

Article

Influence of Sowing Time on *Fusarium* and Fumonisin Contamination of Maize Grains and Yield Component Traits

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Abstract: The main aim of this research was to study the effect of two sowing times (early and late) on *Fusarium* and fumonisin contamination and some yield component traits of two maize hybrids from the FAO maturity groups 500 (ZP 560) and 600 (ZP 666) within a two-year growing season (2016–2017). *F. verticillioides* and *F. subglutinans* have been identified as *Fusarium* ear rot (FER) pathogens and potential producers of B-type fumonisins (FBs), with *F. verticillioides* as the predominant *Fusarium* species in both years. The incidence of *F. verticillioides* and FB levels were affected by sowing time and maize hybridity. With early sowing and the mid-maturity hybrid ZP 560, *F. verticillioides* and FB contamination were lower than with late sowing and the late-maturity hybrid ZP 666. Yield parameters also differed significantly between sowing time and maize hybrid treatments. Early sowing increased ear length (EL), number of grains per ear (NGE), grain weight per ear (GWE), and grain yield per hectare (GY). The late-maturity hybrid ZP 666 had higher yield component traits and GY than the mid-maturity hybrid ZP 560. EL, GWE, thousand-grain weight (TGW), and GY were affected by year. Interactions between sowing times and maize hybrids were highly significant ($p \leq 0.01$) for FB level, GWE, and GY. The obtained results indicate the importance of applying early sowing to achieve high maize grain yields with lower contamination by *F. verticillioides* and FBs. Although mid-maturity hybrid ZP 560 and late-maturity hybrid ZP 666 showed significant differences in terms of levels of *F. verticillioides* and FB contamination, both were susceptible to *F. verticillioides*, with high FB levels. These results should be useful to breeders of maize hybrids to create genotypes more resistant to these fungal contaminants.

Keywords: sowing time; maize hybrids; *Fusarium* spp.; fumonisins; yield component traits



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1. Introduction

After rice and wheat, maize (*Zea mays* L.) is the third most important cereal crop worldwide. It is used for food, feeds, and biofuel production. In Serbia, maize is grown on 996,527 ha, with an average yield of 7.9 t ha⁻¹ and a total production of 7,872,607 tons in 2020. The total production of maize for fodder in 2020 was 746,926 tons grown on 35,663 ha [1]. Maize diseases deteriorate grain yield and quality. Ear rot is the most common maize fungal disease caused by *Fusarium* spp. In Europe, Gibberella ear rot (red ear rot or red fusariosis) is caused by pathogens of the *Fusarium graminearum* species complex (FGSC), with *F. graminearum sensu stricto* (s.s.) being the most studied [2,3], while Fusarium ear rot (FER) (pink ear rot or pink fusariosis) is caused by *Fusarium* species from the *Fusarium fujikuroi* species complex (FFSC), primarily by *F. verticillioides* (Saccardo) Nirenberg (*Gibberella moniliformis* (Wineland)) [4,5]. There are several possible pathways of maize infection. One of the most common is via airborne conidia which mature on the silk. Entering through the silk, conidia infect the grains, but a low percentage of grains show

symptoms; they are mostly asymptomatic. Systemic infection through the seeds is another pathway. From the seeds, the fungus continues to develop in the roots, stem, and cob. However, the infection of grains via silk is more common than through seeds [6]. Although simultaneous contamination of maize grains by more than one *Fusarium* species is common, *F. verticillioides* is the predominant species. It is an endophyte that survives for a long time in the plant. It can be found in both symptomatic and asymptomatic maize grains [7].

