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Original Article

Effects of maize genotypes, nitrogen rates and sources in yield, nutritional status, and fumonisins incidence

Efeito de genótipos de milho, doses e fontes de n na produtividade, estado nutricional e incidência de fumonisinas

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Abstract

The maize yield, nutritional status, and grain fumonisins concentration were evaluated in different genotypes, doses, and nitrogen sources (N) in two years and three locations. Two experiments were carried out in each area and year in an experimental design of a subdivided plot with four replications. One experiment involved a 4x2 factorial treatment: four nitrogen (N) doses (0, 80, 160, and 240 kg ha^{-1}) in coverage and having urea as a source of N and two genotypes. Another experiment involved a 4x2 factorial treatment: four N sources: urea, urea covered with polymer, ammonium nitrate, and ammonium nitrate + urea (UAN), at a dose of 160 kg ha^{-1}, in two genotypes. The genotype generally influenced maize yield more than N doses and sources, mainly due to the bushy stunt/corn stunt tolerance of AG7098 PRO2 and AG8677 PRO2. The N doses linearly increased the N leaf content. However, the N sources did not affect the N leaf content. The N doses and sources had no significant effect on the content of fumonisins, which was affected only by the genotypes in Sete Lagoas in 2016 (N doses experiment) and 2017 (N sources experiment). The hybrids, P3630H and AG8677PRO2 (Sete Lagoas, 2016, N doses experiment and 2017, N sources experiment, respectively) exceeded the Brazilian legislation for Maximum Tolerance Limit for fumonisins in corn grains, which is 5,000 µg kg^{-1}. The best result was obtained with AG7098 PRO2, with yields (above 10,000 kg ha^{-1}) and fumonisins consistently below 5,000 µg kg^{-1}. Therefore, the selection of corn hybrids is a strategy to reduce the occurrence of fumonisins in the grains.

Keywords: *Zea mays*, *Fusarium verticillioides*, fertilization, nitrogen.

Resumo

A produtividade do milho, o estado nutricional e a concentração de fumonisinas no grão foram avaliadas em diferentes genótipos, doses e fontes de nitrogênio (N) em dois anos e três localidades. Foram conduzidos dois experimentos em cada localidade e ano, em delineamento experimental de parcela subdividida com quatro repetições. Um experimento envolveu um tratamento fatorial 4x2: quatro doses de nitrogênio (N) (0, 80, 160 e 240 kg ha^{-1}) em cobertura, tendo uréia como fonte de N e dois genótipos de milho. Outro experimento envolveu um tratamento fatorial 4x2, sendo quatro fontes de N: uréia, uréia recoberta com polímero, nitrato de amônio e nitrato de amônio + urea (URAN), na dose de 160 kg ha-1, em dois genótipos. O genótipo do milho influenciou o rendimento, mais do que as doses e fontes de N, principalmente devido à tolerância ao enfezamento do milho AG7098 PRO2 e AG8677 PRO2. As doses de N aumentaram linearmente o teor foliar de N. No entanto, as fontes de N não afetaram o teor de N foliar. As doses e fontes de N não tiveram efeito significativo sobre o teor de fumonisinas, que foi afetado apenas pelos genótipos em Sete Lagoas em 2016 (experimento de doses N) e 2017 (experimento de fontes N). Os híbridos, P3630H e AG8677PRO2 (Sete Lagoas, 2016, experimento de doses de N e 2017, experimento de fontes de N, respectivamente) ultrapassaram o Limite Máximo de Tolerância permitida pela legislação brasileira para fumonisinas em grãos de milho, que é de 5.000 µg kg^{-1}. O melhor resultado foi obtido com AG7098 PRO2, com produtividade (acima de 10.000 kg ha^{-1}) e fumonisinas consistentemente abaixo de 5.000 µg kg^{-1}. Portanto, a seleção de híbridos de milho é uma estratégia para reduzir a ocorrência de fumonisinas nos grãos.

Palavras-chave: *Zea mays*, *Fusarium verticillioides*, fertilização, nitrogênio.

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1. Introduction

Nitrogen is a component of various nitrogen-containing molecules essential in vital plant growth and development processes. Maize crops require large amounts of nitrogen, and its deficiency compromises the development and yield of the crop. In addition, N is an integral constituent of several secondary compounds of plant defense mechanisms. (Pizolato et al., 2016; Batista et al., 2019). Nitrogen is a dynamic element in the soil-plant-atmosphere system, and several factors influence its plant availability. Living organisms can absorb this element as ammonium (NH4+) or nitrate (NO3-), the first preferred by bacteria and the second by plants since ammonium is toxic to plant cells. Once absorbed in the form of nitrate, nitrogen is immediately converted to nitrite (NO_2^-) by the enzyme nitrate reductase, and the nitrite is then converted to ammonia by nitrite reductase. The ammonia formed in this process then enters the cycle known as GS/GOGAT (Glutamine synthase/ Glutamate synthase), which originates the amino acids glutamine and glutamate, precursors of many of the other amino acids synthesized by plants (Taiz et al., 2017).

