secas e maior produtividade de biomassa fresca (66.368 vs. 62.015kg·ha^{-1}) e seca (20.964 vs. 19.485kg·ha^{-1}) com fungicida. O fungicida reduziu teor de LDA de 5,99% para 5,16%, gerando maior ISDMD para planta inteira (43,14 e 62,57%, para 24 e 48 horas, respectivamente). A associação do fungicida com inoculante promoveu maior concentração de ácido acético do que quando cada um foi utilizado isoladamente, e as perdas de matéria seca da silagem com inoculante foram maiores que as da silagem controle (8,88 vs. 12,78%, respectivamente). Fungicida e inoculante utilizados associados proporcionaram silagens com menor teor fibroso.*

Palavras-chave: bactérias lácticas (BAL), digestibilidade, doenças foliares, piraclostrobina, Zea mays L.

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INTRODUCTION

It is known that corn silage has highly heterogeneous structural and chemical composition and yield (Kalebich and Cardoso, 2017), mainly provided by agronomic variation in the way the crop is conducted, and that is why the use of leaf fungicide has been highlighted in recent studies (Kalebich *et al.*, 2017). The ability to enhance grain yield in corn through the prevention of the main leaf diseases with the use of fungicides seems to have been well clarified (Wise and Mueller, 2011). Kalebich and Cardoso (2017) observed improvements in the characteristics related to the yield and chemical quality of vegetative fraction of corn plant with pyraclostrobin + fluxpyroxade use in a preventive way in vegetative stage.

Due to its good ability to control leaf diseases, pyraclostrobin has also stood out for its greening effect (Haerr *et al.*, 2016). These physiological changes induced by this active principle have already shown the ability to increase plant yield and digestibility (Wise and Mueller, 2011). The improvement in nutritional value may be linked to reduced lignification, higher proportion of grains and greater assimilation of nitrate, increasing content of crude protein in the plant.

In addition, the presence of fungi promotes competition for nutrients between them and the plant, making the plant to express some defense mechanisms to try to control the development of fungus, such as lignification and leaf fall, and this can reduce the dry matter and fiber digestibility (Haerr *et al.*, 2016).

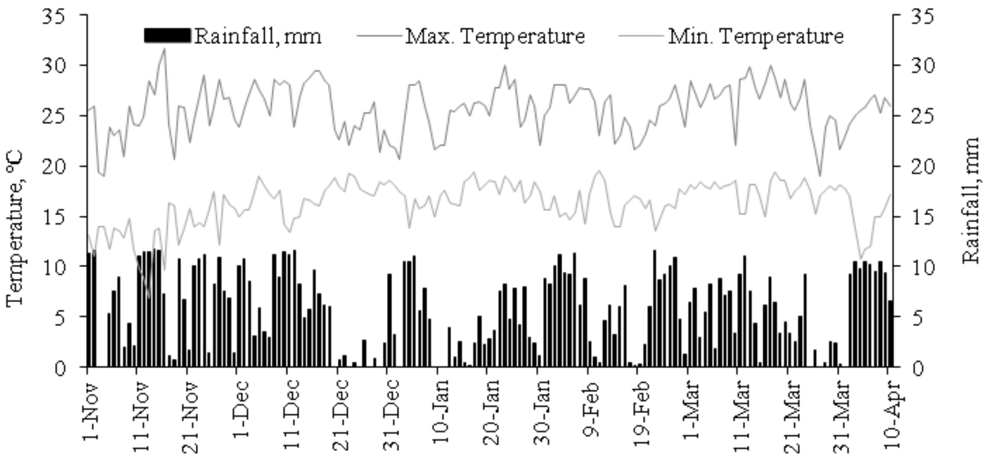
Fungi can also cause innumerable losses in the post-harvest, be they nutritional, physical, or biotic. There is evidence that antimicrobial compounds released by plants against fungal attacks can also reduce the bacterial community, which are essential for silage fermentation (Malinovsky *et al.*, 2014). Queiroz *et al.* (2012a) showed a reduction in lactate levels and an increase in pH in corn silages with medium and high rust conditions, decrease the fermentative quality of silage. Therefore, we believe that the use of fermentation-inducing inoculants can partially compensate for this type of damage. We aim to evaluate the yield, morphometric characteristics, and nutritional value of the corn plant with preventive application of foliar fungicide (pyraclostrobin + fluxpyroxade), and also the association with inoculant on ensiling on the chemical-fermentative characteristics of silage.