Fusarium species produce different mycotoxins (secondary metabolites) affecting the quality of crops and human and animal health. Fumonisin is a major mycotoxin in maize and maize products [8]. There are more than 15 fumonisin homologs (A, B, C, and P), among which B-type fumonisins (FBs) are the most abundant. Fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), and fumonisin B₃ (FB₃) are the most frequent forms. FB₁ is the most toxic compound [9]. FBs are secondary metabolites produced by *F. verticillioides*, *F. proliferatum* (Matsushima) Nirenberg, and related species. They were first isolated in 1988 from *F. verticillioides* (previously known as *F. moniliforme* Sheldon) [10]. A wide range of mycotoxins are produced by *F. subglutinans* (Woll. & Reink.) Nelson, Tousson & Marasas, including fumonisins [11,12]. Fumonisin can cause acute and chronic diseases in animals and humans. The International Agency for Research on Cancer (IARC) classified them as human carcinogens in the 2B group [13]. The crucial enzyme in the biosynthesis of sphingolipids, lipid components in eukaryotic cell membrane structure, ceramide synthase, is inhibited by FB₁ and hydrolyzed FB₁ [14]. Ingestion of fumonisin-contaminated food can lead to mycotoxicosis in humans and animals. Some health issues in humans caused by fumonisins are esophageal and liver cancers, neural tube defects, growth impairment, and birth defects. Fumonisin can also be hepatotoxic and nephrotoxic in animals. Animal diseases caused by fumonisins involve multiple organs (liver, kidneys, lungs, brain, and others) and include developmental disorders and cancer [15,16]. An equine leukoencephalomalacia and a porcine pulmonary edema syndrome are caused by high levels of fumonisins in animal feeds [17,18]. Feed contamination by mycotoxins may cause serious health problems, economic losses, and reduced livestock productivity. Hence, many countries have defined maximum levels of main mycotoxins, including FBs, for human food and animal maize-based feeds. According to European regulations, the maximum permissible levels of FBs (sum of FB₁ and FB₂) are 4000 µg kg⁻¹ in unprocessed maize and 1000 µg kg⁻¹ for human consumption (Commission Regulation 2007/1126/EC) and 60,000 µg kg⁻¹ in maize and maize products intended for animal feeding (Commission Recommendation 2006/576/EC). Mycotoxin levels in grains are not genetically determined since they are connected with agricultural cultivation and practice [19].

The occurrence and prevalence of *Fusarium* spp. and the production of fumonisins can be affected by climatic conditions. FER epidemics occur commonly in dry years and are favored by warm, dry weather during a grain-filling stage. Droughts at the beginning of the growing season and wet weather during pollination and silking stages can favor the growth of FER causative agents as well as fumonisin synthesis in harvested maize grain [20,21]. Commercial maize hybrids vary in their susceptibility to *Fusarium* infection and fumonisins. There are no hybrids resistant to FER. However, hybrids resistant to insect-borne diseases may have lower fumonisin levels [22]. The lack of highly resistant hybrids to fumonisin contamination calls for the application of integrated pest-management strategies. Many agricultural pre-harvest practices, such as tillage, management of crop residues, crop rotation, sowing time, plant density, fertilization, insect control, and harvest time, have a significant impact on *Fusarium* spp. and fumonisin control [20,23,24]. Few data exist on the effect of sowing time on *Fusarium* spp. and fumonisin maize contamination. According to Blandino et al. [25–27], sowing time has a significant effect on the incidence of *Fusarium* spp. and the level of mycotoxins in maize grain. In warm regions, sowing times may be altered in short-season maize hybrids in order to reduce mycotoxin contamination. Depending on the locality and weather conditions during the silking period, the sowing time should be determined so as to reduce the heat stress on maize plants [28]. Blandino et al. [27] have reported that *Fusarium* spp. were more prevalent in maize grown in dryland than in

optimal moisture conditions. Further, late sowing increased *Fusarium* infection in maize grains [27]. Parsons and Munkvold [29] have also stated that maize grain colonization by *F. verticillioides* was more severe in dryland than in irrigated plots. In regions with temperate climates, early sowing time can significantly reduce mycotoxin contamination in maize grains, but annual climatic changes can reverse it [22]. Given that Serbia is a leader in producing and exporting maize among the Balkan countries, high-yielding and high-quality production per unit area is mandatory in modern sustainable maize grain production. In Serbia, there are no data in the literature on the influence of sowing time on fungal and mycotoxin maize contamination, so all relevant data are of great importance. Therefore, the main aim of this research was to assess the effect of two sowing times (early and late) on the incidence of *Fusarium* spp. and FB levels in the grain of the two commercial maize hybrids from the two FAO maturity groups (500 and 600) during two growing seasons (2016–2017). Certain yield component traits and grain yield were evaluated as well.