Soil organic matter (SOM) is the primary source of N to crops through decomposition. However, the plants' demand for nitrogen is often more significant than that supplied by SOM, which implies adding nitrogen fertilizers to the soil. Furthermore, the N-use efficiency of fertilizers is low due to losses caused by volatilization and leaching processes and the plant's preferential absorption of ammonium over nitrate (Duete et al., 2009).

Nitrogen losses can vary over time, depending on several factors, such as soil type, crop, and fertilizer (Sainju, 2017). Different nitrogen fertilizers are on the market, with a predominance of urea, which presents about 45% of N in the amidic form (N-NH_2). Thus, a significant amount of N may be lost through volatilization. Developing N-slow-release technologies, such as polymer-coated urea and other N sources, such as ammonium nitrate and a solution of urea and ammonium nitrate in water (UAN), can reduce N losses and potentially synchronize N release according to crop demand. UAN (32% N) is a fluid fertilizer with ammonium nitrate and urea solubilization (Minato et al., 2020). It results in a solution containing higher N than its components' saturated solutions. UAN's main benefits include reducing the potential N losses by NH_3 volatilization. Besides having three N forms in a single product, it represents a better technology concerning ammonium nitrate. The polymer-coated urea consists of a high CTC polymeric treatment enriched with a urease enzyme inhibitor (NBPT) for reducing losses from amidic sources, such as urea and polymers specific additives or nutritional complexes used as mechanical barriers and the action of urease (Pereira et al., 2009).

An increase in corn grain yield resulting from different doses of nitrogen has been observed (Peruzzo et al., 2015; Portugal et al., 2017; Scotta et al., 2018; Moreira et al., 2019). Many studies demonstrated a linear relationship between grain yield and N doses. Thus, corn productivity increases significantly in applications of different N doses in topdressing, especially with 120 kg ha^{-1} of N (Besen et al., 2020). Work by Lins et al. (2020) showed that when different genotypes and doses of N are used, there is no difference among the genotypes. However, the treatment with nitrogen has a significant influence, with the highest production observed at the dose of 320 kg ha^{-1} of N. Thus, using different doses and sources of nitrogen fertilizers may increase the availability of this nutrient to plants (Xiao et al., 2019; Bortoletto-Santos et al., 2020).

Nitrogen fertilization is also related to disease incidence in maize and other crops, preventing or favoring plant infection and mycotoxin production by fungal pathogens (Blandino et al., 2008; Bouras et al., 2016; Scarpino et al., 2022; Blandino et al., 2022; Battisti et al., 2022; Keszthelyi et al., 2022).

In maize, mycotoxin production occurs through the infection by several *Fusarium* species, such as *Fusarium verticillioides*, *F. graminearum*, *F. subglutinans*, and *F. proliferatum* (Oldenburg et al., 2017; Bocianowski et al., 2019). Mycotoxins are toxic metabolites accumulated mainly in grains and can cause damage to human and animal health. Mycotoxins are ingested directly and indirectly through grain consumption, processed derivatives, milk, eggs, and meats (Portugal et al., 2017). *Fusarium verticillioides* produce fumonisins, the most common mycotoxins in corn, with high incidence and toxicity (Madania et al., 2013; Volkel et al., 2011; Lanza et al., 2014).

Interactions of biotic and abiotic factors induce fumonisin production (Kamle et al., 2019), such as temperature, water stress, osmotic stress, pH, fungicides, the reaction of hybrids to toxigenic fungi, and fertilization (Shim and Woloshuk, 1999; Blandino et al., 2008; Cao et al., 2014; Lanza et al., 2014; Santiago et al., 2015; Lanza et al., 2016; Van Rensburg et al., 2016; Gesteiro et al., 2021; Battisti et al., 2022; Keszthelyi et al., 2022; Scarpino et al., 2022; Blandino et al., 2022). Thus, the control of fungal growth and mycotoxin production through genetic resistance, fungicides, and the management of plant fertility may also be a viable strategy. However, research is needed to assess how different factors may affect mycotoxin synthesis under tropical conditions.

The nitrogen rates and sources have been poorly studied in Brazil, where the climatic conditions are most favorable to grain infection by fungi and fumonisin production. Thus, the influence of N doses and sources on maize hybrids was assessed regarding the leaf nutrition, yield, and fumonisins concentration.