MATERIAL AND METHODS

The study was carried out in the city of Guarapuava, Paraná, Brazil, located at geographic coordinates 25°38’09” S and 51°48’49” W and altitude of 1,100 m. According to Köppen's classification, the climate of the region is Cfb (Subtropical humid mesothermal), with no dry season, with cool summers and moderate winter. Fig. 1 shows the maximum and minimum temperatures (ºC) and rainfall (mm), in ten days, during the period of conduction of the crop.

The soil of the experimental area was classified as Latossolo Bruno Típico and previously the tillage presented the following chemical characteristics in profile from 0 to 20cm:$CaCl_2$ 0,01M:4,91; P:12,06mg dm^{-3}; K^+:0,37cmolc dm^{-3}; Al^{3+}:0,20cmolc dm^{-3}; H^+ +Al^{3+}:5,80cmolc dm^{-3}; Ca^{2+}:4,09cmolc dm^{-3}; Mg^{2+}:1,82cmolc dm^{-3} and base saturation (V%):51,90%.

The corn sowing (*Zea mays*, L.) was carried out on November 2, 2017 under a no-tillage system, in succession to the ryegrass (*Lolium multiflorum*) crop, freshly dried with glyfosate herbicide (Roundup®WG: 2kgha^{-1}). The hybrid used was the Maximus VIP3 (Syngenta®), implanted with spacing between lines of 80cm, sowing depth of 4 cm and linear distribution to reach 60,000 seeds·ha^{-1}. At 25 days after sowing (DAS) the plant population was estimated at 59,000 plants·ha^{-1}. The experimental area consisted of eight lines and ten meters in length, totaling 64m^2 (6.4m×10.0m), with the useful area of each plot consisting of the four central lines (3.2m×10.0m).



Source: Simepar/Unicentro station, Guarapuava, PR.
Figure 1. Maximum and minimum temperatures (ºC) and rainfall (mm), in ten days, during the conduction period of the crop (2017/2018).

The fertilization was carried out with 500kgha^{-1} of fertilizer N-P_2O_5-K_2O in the formula 12-31-17. The nitrogen in cover fertilization was made during the V4-V5stage with 500kgha^{-1} of urea (45% of N). On December 7, 2017, undesirable plants and pests were controlled by the chemical method using glyphosate herbicide (Roundup®WG: 2kgha^{-1}) and imidacloprid + beta-cyfluthrin insecticide (Connect®: 0.75L ha^{-1}) with mineral oil (Nimbus®: 0.5L ha^{-1}).

The harvest of corn for silage was on March 31, 2018 (149 DAS) when they were in dough grain stage - R4, 30 cm above the ground with manual equipment to preserve the integrity of the plants, and after the harvest they were sent to the laboratory for the corresponding analyzes.

In experiment 1 were evaluated the fresh and dry biomass yield, morphometric composition, chemical composition, and digestibility of corn plants submitted or not to foliar fungicide. The experimental design was randomized blocks, consisting of two treatments: with (FUN) and without (CON) use of leaf fungicide and five replications.

To ensure the control of fungal diseases, on December 26, 2017 and January 12, 2018, in vegetative V8 and tasseling stage (VT), respectively, applications were made with pyraclostrobin (strobilurin) fungicide + fluxpyroxade (carboxamide) (Orkestra® SC) at a dose of 0.35Lha^{-1}. The spraying was carried out with the aid of a motorized backpack sprayer, equipped with a bar containing four fan-shaped double spray tips (Twinjet TJ 60 110.02).