2. Materials and Methods

2.1. Field Trials and Treatments

Field trials were conducted at the Institute for Animal Husbandry, Belgrade-Zemun, Serbia (44°84' N, 20°40' E; 88 m a.s.l.) during two growing seasons (2016–2017). The two maize hybrids, ZP 560 (FAO maturity group 500) and ZP 666 (FAO maturity group 600), were tested at two sowing times (8 April—early time of sowing and 26 April—late time of sowing). The hybrids were selected based on their high prevalence in maize production in the area where the experiment was performed.

The field trials were arranged in a split-split plot design with four replicates. The main plots were maize hybrids and the subplot treatments were the two sowing times. The total plot size was 16.8 m², and the sub-plot sizes were 6 m × 2.8 m, with a 70 cm inter-row spacing. Every sub-plot consisted of four rows. The soil type was chernozem with 1.41% CaCO₃, >5% soil organic matter, pH 7.8 in KCl, 0.258% total N, 19.08 mg 100 g⁻¹ of soil phosphorus, and 17 mg 100 g⁻¹ of soil potassium. The preceding crop was wheat in both experimental years. The fertilization with ammonium nitrate 120 kg N ha⁻¹ was applied at the five-leaf to six-leaf maize stages. Pre-emergence and post-emergence herbicides were used to control weeds.

2.2. Evaluation of Qualitative and Productive Traits of Maize Grain Samples

In early October 2016 and 2017, the maize harvest was performed manually when the grain moisture content was below 26%. Harvested ears were manually dehusked and shelled. The sample size was about 2 kg per each sub-plot. Before analyses, maize grain samples were kept at 4 °C.

According to the previously described method of Krnjaja et al. [30], the incidence of fungal species was evaluated using 50 maize grains sampled from each replication. Grains were firstly disinfested in 1% sodium hypochlorite (NaOCl) for 3 min, then rinsed with sterile distilled water, dried on Whatman filter paper, and plated on potato dextrose–7.5% salt agar in Petri dishes (5 grains per plate). Incubation of plates was for 14 days at room temperature. Based on the morphological characteristics of the fungal colonies that grew around the maize grains, fungal species were identified using fungal keys by Watanabe [31] and Leslie and Summerell [32]. The appearance and color of the colony, conidiophore branching, formation of phialide, and presence or absence of micro- and macroconidia, chlamydospores, sporodochia, etc., were the main criteria for fungal species identification. The percentage incidence of *Fusarium* spp. was calculated as the ratio of the number of grains infected by *Fusarium* spp. and the total number of grains multiplied by 100.

Maize grain sub-samples of about 200 g from each sub-plot were dried for 72 h at 60 °C, then ground in an analytical mill (IKA A11, Staufen, Germany) and analysed for FBs. By enzyme-linked immunosorbent assay (ELISA), using the kit *Celer FUMO* (Tecna, Rimini, Italy), FBs in grain samples were quantified according to the assay procedure. The ground sample was mixed with sodium chloride and 70% methanol and shaken in

a blender for 3 min. After extraction, the sample was added to a premix microwell with enzyme-conjugated fumonisin, mixed, and transferred to anti-fumonisin antibody-coated microwells. Incubation of these microwells was performed in the dark for 10 min at room temperature. During incubation, fumonisin from the extracted sample and enzyme-conjugated fumonisin competed to bind with the anti-fumonisin antibody that coated the microwells. After incubation, microwells were washed three times with buffer to remove unbound conjugate and non-specific reactants. In washed microwells, the chromogen solution was added and incubated for 10 min. At that time, the blue color developed from chromogen as an enzymatic reaction between the bound conjugate with chromogen. The intensity of the blue color is indirectly proportional to the fumonisin level in the sample. If the level of fumonisin increases, the intensity of the blue color will decrease. Finally, the enzyme reaction was stopped by adding an acidic solution, changing the chromogen color from blue to yellow. The absorbances of microwells were read optically by an ELISA reader (Biotek EL × 800TM, Winooski, VT, USA) at a short wavelength of 450 nm. The detection limit for FBs in maize was 0.75 mg kg⁻¹.