2. Material and Methods

2.1. Experimental conditions

Two experiments using N fertilizer rates (0, 80, 160, and 240 kg N ha^{-1}) and N sources (ammonium nitrate - AN, urea - Ur, polymer-coated urea - Ur Pol, and UAN) were conducted under irrigation in the experimental area of Embrapa Maize and Sorghum at Sete Lagoas City, Minas Gerais State (MG), in the years of 2016 and 2017. Additionally, in irrigated conditions, the N-rates experiment was conducted in Paracatu, MG (2016) and N sources in Patos de Minas, MG (2017). Before implementing the experiments, soil samples were collected for chemical analysis and determination of clay content. The results of the soil analysis are shown in Table 1.

The experimental design was subdivided into plots with four replicates. The N rates experiments consisted of a 4x2 factorial, with four N rates (0, 80, 160, and 240 kg ha^{-1}) in a topdressing application, with a urea source and two genotypes of maize. The treatments of the N sources experiment comprised a 4x2 factorial, four sources of N (ammonium nitrate, urea, polymer-coated urea, and UAN) at a 160 kg ha^{-1} of N in a topdressing application, and two maize genotypes. The 160 kg ha^{-1} was used because it is a good value related to the maize demand for high yields. In the experiments of N rates in Sete Lagoas, the genotypes used were P3630H and AG7098 PRO2 in 2016 and AG8677 PRO2 and P30F53 (conventional) in 2017. In the N sources experiments, the genotypes were AG7098 PRO2 and P30F53YH in Paracatu and Patos de Minas in both years. All genotypes are early-cycle single hybrids.

The experimental plot was four lines five meters long, spaced 0.7 m in Sete Lagoas and spaced 0.5 m in Paracatu and Patos de Minas. The valuable data were collected from the three-meter-long central lines, excluding one meter at each end.

In both experiments (N rates and N sources) in 2016, the planting in Sete Lagoas occurred on December 2, 2016 (off-season corn). The fertilization carried out during planting was 400 kg ha^{-1} of the formulated 08-28-16 and 50 kg ha^{-1} of FTE BR12. In V4, about 20 days after planting (DAP), cover fertilization was performed according to the proposed treatments. During the flowering period, 30 leaves on the opposite side and below the primary ear were collected for levels of leaf N analysis. The experiments were harvested on July 14, 2016.

In the second year, in the two experiments, the corn was planted in Sete Lagoas on December 2, 2016, with 370 kg ha^{-1} of the formulated on August 28, 2016. The same N rates and source treatments were used in coverage at 20 DAP. According to the abovementioned methodology, the leaves were harvested for nutritional diagnosis during flowering. The data were collected on April 20, 2017.

In Paracatu, maize was planted on February 22, 2016, in the N rates experiment (off-season corn). In pre-planting and on all plots, 150 kg ha^{-1} 00-00-52 + 1.33% B was applied. 300 kg ha^{-1} of Robustto 13-34-00 and 2 L ha^{-1} of Quimifol Arrank was applied at planting. In this case, the treatments with N doses occurred on the planting day. For disease control, two Pyraclostrobin + Epoxiconazole applications were performed (0.75 L ha^{-1} / application) in the V7 and VT stages. According to the methodology described, the leaves were collected for nutritional evaluation during the flowering period. The experiments were harvested on July 19, 2016.

Table 1. The soils' chemical characteristics and clay content before the installation of field experiments in Sete Lagoas, Paracatu, and Patos de Minas.

Sete Lagoas								
Depth	**pH**	**MO**	**P**	**K**	**Ca**	**Mg**	**Al**	**H+Al**
cm	**H_2O**	**dag kg^{-1}**	**----mg dm^{-3}----**		**--------------cmol$_c$ dm^{-3}--------------**			
0-20	5.50	3.78	7.71	45.46	3.69	1.09	0.07	5.33
20-40	5.45	3.15	3.57	38.34	2.60	0.79	0.15	5.26
Depth	SB	T	V	Cu	Fe	Mn	Zn	Clay
cm	---cmol$_c$ dm^{-3}---		%	--------------mg dm^{-3}--------------				dag kg^{-1}
0-20	4.89	10.22	47.88	0.80	32.20	27.09	1.06	65.00
20-40	3.48	8.74	39.92	0.87	31.26	19.70	0.70	
Paracatu								
Depth	pH	MO	P	K	Ca	Mg	Al	H+Al
cm	H_2O	dag kg^{-1}	----mg dm^{-3}----		--------------cmol$_c$ dm^{-3}--------------			
0-20	5.40	2.23	7.87	97.00	3.16	1.21	0.18	3.62
Prof.	SB	T	V	Cu	Fe	Mn	Zn	clay
cm	---cmol$_c$ dm^{-3}---		%	--------------mg dm^{-3}--------------				dag kg^{-1}
0-20	4.62	8.24	56.07	1.90	27.10	22.80	4.40	50.00
Patos de Minas								
Depth	pH	MO	P	K	Ca	Mg	Al	H+Al
cm	H_2O	dag kg^{-1}	----mg dm^{-3}----		--------------cmol$_c$ dm^{-3}--------------			
0-20	5.64	3.45	2.69	156.00	2.00	0.89	0.05	3.43
Depth.	SB	T	V	Cu	Fe	Mn	Zn	Clay
cm	---cmol$_c$ dm^{-3}---		%	--------------mg dm^{-3}--------------				dag kg^{-1}
0-20	3.30	6.72	48.99	2.60	29.95	14.30	1.15	85.00