The presence and severity of the main corn leaf diseases were also evaluated, namely: Rust (*Puccinia sorghi* Schw.), Macrospora leaf spot (*Diplodia macrospora* Earle), Leaf blight (*Setosphaeria turcica*), Cercosporiosis (*Cercospora zeae-maydis*) and White spot (*Phaeosphaeria maydis*). For this, the diagrammatic scale proposed by Azevedo (1998) was considered.

Simultaneously the harvest was measured the height of plant and ear insertion (m), as well as the count of number of dry leaves per plant. After harvesting, the homogeneous and representative plants of each plot were taken to the laboratory for segmentation of the components: stem, leaves, husk, corn cob, grains and vegetative fraction. It corresponds to the vegetative fraction of the plant without grains, which were carefully removed so as not to injure the ear.

First, each component was coarsely chopped and transferred to a forced air oven at 55°C for partial drying for 72 hours. Sequentially the samples were 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. The crude protein (CP) was determined by the micro Kjedahl method and ether extract (EE) according to methodologies described by AOAC (Official…, 1995). The levels of neutral detergent fiber (aNDF) were obtained using thermostable α-amylase (Termamyl 120L, Novozymes Latin América Ltda.) but not using sodium sulfite (Van Soest *et al.*, 1991). The content of acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970) sequentially. Hemicellulose was estimated by the difference between NDF and FDA, and cellulose by the difference between FDA and LDA.

The dry matter ruminal disappearance rate was determined by the *in situ* technique using nylon bags measuring 12cm×8cm and pores of 50µm, containing 5g of dry and ground sample (Nocek, 1988). The incubation times were 1h, 6h, 12h, 24h, 36h and 48 hours. Undegradable NDF (NDFU) was the residual fraction of this after 240 hours of incubation. For this purpose, were used two bovines with permanent ruminal cannula, male, with five years old and an average of 650kg liveweight.

After harvesting the useful area in experiment 2, the corn plants were chopped into a stationary forage (EM 6400 from the Nogueira® brand) and were then added with an inoculant diluted in non-chlorinated water, according to the manufacturer's instructions, to obtain a concentration of $20\times10^{10}UFCg^{-1}$ of product per ton of natural matter. The inoculant used was the Pioneer® brand 11CFT, consisting of strains of *Lactobacillus buchneri* ($1.1\times10^{11}UFCg^{-1}$) and *L. casei* ($1.1\times10^{11}UFCg^{-1}$). With this we formed a randomized blocks design, in a 2×2 factorial scheme, with (FUN) and without (CON) use of leaf fungicide; and with (INO) and without (NO) use of inoculant, and five replications.

The chopped material was placed in mini-silos of PVC with 20cm in diameter and 40cm in height, compressed to generate density of 600kg of natural material m^{-3}. The silos were stored in the laboratory for a period of 60 days. After opening, 500g of sample of each treatment was collected, which were weighed and pre-dried in a forced air oven at 55℃ for 72 hours to determine the partial dry matter content (Official…, 1995) and then were ground in a Wiley mill, with a 1mm mesh sieve.

The chemical and digestibility analyzes followed the same practices and methodologies previously described. The content of soluble sugars was determined according to AOAC (Official…, 1995), and the NDIP and ADIP according to Silva and Queiroz (2009). The concentration of ammoniacal nitrogen ($N\text{-}NH_3$) was analyzed by distillation with magnesium oxide (Official…, 1995). The contents of total digestible nutrients (TDN) were obtained according to Bolsen *et al.* (1996). The estimate of dry matter losses in anaerobiosis was calculated according to Jobim *et al.* (2007). The concentration of lactic acid was performed according to the methodology described by Price (1969). The concentration of acetic acid was determined by gas chromatography using a Shimadzu® GC-2010 Plus chromatograph equipped with an automatic AOC-20i injector, Stabilwax-DA™ capillary column (30m, 0.25mm ID, 0.25µm df, Restek®) and flame ionization detector (FID). The injector and detector temperatures were 250 and 300℃ respectively, and the initial column temperature was 40℃. The detection and integration of the peaks were made using the GCsolution software v. 2.42.00 (Shimadzu®).