Yield component traits, ear length (EL), number of grains per ear (NGE), grain weight per ear (GWE), and thousand-grain weight (TGW) were analysed in the grains of ten ears per each replication. Grain yield (GY) was calculated for 14% ear moisture content from the two central maize rows in each sub-plot.

2.3. Statistical Analysis

Statistical analysis of experimental data was performed using SPSS software (IBM SPSS Statistic 20). The effects of two sowing times on *Fusarium* incidence, FB levels, and yield parameters in the two maize hybrids were evaluated by ANOVA (analysis of variance) according to the random block design with four replications. Statistical significance was determined by the Fisher test (*F*-test) at $p \leq 0.05$ and $p \leq 0.01$ levels. In pairwise comparisons, the treatment means were determined using Tukey's test at $p \leq 0.05$. Pearson correlations were calculated to evaluate relationships between the investigated parameters.

3. Results

3.1. Climatic Data

Based on the climatic data of the Republic Hydrometeorological Service of Serbia (Belgrade-Surčin area), in the maize growth period (April–October), the total rainfall (488.3 mm) and mean relative humidity (RH) (69.6%) were higher, while the mean monthly temperature was lower (18.5 °C) in 2016 compared to 2017 (337.6 mm, 61.6%, and 19.3 °C). The mean monthly temperatures during the maize silking in July 2016 and 2017 were 23.6 °C and 25 °C, respectively (Figure 1). These temperatures above 20 °C were very suitable for maize infection by FER pathogens. High temperatures continued in August 2016 (21.9 °C) and 2017 (25.2 °C). Mean temperatures, mean RH, and total rainfall were 19.2 °C, 60.1 mm, and 68% and 18 °C, 58.2 mm, and 65% during the grain filling and maturity stages in September 2016 and 2017, respectively (Figure 1).

3.2. Mycological and Fumonisin Analyses

Species within the *Fusarium graminearum* species complex (FGSC), *F. subglutinans*, and *F. verticillioides* were identified on the maize grains in both years, except for FGSC species in 2017. *F. verticillioides* and *F. subglutinans* belong to the *Fusarium fujikuroi* species complex (FFSC) and were potential producers of FBs. *F. verticillioides* was prevalent in all maize treatments tested, while FGSC species and *F. subglutinans* were isolated sporadically with a low incidence. The significance of the tested factors on the incidence of *F. verticillioides* and FB levels is shown in Table 1. The incidence of *F. verticillioides* and FB levels were significantly ($p \leq 0.01$) affected by both hybridity and sowing time. In early sowing treatments, mid-maturity hybrid ZP 560 had lower *F. verticillioides* incidence and FB levels than late-maturity hybrid ZP 666. *F*-values of year × hybrid, year × sowing time, hybrid × sowing

time, and year \times hybrid \times sowing time interactions were highly significant ($p \leq 0.01$) for FB levels.