NOTE: pH in water, ratio 1:2.5 ADFS (Air-Dryied Fine Soil); P, K, Cu, Mn, Fe, and Zn extracted by Mehlich-1 method; Ca, Mg, Al - extracted by KCl method 1 mol L^{-1}; H+Al - extracted by Calcium acetate 0.5 mol L^{-1} - pH 7.0; MO - Walkey & Black method (MO = 1.724 x CO).

Before planting, in Patos de Minas, in the N sources experiment, two tons ha^{-1} of dolomitic limestone (32% Ca, 18% MgO, and 95% PRNT), one ton ha^{-1} of Agricultural Plaster, and 200 kg ha^{-1} of 00- 00-48 with 2% B was applied. Planting took place on December 7, 2016, with an application of 230 kg ha^{-1} of MAP, when the proposed nitrogen fertilization treatments were also applied.

2.2. Data collection and statistical analysis

The nutritional status of plants was evaluated in the flowering stage, and the grain yield (13% humidity) and the total fumonisins concentration (TF) at harvest time. The total fumonisins concentration (TF) was calculated by adding fumonisins FB1 and FB2 (Teixeira et al., 2017). The 500 g of grains of maize were submitted to fumonisins analysis performed with the HPLC-MS/MS technique. The quantification limits being 125.0 µg kg^{-1} and the uncertainty limits for FB1 (± 33.2 µg kg^{-1}; K = 2.18) and FB2 (± 35.2 µg kg^{-1}; K = 2.52). The fumonisin concentration was given in µg kg^{-1} (ppb). Samples with more than 5,000 µg kg^{-1} were considered above the tolerance limit following RDC No. 07, February 18, 2011, in full force since January 1, 2017 (Brasil, 2011).

The variables Yield, N leaf content, and total fumonisins were subjected to analysis of variance for each crop season and location. When necessary, treatment means were compared using the Tukey test to compare the averages of the experiment with nitrogen sources at a 5% probability. The experiment with nitrogen doses was adjusted using linear regression analysis, with a 5% error probability using the Sisvar program (Ferreira, 2011).

Data on humidity and temperature were collected to assess water availability during the crop cycle, which interferes with plant development and disease incidence. These data were used to calculate the hydrothermal coefficient of water supply (K), according to the Sielianinov hydrothermal index: K = (10P) / (DT), where P is the monthly amount of precipitation [mm], D is the number of days, and T is the average daily air temperature for the month [°C]. The outstanding value of the coefficient was one (1). The Sielianinov index (K) is the water coefficient that guarantees the water or moisture balance (Radomski et al., 1977). The K index is used in agricultural climatology to assess the duration and intensity of drought, with K> 1 = wet period and K <1 = dry period.

3. Result and Discussion

3.1. Experiments with genotypes and nitrogen rates

The analysis of variance of data from experiments on maize genotypes and N rates showed no effect of the N rates and the interaction of the genotype for all variables analyzed (P>0.05). The three experiments' genotypes influenced the production variable (P<0.005). N doses influenced the leaf N content in the three experiments (P<0.001). The total Fumosins content was influenced only by the genotypes in the experiment conducted in Sete Lagoas in 2016 (P=0.0001).

Considering the genotypes, the hybrids AG7098 PRO2 (11,497 kg ha^{-1}, Sete Lagoas, and 10,892 kg ha^{-1}, Paracatu, the Year 2016) and AG8677 PRO2 (7,662 kg ha^{-1}, Sete Lagoas, Year 2017) were more productive than the hybrids P3630H (9,478 kg ha^{-1}, Sete Lagoas, Year 2016), P30F53 (2,117 kg ha^{-1}, Sete Lagoas, Year 2017), and P30F53YH (8,861 kg ha^{-1}, Paracatu, Year 2016). However, possibly due to dilution, the relationship was equal or reversed regarding the nutritional aspect by measuring the nitrogen leaf contents.