The data were submitted to the Shapiro-Wilk and Bartlett tests, in order to verify the normality and homogeneity of variance, respectively. Once these assumptions were met, the F test was applied through ANOVA using the SAS program (Statistical…, 1993). The results were presented as significant ($p<0.05$) and trends ($p<0.10$). The data related to rumen disappearance were submitted to polynomial regression analysis considering the variable stage of maturity, using the *Regression* procedure (PROC REG). The choice of regression models was made by their degree of adjustment, based on the coefficient of determination (R^2).

Data from experiment 1 following the statistical model: $Y_{ij}=\mu+\alpha_i+\beta_j+\varepsilon_{ij}$, where: Y_{ij} = Variable response related to fungicide *i* in block *j*; µ = Overall; α_i = Fungicide effect *i*; *i* = CON and FUN; β_j = Block effect *j*; *j* = 1, 2, 3, 4, 5; ε_{ij} = Random error associated with each observation Y_{ij}.

Data from experiment 2 following the statistical model: $Y_{ijk}=\mu+\alpha_i+\beta_j+\gamma_{ij}+\delta_k+\varepsilon_{ijk}$, where: Y_{ijk} = Variable response related to fungicide *i* with inoculant *j* in block *k*; µ = Overall; α_i = Fungicide effect *i*; *i* = CON and FUN; β_j = Inoculant effect *j*; *j* = INO and NO; γ_{ij} = Effect of the interaction of fungicide *i* with inoculant *j*; δ_k = Block effect *k*; *k* = 1, 2, 3, 4, 5; ε_{ijk} = Random error associated with each observation Y_{ijk}.

RESULTS AND DISCUSSION

Rust and leaf blight are important diseases of corn, and we observed a low incidence of them, with no differences for both in relation to the use of fungicide (Tab. 1). According to Kalebich and Cardoso (2017), for the development of the disease a susceptible plant, a pathogen and a favorable environment are required, and as *Puccinia sorghi* (Schw.) and *Setosphaeria turcica* are prevalent in tropical regions and as we have hybrids with good resistance to them, this was sufficient for such results.

Table 1. Leaf area affected (%) by leaf diseases in corn grown with or without fungicide

Fungus / Disease	Fungicide		CV, %	*P*
	CON	FUN		
Puccinia sorghi Schw. Rust	4.1	3.2	32.94	0.6213
Diplodia macrospora Earle Macrospora leaf spot	20.1	4.2	11.50	0.0111
Setosphaeria túrcica Leaf blight	8.3	5.2	67.91	0.6877
Cercospora zeae-maydis Cercosporiosis	22.0	2.3	55.23	0.0097
Phaeosphaeria maydis White spot	50.0	26.0	31.14	0.0327

FUN: Application of $0.35L \cdot ha^{-1}$ of pyraclostrobin + fluxpyroxade (Orkestra®SC) in vegetative V8 and tasseling stage (VT). CON: Control.

On the other hand, there was a decrease in percentage of leaf area affected by macrospore leaf spot, cercosporiosis and white spot ($p<0.05$) in FUN corn. The white spot was highlighted by the greater predominance among the highlighted diseases, with 50% for CON and 26% for FUN. Despite the good reduction, the high severity even with the use of fungicide is due to the fact that in addition to the fungus *Phaeosphaeria maydis*, recent studies have identified as a potential cause of *Pantoea ananatis*, a gram-negative bacteria that it is not controlled by strobilurins and / or carboxamides.