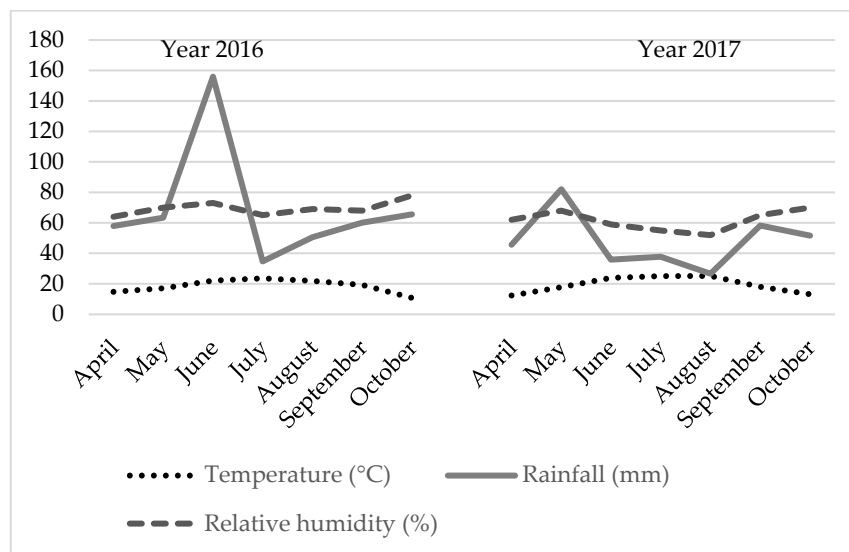


Figure 1. Mean monthly temperature (°C), total monthly rainfall (mm), and mean relative humidity (%) during the period April–October in 2016 and 2017 (Belgrade-Surčin area, Serbia).

Table 1. Effects of year, hybrids, and sowing time on the incidence of *F. verticillioides* and levels of FBs.

Factor	Incidence of <i>F. verticillioides</i> (%)	FBs ($\mu\text{g kg}^{-1}$)
Year effect (Y)		
2016	22.6	6793.1
2017	19.5	7057.5
F-test	ns	ns
Hybrid effects (H)		
ZP 560	15.1 ^b	5778.7 ^b
ZP 666	27.0 ^a	8071.9 ^a
F-test	**	**
Sowing time (ST)		
Early	15.9 ^b	2397.5 ^b
Late	26.2 ^a	11,453.1 ^a
F-test	**	**
Interactions (F-test)		
Y \times H	ns	**
Y \times ST	ns	**
H \times ST	ns	**
Y \times H \times ST	ns	**
Mean	21.1	6925.3

Means followed by the same letter are not significantly different by Tukey's test at $p \leq 0.05$. ns, not significant; **, significant at the 0.01 level of probability.

In addition, saprophytic species of *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Chaetomium*, *Nigrospora*, *Penicillium* and *Rhizopus* genera were also isolated on the maize grains in both years, except for *Chaetomium* spp. in 2016 and *Cladosporium* spp. in 2017 (data not shown).

3.3. Yield Parameters

The effect of the studied factors on the EL, NGE, GWE, TGW, and GY has shown in Table 2. The sowing time effect was statistically significant ($p \leq 0.01$) for EL, NGE, GWE, and GY, with higher values obtained in early sowing. There was a significant difference

($p \leq 0.01$) observed between investigated hybrids for EL, NGE, GWE, and GY. The late-maturity hybrid ZP 666 had higher EL, NGE, GWE and GY than the mid-maturity hybrid ZP 560. Year effect was significant ($p \leq 0.01$) on EL, GWE, TGW, and GY with higher values in 2016. Significant differences were observed in the interactions between year \times hybrid on EL ($p \leq 0.05$), NGE ($p \leq 0.01$), and GWE ($p \leq 0.05$), year \times sowing time on EL ($p \leq 0.05$), hybrid \times sowing time on GWE and GY ($p \leq 0.01$), and year \times hybrid \times sowing time on EL and GY ($p \leq 0.01$).

Table 2. Effects of year, hybrids, and sowing time on the yield component traits and grain yield.