In 2017, at Sete Lagoas, there was a high incidence of Maize Bushy Stunt Phytoplasma and Corn Stunt Spiroplasma, drastically reducing the susceptible genotype's productivity P30F53 (2,117 kg ha^{-1} of grains) (Cota et al., 2018). On the other hand, the hybrid AG8677 PRO2 showed a lower stalking incidence, and its productivity was 7,662 kg ha^{-1}.

Regarding the total fumonisins, the hybrid AG7098 PRO2 showed lower fumonisins concentrations than hybrid P 3630H in 2016 at Sete Lagoas (Figure 1). The total fumonisins content for AG7098 PRO2 and P3630H was 1,239 µg kg^{-1} and 11,254 µg kg^{-1}, respectively (Figure 1). Therefore, the total fumonisins value for the genotype P3630H was above the Maximum Tolerance Limit (MTL) for corn grains in Brazil, corresponding to 5,000 µg kg^{-1} (Brasil, 2011).

According to the European Commission (2006), choosing the hybrids or varieties most suitable for edaphoclimatic conditions and agronomic practices is essential to reduce plant stress and make crops less susceptible to fungal infections. Likewise, developing genotypes for resistance to fungi and insects that contaminate seeds under Brazilian climates must also be prioritized.

Considering the N rates, the yield of the hybrids in Sete Lagoas in 2016 and the foliar levels of N in the two locations over two years increased linearly (Figure 2). This result corroborates several studies showing the high potential of corn grain yield and N leaf contents in response to nitrogen fertilization (Peruzzo et al., 2015; Portugal et al., 2017; Scotta et al., 2018; Moreira et al., 2019). There was no effect for total fumonisins, and the two hybrids' average values were below the LMT, except at Sete Lagoas in 2016 for the N rates of 0, 80, and 240 kg ha^{-1}.

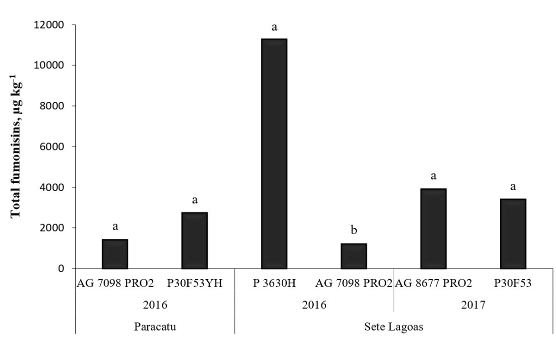

Figure 1. Average values of total fumonisins (TF, µg kg^{-1}) as a function of the corn genotypes (AG 7098PRO2, P30F53YH, P3630H, AG8677PRO2) in experiments of N doses conducted at Paracatu, Year 2016, and at Sete Lagoas, years 2016 and 2017. Maize genotypes means followed by the same letters each year, and location do not differ at the 5% probability level by the Tukey test.

This result differs from previous studies showing a higher incidence of fumonisins in insufficient nitrogen doses. On the contrary, Marocco et al. (2008) reported an increase of 70 to 99% in fumonisins in corn in two years due to increased nitrogen fertilizer levels. Nevertheless, Miguel et al. (2015) used 80, 160, and 240 kg ha^{-1} of nitrogen fertilizer and two sources of urea (urea and urea + NBPT urease inhibitor) to determine the effects of nitrogen fertilization on corn contamination by fungi and fumonisins incidence. The authors found high concentrations of fumonisins (19.49 µg/g) in the treatment without nitrogen application and lower levels of fumonisins with 160 kg ha^{-1} of urea. Therefore, the correlation between nitrogen fertilizer dosages and fumonisin contamination (-03780; p<0.05) was negative and significant. Also, nitrogen rates reduce fumonisins in maize plants (Blandino et al., 2008). Scarpino et al. (2022) evaluated the effect of nitrogen fertilization rates ranging from 0 to 400 kg ha^{-1}. They concluded that the contamination by fumonisins increased in soils with low fertility. However, it was observed that higher levels of N favored the production of deoxynivalenol (DON) and zearalenone in corn. The authors recommend a balanced application of N to reduce contamination by various types of mycotoxins. Other mycotoxins and toxic metabolites that may pose risks to public health due to their presence in food and feed were also evaluated. These mycotoxins are produced by *Fusarium* spp session Liseola (beauvericin, bikaverin, fusaric acid, fusaproliferin, fusarin C, and moniliformin) and *Fusarium* spp. sections Discolor and Roseum (aurofusarin, butenolide, culmorin and nivalenol). The results show that mycotoxins produced by species of *Fusarium* spp. from the Liseola session increased with low N fertility and that mycotoxins produced by *Fusarium* spp. from the Discolor and Roseum sessions increased with the increase in the applied N rate. The occurrence of multiple mycotoxins can result in additive or synergistic effects (Scarpino et al., 2022). As a recommendation, the authors suggest a balanced application of N, considered between 200 and 300 kg N ha^{-1}, when ensuring a lower risk of contamination by mycotoxins.