The satisfactory result obtained in the control of cercosporiosis also deserves to be highlighted, since the increase of a percentage unit of leaf area affected by it can cause a reduction of 47kg of grains$\cdot ha^{-1}$ in the yield of generic hybrids and of 35kg of grains$\cdot ha^{-1}$ in moderately tolerant hybrids (Kalebich *et al.*, 2017).

The preventive application of fungicide did not express differences for plant height and ear height, with mean of 2.27m and 1.32m, respectively (Tab. 2). The plant morphometric characteristics seem to be more subject to variations between genotypes than to the presence of fungal diseases (Kalebich *et al.,* 2017).

Table 2. Agronomic characteristics of corn grown with or without fungicide

	Fungicide		CV, %	*P*
	CON	FUN		
Plant height, m	2.28	2.27	1.25	0.5415
Ear height, m	1.33	1.31	3.81	0.6832
Number of dry leaves	1.93	1.57	17.57	0.0372
Fresh biomass yield, $kg \cdot ha^{-1}$	62,015	66,368	8.42	0.0719
Dry biomass yield, $kg \cdot ha^{-1}$	19,485	20,964	6.53	0.0504

FUN: Application of $0.35L \cdot ha^{-1}$ of pyraclostrobin + fluxpyroxade (Orkestra®SC) in vegetative V8 and tasseling stage (VT).CON: Control.

The lower number of dry leaves observed in FUN corn ($p<0.05$) agrees with several other studies, either for corn or for other grasses (Kalebich *et al.*, 2017). Pyraclostrobin is recognized for its ability to cause delay in senescence due to reduction of oxidative stress, increased photosynthetic capacity, inhibition of ethylene biosynthesis and reduction of stomatal opening and loss of water by sweating (Paul *et al.*, 2011). This would result in higher total biomass yields.

According to Wise and Mueller (2011), the use of fungicides can increase the yield by more than 20%, but maybe in Brazil these differences are more modest due to the cultivated hybrids (Paul *et al.*, 2011). We observed higher yields of fresh (66,368vs. 62,015kg·ha^{-1}; p=0.0719) and dry (20,964vs. 19,485kg·ha^{-1}; p=0.0504) biomass for FUN corn compared to CON. Wise and Mueller (2011) affirm that above 5% of the affected leaf area, it is already possible to observe significant differences in yield, be it for grains or silage. Some researchers have also shown that even in the absence of disease, the application of fungicide can lead to higher yields and this would be due to the greater activity of nitrate reductase and consequently greater assimilation of nitrogen by the plant during its development (Kalebich and Cardoso, 2017).

The use of fungicide did not promote any change in the dry matter content of the plant fractions, as well as of the whole-plant and the vegetative fraction (Tab. 3). Likewise, there were no differences in centesimal participation for stem, husk and grains in the plant. The share of leaves and corn cob was lower in FUN corn (16.19% and 8.31%, respectively) compared to CON (17.03% and 9.28%, respectively). We believe that the use of fungicide may have helped to control stalk diseases, which, even though this was not our study objective, nor had any significance in participation, there was a difference that may justify its overlap in composition in relation to leaves and corn cob.

Table 3. Morphometric characteristics (% of the whole-plant) and dry matter contents of the plant fractions of corn grown with or without fungicide

Plant fraction	Morphometric composition, % DM				Dry matter, %			
	CON	FUN	CV, %	*P*	CON	FUN	CV, %	*P*
Stem	17.96	19.22	8.51	0.2747	19.40	20.42	5.49	0.2170
Leaves	17.03	16.19	2.99	0.0453	25.85	25.47	3.14	0.4912
Husk	10.14	10.55	5.31	0.3056	29.37	28.90	10.26	0.8158
Corn cob	9.28	8.31	6.33	0.0505	36.56	36.20	4.60	0.7468
Grains	45.58	45.02	5.56	0.7429	59.52	58.95	2.19	0.5213
Whole-lant					31.50	31.61	3.03	0.8571
Vegetative fraction					24.25	25.92	5.82	0.1440

FUN: Application of 0.35L·ha^{-1} of pyraclostrobin + fluxpyroxade (Orkestra®SC) in vegetative V8 and tasseling stage (VT). CON: Control.