Factor	EL (cm)	NGE	GWE (g)	TGW (g)	GY (kg ha ⁻¹)
Year effect (Y)					
2016	23.7 ^a	721.4	261.9 ^a	396.7 ^a	12,679.6 ^a
2017	21.4 ^b	719.0	160.6 ^b	273.9 ^b	8911.1 ^b
F-test	**	ns	**	**	**
Hybrid effects (H)					
ZP 560	22.2 ^b	695.1 ^b	192.9 ^b	334.1	9950.7 ^b
ZP 666	23.0 ^a	745.3 ^a	229.6 ^a	336.4	11,640.1 ^a
F-test	**	**	**	ns	**
Sowing time (ST)					
Early	23.1 ^a	733.4 ^a	221.9 ^a	345.8	12,097.0 ^a
Late	22.1 ^b	707.0 ^b	200.7 ^b	324.7	9493.8 ^b
F-test	**	*	**	ns	**
Interactions (F-test)					
Y \times H	*	**	*	ns	ns
Y \times ST	*	ns	ns	ns	ns
H \times ST	ns	ns	**	ns	**
Y \times H \times ST	**	ns	ns	ns	**
Mean	22.6	720.2	211.3	335.3	10,795.4

EL, Ear length; NGE, Number of grains per ear; GWE, Grain weight per ear; TGW, Thousand-grain weight; GY, Grain yield per hectare. Means followed by the same letter are not significantly different by Tukey's test at $p \leq 0.05$. ns, not significant; *, significant at the 0.05 level of probability; **, significant at the 0.01 level of probability.

3.4. Correlational Analyses

A strong positive correlation was determined between the incidence of *F. verticillioides* and FB levels ($r = 0.60$ **). Yield parameters (EL, NGE, GWE, TGW, and GY) were positively correlated with the incidence of *F. verticillioides*. Positive correlations between all the yield parameters (EL, NGE, GWE, TGW, and GY) were observed as well. EL was positively significantly correlated with GWE ($r = 0.86$ **), TGW ($r = 0.79$ **), and GY ($r = 0.85$ **); NGE with GY ($r = 0.41$ *); GWE with TGW ($r = 0.88$ **) and GY ($r = 0.86$ **); and TGW with GY ($r = 0.69$ **). There were positive non-significant correlations between EL and NGE ($r = 0.23$) and NGE with GWE ($r = 0.29$) and TGW ($r = 0.05$) (data not shown).

4. Discussion

Timely sowing and the use of maize hybrids resistant to FER can be quite effective agricultural measures for reducing *Fusarium* and fumonisin contamination. Weather conditions at the beginning of the growing season often determine sowing time. Climatic factors, such as temperature and precipitation, vary from year to year. Therefore, the period for the optimal sowing time can be extended. The examinations of more maize hybrids under different sowing times and climatic conditions can contribute to determining and recommending high-yielding hybrids less susceptible to ear rot. The purpose of this study, therefore, was to estimate the effect of sowing time on *Fusarium* incidence, FB levels, and some productive traits (EL, NGE, GWE, TGW, and GY) in two maize hybrids (ZP 560 and ZP 666) during two growing seasons (2016–2017) in Serbia.

In grains of maize hybrids ZP 560 and ZP 666, species within the FGSC, *F. subglutinans*, and *F. verticillioides* have been identified. FGSC species were isolated only in 2016, while potentially FB-producing species, *F. verticillioides* and *F. subglutinans*, were isolated

in both years. *F. verticillioides* was predominant, with an average incidence of 21.1% for all treatments tested (Table 1), while FGSC species and *F. subglutinans* were isolated sporadically and with low incidence. This was in line with the results of Aguin et al. [11], who reported that *F. verticillioides* was the most commonly isolated species from maize grains and emphasized that *F. verticillioides* in association with *F. subglutinans* is becoming the dominant species. Similarly, by investigating the effect of sowing time on fungal and mycotoxin contamination of maize grains, Blandino et al. [26] have established higher grain infection with *F. verticillioides* than with *F. graminearum* strains. In addition, Picot et al. [33] have established a significantly increased level of DNA of *F. verticillioides* compared to *F. graminearum* strains isolated from maize grains. This was hypothetically explained by the ability of *F. verticillioides* to grow in a wide range of temperatures, together with minimum requirements for water activity in the silking stage. Silva et al. [34] also found *F. verticillioides* to be the most prevalent species in maize grains and determined that 95% of strains had essential genes for the biosynthesis of FBs.