3.2. Experiment with genotypes and nitrogen sources

The analysis of variance of the data from maize genotypes and N source experiments showed no effect of the N source and the interaction of the genotype for all variables analyzed: on yield (P>0.54), N leaf content (P>0.23), and total fumonisins (P>0.54).

Considering the genotypes and following the same trend as the experiments with genotypes and N rates, hybrid AG8677 PRO2 showed a superior yield (8,321 kg ha^{-1}) than the hybrid P30F53 (2,486 kg ha^{-1}) in the year 2017 at Sete Lagoas (P=0.0001) and AG7098 PRO2 (13,715 kg ha^{-1}) than the hybrid P30F53YH (10,829 kg ha^{-1}) in the year of 2017 at Patos de Minas (P=0.0001). However, in 2017, at Sete Lagoas, like the N rate test, there was a high incidence of plant stunting, drastically reducing the productivity of P30F53, a susceptible genotype.

The N leaf content differed between the hybrids AG7098 PRO2 (35,3 g kg^{-1}) and P 3630H (39,0 g kg^{-1}) at Sete Lagoas in 2016 (P=0.0001). However, the average values of N leaf contents indicate adequate corn nutrition, that is, within the sufficiency range (Martinez et al., 1999).

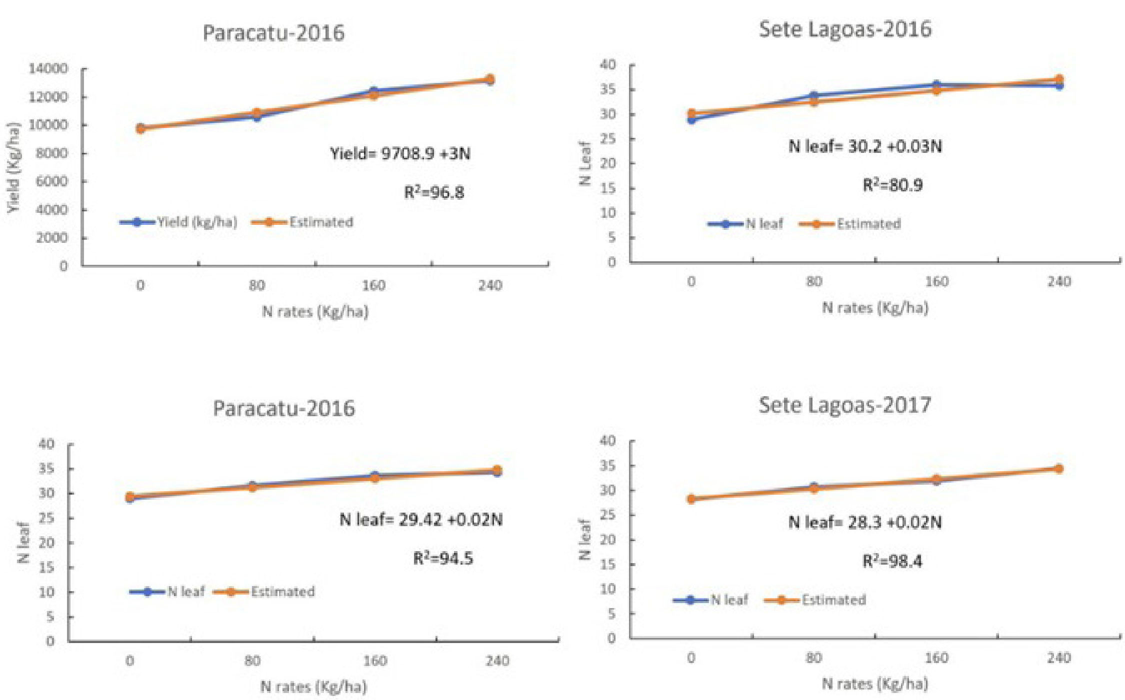

Figure 2. Simple linear regression analysis for the production variables (Paracatu-2016) and N foliar (Paracatu-2016, Sete Lagoas-2016/2017) depending on the N dose applied.

There was a significant difference in their total levels of fumonisins only for the corn genotypes factor in Sete Lagoas in 2017 (P=0.0001) (Figure 3). The total fumonisins values were 8,440 and 1,046 µg g^{-1} for AG8677 PRO2 and P30F53, respectively. The first value was above the LMT value for fumonisins (Brasil, 2011). In 2016, fumonisins were not evaluated in Sete Lagoas. However, in Patos de Minas, the total fumonisins level was below the LMT for the two hybrids tested, with 1,497 µg kg^{-1} for AG 7098 PRO 2 and 1,361 µg kg^{-1} for P30F53YH, which did not differ from each other (P=0.82) (Figure 3).