Although we have not reported any difference between the dry matter content, it is important to say that the composition of the dry matter may change due to fungal action, especially that there is a partial substitution of nitrogenous compounds for fibrous compounds (Macome *et al.*, 2017). These changes mainly affect the vegetative fraction. According to Haerr *et al.* (2016), competition for nutrients with fungi make the plant to express some defense mechanisms to try to control the development of the fungus, such as lignification.

Regarding the chemical composition of the plant (Tab. 4), there were trends of decrease in ether extract content (2.23 and 1.93% for CON and FUN, respectively) and an increase in cellulose content (22.32 and 24.24% for CON and FUN, respectively) using foliar fungicide. These data are in accordance with those described by Kalebich *et al.* (2017). We expected to find differences for NDF and ADF between treatments, but that was not seen. Hollis *et al.* (2019) obtained an increase in NDF, FDA and hemicellulose contents of the corn treated with fungicide in a vegetative stage, in addition to a reduction in lignin concentration. In our study, FUN also significantly reduced the ADL content from 5.99% to 5.16%. This reduction is described by countless authors as the main factor responsible for the increase in the digestibility of DM and NDF in plants and silages submitted to the application of pyraclostrobin (Kalebich *et al*., 2017; Kalebich and Cardoso, 2017; Hollis *et al.*, 2019; Cardoso, 2020). In addition, after the fungus installed in the plant and this initiates the lignification of the secondary cell wall as a defense mechanism, some fungi release secondary metabolites, which can often be toxic to animals (Hollis *et al.*, 2019). This emphasizes the need for preventive rather than curative applications of foliar fungicides.

Table 4. Chemical composition, *in situ* dry matter digestibility (ISDMD) in 24 and 48, and undegraded NDF (NDFU) of the whole-plant and vegetative fraction

	CON	FUN	CV, %	*P*
Chemical composition, % DM				
Ash	3.48	3.51	6.76	0.8508
Crude protein	6.55	6.52	7.98	0.9274
Ether extract	2.23	1.93	4.08	0.0846
NDF	57.20	57.83	2.32	0.4937
Hemicellulose	28.88	28.43	2.33	0.3419
ADF	28.31	29.41	4.93	0.2911
Cellulose	22.32	24.24	5.66	0.0823
ADL	5.99	5.16	5.01	0.0094
ISDMD-24h, % DM				
Whole-plant	41.57	43.14	8.17	0.0507
Vegetative fraction	27.60	29.06	4.89	0.0435
ISDMD-48h, % DM				
Whole-plant	58.22	62.57	1.34	0.0030
Vegetative fraction	41.28	43.32	8.48	0.0205
NDFU, % NDF				
Whole-plant	34.35	33.96	3.53	0.8009
Vegetative fraction	43.67	41.91	6.99	0.5229

FUN: Application of 0.35L·ha^{-1} of pyraclostrobin + fluxpyroxade (Orkestra®SC) in vegetative V8 and tasseling stage (VT). CON: Control.

The ISDMD was higher for FUN compared to CON, both for whole-plant (43.14 and 62.57%, for 24 and 48 hours) and for the vegetative fraction (29.06 and 43.32%, for 24 and 48 hours). These differences highlight the strong impact caused by the increase in the lignin content in the plant. Similar results were observed by Haerr *et al*. (2016). Kalebich *et al.* (2017) described an increase in NDF digestibility even with higher content of lignin with two applications of fungicide, something that we did not observe in our study. According to these authors, the time of application of the fungicide can directly influence fiber digestibility.