In this study, there were significant effects of sowing time and maize hybridity on the incidence of *F. verticillioides* and FB levels. Late sowing treatments were associated with a significantly higher incidence of *F. verticillioides* and FB levels in late-maturity hybrid ZP 666. Mid-maturity hybrid ZP 560 had about two times less incidence of *F. verticillioides* than ZP 666. Further, FB contamination was about one-and-a-half times lower in ZP 560 than in ZP 666 (Table 1). This report is in agreement with the published results by Abbas et al. [28], Blandino et al. [23,26,27], and Parsons and Munkvold [29,35]. Blandino et al. [26] have reported a significant effect of sowing time on *F. verticillioides* and FB contamination of maize grains, with the highest contamination at the latest sowing time. The maize hybrid effect was significant regarding *F. verticillioides* infection grains but not FB levels in all of the three study years [26]. Parsons and Munkvold [35] also demonstrated that FER and FB contamination depended on year, sowing time, and maize hybridity. According to these authors, early sowing time treatments had a lower percentage of both FER symptomatic maize grains and FB levels than late sowing treatments. The hybrids susceptible to FER showed higher FER and FB contamination of maize grains than resistant ones in all sowing treatments. These contamination parameters were lower in early sowing than in later sowing times. Similarly, the early sowing time resulted in lower FB levels in Bt and non-Bt maize hybrids, with lower FB levels in Bt hybrids [28]. Furthermore, FBs produced by *F. verticillioides* and *F. proliferatum* had significantly higher levels in late sowing time treatments, especially in maize hybrids with prolonged cycle lengths (FAO maturity group 600) [27].

The year effect was not significant regarding the incidence of *F. verticillioides* and FB levels (Table 1). This is in contrast with the results of Blandino et al. [26] and Krnjaja et al. [30]. These authors have emphasized that the extraordinary differences in total rainfall during the reproductive maize stage between the two growing seasons significantly influenced the incidence of FB-producing *Fusarium* spp. and FB levels in grains. In this research, total rainfall and mean air temperature values were approximately the same in July 2016 (34.7 mm and 23.6 °C) and July 2017 (37.7 mm and 25 °C) (Figure 1) during the silking stage, which may explain the lack of a significant year effect observed for *F. verticillioides* and FB contamination. Similarly, Berardo et al. [36] pointed out that FB levels in maize grains originating from different growing areas were influenced by some crucial climatic factors, such as high temperatures during the flowering stage and wet weather during the maturity stage. Abbas et al. [37] have concluded that minimum air temperature above 20 °C in the growing season (May–July) was of particular significance for high maize contamination with FBs stimulating faster fungal growth. Accordingly, favorable weather conditions in both tested years (Figure 1) contributed to high FB levels in maize grains (Table 1). Considering all the treatments tested, the mean FB level was 6925.3 µg kg⁻¹, which is above the maximum limit set by the European Commission (4000 µg kg⁻¹ in unprocessed maize and 1000 µg kg⁻¹ in maize for direct human consumption; Directive 2007/1126/EC).

Optimal sowing time is one of the most crucial agronomic factors for achieving high maize yields and ensuring food security. It is determined by different climatic, agroecological, and environmental conditions. Early sowing and late-maturity hybrid ZP 666 influenced higher yield component traits and grain yield. Grain yield was about 1.7 times lower in mid-maturity hybrid ZP 560 than in ZP 666, while early sowing treatments had about 1.3 times more grain yield than late sowing treatments (Table 2). In similar studies, Baum et al. [38], Liaqat et al. [39], Sab et al. [40], and Ke and Ma [41] have also reported that early sowing treatments resulted in higher grain yields in late-maturity hybrids than in early- and mid-maturity hybrids. In addition, Liaqat et al. [39] and Sab et al. [40] have found an inconsistent effect of interaction between maize hybridity and sowing time for tested maize growth parameters. Then, Baum et al. [38] pointed out that the effect of hybrid maturity was lower compared to the sowing time on maize grain yield and phenology, explaining that hybrid maturity depended on climatic conditions in some of the localities. Similarly, Djaman et al. [42] have established that late sowing caused decreased maize yield due to low temperatures during grain filling and physiological maturity stages. In our research, late sowing caused a reduction in yield parameters due to low rainfall in August and less grain filling.