There is variability among corn hybrids regarding susceptibility to fumonisin-producing *Fusarium* species and also to mycotoxin synthesis (Gesteiro et al., 2021; Lanza et al., 2016; Van Rensburg et al., 2016; Santiago et al., 2015; Cao et al., 2014). Lanza et al. (2016) observed variations in the percentage of burnt grains, the incidence of mycotoxin-producing species, and the concentration of total fumonisins for four maize hybrids. The hybrid's effect on fumonisins' occurrence was also found in other studies (Santiago et al., 2015; Cao et al., 2014).

Considering the N sources, the sources tested did not affect the yield, N leaf content, and total fumonisins. In Sete Lagoas, in 2016 and 2017, the treatments with N sources were in a cover application (topdressing applications) 20 days after planting. Differently, in Patos de Minas 2017, the treatments were applied during the planting period. For N alternative sources, the anticipation of fertilizer application is an important option that can favor the expression of the aggregated technology, leading to a slower or delayed release of N, which reduces the N volatilization. Almeida (2016) showed that plants' responses to N sources are quite variable, with situations in which the aggregated technology stands out concerning the urea sources, and others do not. Furthermore, in several studies (Shim and Woloshuk, 1999; Blandino et al., 2008; Bouras et al., 2016), it has been shown that the sources of N and Carbon can control mycotoxin biosynthesis.

Although without significant effect, the low values of total fumonisins obtained from UAN sources in Sete Lagoas and Patos de Minas are worth mentioning. Thus, it can be inferred that if the standard deviation for fumonisins was not high, a significant difference could be detected between the N sources.

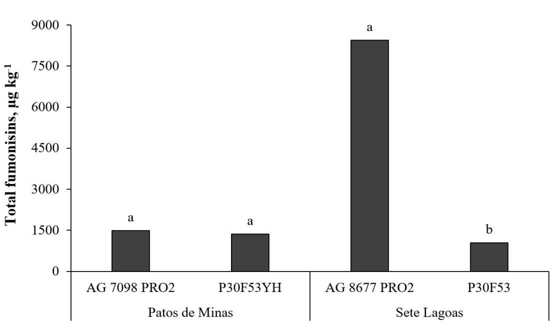

Figure 3. Average values of total fumonisins (TF, µg kg^{-1}) as a function of the corn genotypes (AG7098PRO2, P30F53YH, AG8677PRO2) in experiments of N sources conducted at Patos de Minas and at Sete Lagoas in 2017. Means followed by the same letters in each location do not differ at the 5% probability level by the Tukey test.

Different results have been observed in other studies. For example, Bocianowski et al. (2019) evaluated the mycotoxin production in two maize hybrids fertilized with different N sources. According to these authors, different N sources can reduce or increase grain contamination fungi but prevent mycotoxin synthesis.

Blandino et al. (2008) suggested that the slow release of nitrogen fertilizer might result in mycotoxin production by favoring the plant to stay green until the grain maturation, thus providing an ideal condition for fungi development. In contrast, Bocianowski et al. (2019) stated that the stay-green condition fertilized with urea leads to a lower accumulation of mycotoxins than cultivars without this characteristic. Therefore, the authors recommended plant fertilization with slow-release nitrogen fertilizer.

Adequate mineral fertilization, particularly nitrogen fertilization and sowing periods, can reduce the infestation of plants by *Fusarium* spp. (Bocianowski et al., 2019). Although there are recommendations that different N sources reduce fungi contamination, excess N in the soil increases the grain infection incidence.

This work and others showed that several factors could influence maize infection by toxigenic fungi and determine the type of prevalent mycotoxin. Among those factors, climatic conditions, different stresses, planting time, harvest time, plant density, and hybrid resistance are predominant (Shuman, 1994; Blandino et al., 2008; Bocianowski et al., 2019; Kamle et al., 2019).

3.3. Hydrothermal conditions

The thermal and humidity conditions varied during the crop seasons (Table 2). In Sete Lagoas, the hydrothermal conditions were favorable for the maize crop until April 2016. The planting and harvest were on 12/02/2016 and 07/14/16, respectively; thus, the hydrothermal conditions were better at the beginning of the crop development, up to 30 DAS. In the 2016/17 harvest, better conditions were observed between December 2016 and February 2017 (K> 1), when the weather was drier until the harvest on 04/20/2017. In this area, there was supplemental irrigation in the driest periods. In Paracatu, between sowing (02/22/2016) and harvest, the hydrothermal conditions were less favorable to the culture. However, irrigation was used in this location (Table 2).