For corn silage, the use of fungicide did not affect the dry matter content (33.07 and 33.69% for CON and FUN, respectively), but the use of inoculant reduced ($p<0.10$) 1.06 percentage units of dry matter (Tab. 5). That was expected, because the lactic acid bacteria produce fermentation-promoting organic acids that result from the conversion of the substrate consumed by them.

As the crop conditions were optimal, we did not obtain higher concentrations of lactic acid with the use of fungicide and / or inoculant, but the use of fungicide associated with the use of inoculant promoted a higher concentration of acetic acid than when each was used alone ($p<0.05$). The higher production of this acid at the expense of consumption of substrates made the dry matter losses of the INO silage to be higher than the NO silage (8.88vs. 12.78%, respectively). Haerr *et al*. (2016) also did not observe differences with the use of fungicide for any of the mentioned acids, perhaps because the main chemical changes occurred in the plant are in the cell wall, and not in the cell medium where the highest concentration of soluble carbohydrates are deposited (Cardoso, 2020) required for lactic acid bacteria.

Table 5. Chemical-fermentative composition of corn silage grown with or without fungicide and with or without microbial inoculants on ensiling

	Fungicide (F)		Inoculant (I)		*P*		
	CON	FUN	NO	INO	F	I	F×I
Dry matter, % NM	33.07	33.69	33.91	32.85	ns	*	ns
Ash, % DM	3.18	3.31	3.21	3.28	**	ns	ns
Crude protein, % DM	6.62	6.54	6.82	6.33	ns	**	ns
NDIP, % CP	12.79	11.77	11.94	12.62	**	ns	ns
ADIP, % CP	7.05	6.44	6.66	6.83	ns	ns	*
Ether extract, % DM	2.88	3.37	3.31	2.94	**	**	**
NDF, % DM	45.95	48.35	47.67	46.64	ns	ns	ns
Hemicellulose, % DM	20.21	22.33	21.33	21.20	ns	ns	*
ADF, % DM	25.74	26.03	26.33	25.43	ns	ns	*
Cellulose, % DM	21.92	22.59	22.88	21.63	ns	ns	*
ADL, % DM	3.83	3.42	3.46	3.80	ns	ns	ns
Soluble sugar, % DM	1.65	1.68	1.75	1.58	ns	ns	ns
TDN, % DM	69.52	69.62	69.41	70.03	ns	ns	*
Lactic acid, % DM	5.94	5.67	5.97	5.63	ns	ns	ns
Acetic acid, % DM	1.96	2.21	2.00	2.17	ns	ns	**
$N\text{-}NH_3$, % CP	7.36	7.36	7.52	7.04	ns	ns	ns
Dry matter loss, % DM	11.38	10.28	12.78	8.88	ns	**	ns

**p<0,05; *p<0,10; ns: not significant
FUN: Application of $0.35L \cdot ha^{-1}$ of pyraclostrobin + fluxpyroxade (Orkestra®SC) in vegetative V8 and tasseling stage (VT). CON: Control.

The higher content of ash that FUN silage had in relation to CON silage (3.31vs. 3.18%, respectively; p<0.05) has also been documented in other similar studies (Kalebich *et al*., 2017; Hollis *et al*., 2019). One explanation could be the greater stalk participation in this treatment, and another that the leaves are the main site of photosynthesis of the plant and, the healthier they are, the greater the metabolic activity (Hollis *et al*., 2019). This can also be a plausible explanation for the higher content of ether extract in FUN silage compared to CON silage (p<0.05). In addition, Williams *et al.* (1992) found that the energy content between infected and uninfected plants would not differ but noted that there was more protein and less fat in fungus-damaged corn. This difference in the composition of nutrients is due to the fungus using compounds that are more easily accessible as the primary source of nutrients.