The incidence of *F. verticillioides* was significantly positively correlated with FB levels; therefore, high FB levels were expected (Table 1). A significant positive correlation between FER parameters and FB levels was also observed in reports by Presello et al. [43] and Parsons and Munkvold [29]. The relationship between sowing time and FER parameters depends on both abiotic and biotic factors, including pests as biological factors. Insect injuries on maize ears are sites for *Fusarium* infection, where spores of *F. verticillioides* germinate and colonize grain, contributing to FER development.

The tested maize yield parameters were also positively correlated. There were positive correlations between EL with NGE, GWE, TGW, and GY; GWE and TGW with GY; and TGW with GY. Similar results were obtained by Tsimba et al. [44], Bonelli et al. [45], and Zhou et al. [46]. In the present study, there was a higher positive correlation between GWE and GY than between NGE and GY. However, Tsimba et al. [44] and Bonelli et al. [45] have emphasized a lower correlation between GWE and GY than between NGE and GY in late sowing. This was explained by the fact that GWE is more sensitive to climate variations. Hence, climatic factors associated with sowing time influenced maize grain weight variations from silking stage to maturity. On the contrary, Coelho et al. [47] have determined a high positive correlation between NGE with GY regardless of sowing time or maize hybridity.

Considering the qualitative traits of maize in relation to sowing time tested, early and late, early sowing was associated with 1.7 and 4.8 times lower *F. verticillioides* incidence and FB levels in maize grains, respectively. In contrast, yield component traits and grain yield per hectare were 1.0 (EL), 1.0 (NGE), 1.1 (GWE), and 1.1 (TGW) to 1.3 times higher, respectively, in early than in late sowing maize treatments (Tables 1 and 2). Early sowing significantly reduced *F. verticillioides* and FB contamination of maize and significantly increased the yield parameters EL, NGE, GWE and GY. This result indicates the importance of applying early sowing in reducing the risk of fumonisin contamination and fumonisin-producing *Fusarium* species, as well as achieving high grain yields in maize production. Similar results for qualitative characteristics of maize grains have been reported by Blandino et al. [25–27] and Parsons and Munkvold [29,35] and for productive traits by Baum et al. [38] and Djaman et al. [42]. Given the results of very high FB levels, relatively high *F. verticillioides* incidence in maize grains, and lower yield parameters associated with late sowing obtained in this study, it could be recommended for maize growers to avoid late sowing in the region where the field trials were performed. Additionally, late sowing of late-maturity hybrids is not recommended. Generally, early sowing was associated with a better quality of maize grains and higher yields per hectare.

5. Conclusions

Based on the obtained results, sowing time and hybrid susceptibility could be considered crucial preventive agricultural measures to control FER and FB contamination of maize grains. These control measures should be mandatory in integrated pest management in maize. The incidence of *F. verticillioides* and FB levels were reduced with early sowing and the mid-maturity hybrid ZP 560. On the other hand, yield parameters were increased with early sowing and the late-maturity hybrid ZP 666. Although ZP 560 hybrid was less contaminated with *F. verticillioides* and FBs than ZP 666, both hybrids had high FB levels. *Fusarium* spore production and mycotoxin contamination are affected by temperature, relative humidity, and water activity. The impact of environmental factors is considered crucial for the level of contamination. Hence, research on the relationship between *Fusarium* pathogens, maize genotypes, and environmental factors must be constant in order to improve maize protection. In any case, the application of individual measures can be somewhat effective in reducing grain contaminants, while an integrated pest-management strategy provides better grain quality and yield, especially under agro-ecological and weather conditions favorable to the growth of *Fusarium* spp. Therefore, early sowing and hybrids resistant to FER and FBs can be recommended for healthy and high-yielding maize production.

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