According to the K coefficient score, in Patos de Minas, there was good water availability for the plants from December to March, the period indicated for planting (12/07/2016). Until 90 days after sowing, the hydrothermal conditions were favorable to crop growing, followed by drier periods starting in April, the period closest to the harvest (Table 2). Therefore, in this area, there was no supplemental irrigation.

In this work, hydrothermal stress was observed mainly in the final stages of crop development, which is a common characteristic, mainly in second crops. This situation may have favored the synthesis of mycotoxins. In addition, the high humidity and relative humidity levels favored the fungal infection and their development on the ears, mainly during the flowering period to maturation. Additionally, N applications at high concentrations may favor mycotoxin production due to prolonged vegetative growth and leaf expansion (Logrieco et al., 2002; Blandino et al., 2008; Scarpino et al., 2022).

Table 2. Monthly rainfall, average temperature, and K index during the corn seasons.

Place	Year	Month	Precipitation (mm)	Temperature (°C)	Days	Index K	Cultural stage
Sete Lagoas	2016	February	67.0	23.5	29	0.99	Planting 02/12
		March	112.0	22.8	31	1.59	30 DAS
		April	34.0	23.4	30	0.48	60 DAS
		May	5.0	21.4	31	0.08	90 DAS
		June	27.0	19.2	30	0.46	120 DAS
		July	0.0	20.4	30	0.00	Harvest 07/14
Sete Lagoas	2016	December	227.0	22.4	31	3.39	Planting 12/02
	2017	January	110.0	23.2	31	1.53	30 DAS
		February	127.0	22.5	28	2.01	60 DAS
		March	93.0	23.0	31	1.30	90 DAS
		April	9.0	23.1	30	0.13	Harvest 04/20
Paracatu	2016	February	46.0	26.8	29	0.59	Planting 02/22
		March	154.0	25.7	31	1.92	30 DAS
		April	2.0	25.4	30	0.03	60 DAS
		May	3.0	23.6	31	0.04	90 DAS
		june	0.0	21.9	30	0.00	120 DAS
		July	0.0	22.4	31	0.00	Harvest 07/19
Patos de Minas	2016	December	241.0	17.9	31	4.4	Planting 12/07
	2017	January	119.0	18.5	31	2.06	30 DAS
		February	292.0	18.1	28	5.75	60 DAS
		March	100.0	18.0	31	1,80	90 DAS
		April	4.0	17.9	30	0.08	120 DAS
		May	40.0	15.3	31	0.85	Harvest 05/05

The K index is based on the number of monthly days. DAS = days after sowing.

However, stress due to drought or during fertilization can make the corn crop more vulnerable to mycotoxin-producing pathogens (Munkvold and Desjardins, 1977; Miller, 2001; Blandino et al., 2008). Scarpino et al. (2022) found a much higher level of contamination by fumonisins in 2012 (1,847 µg kg^{-1}) than in 2013 (377 µg kg^{-1}). Thus, as in this work, there were different precipitation and temperature trends during 2012 and 2013, with drier conditions prevailing in 2012 during the maturation phase. Considering the doses, there was a significant increase (+134%) in the contamination by fumonisins in 2012 when N was not applied, compared to the other doses, which did not differ. In 2013, there was no difference between doses and more significant precipitation during maturation. These results suggest that water stress during grain maturation may favor the production of mycotoxins.

Using cultural management, controlling *Fusarium* contamination in the field is challenging since several factors influence the fungus/plant interactions. Among them are climatic conditions, biological interactions between plants and other organisms in the soil, and the natural conditions providing good development of the plants (Ferrigo et al., 2016; Oldenburg et al., 2017). Nevertheless, the results and other studies show that adequate management in the field is relevant for reducing mycotoxin contamination produced by *Fusarium* species (Diniz et al., 2021, 2022). Among these management practices, balanced fertility and good soil practices, crop rotation, insect control, choice of genotypes, reduction of water stress, sowing planning, pre-harvest health assessment, and proper storage are relevant (Scarpino et al., 2022; Battisti et al., 2022; Blandino et al., 2022; European Commission, 2006). Thus, many variables affecting mycotoxin production turn into difficult decisions about the most appropriate disease management. Also, multi-toxins should be considered due to the possibility of a specific management strategy while decreasing one mycotoxin incidence, which may favor the synthesis of another in the function of positive or negative interactions between them.

4. Conclusions

The adequate soil fertility levels in the experiments may have favored the lack of yield response to N doses and sources in most areas and the manner of N application in coverage at 20 days after planting.

The nitrogen doses and sources did not affect fumonisins accumulation, which varied between the tested sites.

There was a genotype effect on the incidence of total fumonisins. Therefore, the control strategy recommended for the maize crop is genotype resistance to fumonisin accumulation. The hybrid AG7098 PRO2 showed the highest yield (above 10,000) and fumonisins values below the maximum tolerated limit in Brazil (5,000).

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