The INO silage had a lower crude protein content compared to NO silage, and this is in accordance with the results of Ribas *et al*. (2021) using the same inoculant. The use of fungicide reduced the NDIP from 12.79% to 11.77%, while the FUN×NO silage had a good association in reducing ADIP.

The effect caused by the association between the fungicide and inoculant (FUN×INO) provided silages with lower contents of hemicellulose, ADF and cellulose. While the use of fungicide prevents the early lignification of cell wall tissues (Cardoso, 2020), the chemical action caused by acids produced by lactic acid bacteria causes partial hydrolysis of hemicellulose (Abdel-Rahman *et al.*, 2011). We suppose that this same cause was the reason for the increase in TND of FUN×INO silage (p<0.05).

With the data presented in Tab. 6, note an increase in the dry matter digestibility of FUN silage in relation to CON silage in 24 hours (47.97vs. 45.28%) and in 48 hours (61.17vs. 59.79 %). In a similar study, Queiroz *et al*. (2012b) ensiled healthy corn plants, in medium concentration and in high concentration of leaves affected by rust. The authors observed a decrease in *in vitro* dry matter digestibility, with the following values: without rust: 66.9%, average concentration: 63.2%, and high concentration: 60.1%. This change in digestibility in plants treated and not treated with fungicide is perhaps the most evident result among the works published on the subject (Cardoso, 2020; Hollis *et al.*, 2019; Kalebich *et al.*, 2017). The presence of the fungus in the plant generates competition among them for nutrients. To try to prevent the growth of fungal infestation, the plant adopts mechanisms such as lignification, and this can potentially decrease the digestibility of the plant. Fungal infestation itself can also alter the chemical composition of the plant in the process of competition for nutrients (Kalebich and Cardoso, 2017).

The use of inoculant did not interfere with ISDMD, being in line with the data presented by Oliveira *et al.* (2017) in meta-analysis on inoculants composed of optional homo and heterofermentative bacteria. However, we had a reduction in ISNDFD in 48 hours in the INO silage in relation to the NO silage (53.30 vs. 56.00%). This may have been due to the more extensive partial hydrolysis of hemicellulose caused by the action of acids produced by lactic acid bacteria in the inoculant. This breakdown of hemicellulose into hexoses and pentose sugars (Abdel-Rahman *et al*., 2011) increases the soluble nutrients and causes the NDF fraction to be reduced, however it becomes less digestible portions, basically cellulose and lignin. The NDF of silage was not affected either by the use of fungicide or by the addition of inoculants, but the results obtained generated good reference values.

Table 6. *In situ* digestibility od dry matter (ISDMD) and NDF (ISNDFD), and undegraded NDF (NDFU) of corn silage grown with or without fungicide and with or without microbial inoculants

	Fungicide (F)		Inoculant (I)		*P*		
	CON	FUN	NO	INO	F	I	F×I
ISDMD-24h	45.28	47.97	46.48	46.78	**	ns	ns
ISDMD-48h	59.79	61.17	60.14	60.81	**	ns	ns
ISNDFD-24h	38.30	36.20	37.70	36.80	ns	ns	ns
ISNDFD-48h	55.20	54.30	56.00	53.50	ns	**	ns
NDFU	14.74	14.90	14.46	15.18	ns	ns	ns

**p<0,05; *p<0,10; ns: not significant
FUN: Application of 0.35L ha^{-1} of pyraclostrobin + fluxpyroxade (Orkestra®SC) in vegetative V8 and tasseling stage (VT). CON: Control.

CONCLUSION

The preventive application of fungicide (pyraclostrobin + fluxpyroxade) reduced the leaf area affected by the main fungal diseases of corn, and this intervened for a lower number of dry leaves and higher yield for silage. The quality of fibrous fraction, the plant and silage digestibility were improved with the use of fungicide. In addition, its association with inoculants generated satisfactory results, increasing the TND content of corn silage. However, the use of inoculant alone did not promote significant improvements.

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