DOI: 10.34101/ACTAAGRAR/1/15272

Effects of cultivation practices and hybrid selection on endofusariosis and mycotoxin contamination in maize

István Kecskés[1] – Károly Pál[2] – Adrián Pápai[3] – Tibor Bartók[4] – Levente Horváth[5] – András Csótó[6]*

[1]Kerpely Kálmán Doctoral School, University of Debrecen, 138. Böszörményi St., 4032 Debrecen, Hungary

[2]Institute of Food Science, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, H-4032 Debrecen, Hungary

[3]Kunmag Kft., 5321 Kunmadaras, Hungary

[4]Fumizol Ltd., 6/B. Kisfaludy st., 6725, Szeged, Hungary

[5]Department of Physiology and Animal Health, Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences, Kaposvar

[6]Institute of Plant Protection, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, H-4032 Debrecen, Hungary

*Correspondence: csoto.andras@agr.unideb.hu

SUMMARY

The aim of this study was to determine the levels of deoxynivalenol (DON) and fumonisin mycotoxins in tillage and no-till systems in 2020 and 2021. Additionally, we sought to establish the levels of internal Fusarium infection in different tillage systems. We examined four tillage systems for two years (conventional tillage (plowing), conservation tillage, reduced tillage, and strip-till). The results indicate that toxin levels varied between the two years, with fumonisin production dominant in 2021, while DON toxin production was dominant in 2020. Regarding internal Fusarium infection, the lowest levels were observed in the plowing system in 2020, whereas in 2021, the lowest levels were measured in the reduced tillage system. Like the low DON levels in 2020, the plowing-based tillage system resulted in the lowest fumonisin levels in 2021. Throughout our experiment, the toxin levels were below the permissible limits for unprocessed corn, with DON levels under 1750 μg kg^{-1} and combined fumonisin B1 and B2 levels under 4000 μg kg^{-1}. However, in areas where toxin contamination is typically problematic, considering the beneficial impact of plowing on reducing toxin contamination might be advisable when planning tillage.

Keywords: *Fusarium ear rot; mycotoxins; maize yield loss; agricultural practices; sustainable farming*

INTRODUCTION

Agricultural practices, such as tillage systems, are critical in improving soil health and crop yields. This study reviews the impact of these systems on crop cultivation, highlighting the importance of optimal practices for sustainable agriculture, with particular emphasis on the significance of *Fusarium* infection, which can lead to substantial yield losses and health risks. The *Fusarium* ear rot of maize is a global phenomenon that causes significant yield losses and poses health risks to humans and animals. This disease is a worldwide concern among maize growers due to its substantial impact on production, reducing potential revenue (Masibonge et al., 2015). The most significant risks are posed by the mycotoxins produced by *Fusarium* species, including fumonisins, trichothecenes, and zearalenone (Kovács, 2010). The economic impacts of mycotoxins in animal feed are challenging to measure. A study on the fumonisin content of maize intended for feed in the United States considered two categories of losses: losses related to animal health and commercial losses associated with grain rejected by the market. It is estimated that in an average year, losses caused by fumonisins in feed amount to 1–20 million USD, while in a year with significant *Fusarium* ear rot, losses range from 31–46 million USD (Wu, 2007).

Plant pathogens, insect pests, weeds, abiotic stress, and other pre-harvest losses account for approximately 35% annually. By reducing field losses and food waste by 50%, it would be possible to sustainably feed an additional 3–4 billion people, addressing this challenge without increasing the genetic yield of crops (Mesterházy et al., 2020a). Agricultural practices can help reduce *Fusarium* infection. It is recommended to perform primary tillage with plowing during soil preparation or to prepare seedbeds after removing stubble residues, which provide a favorable environment for toxin-producing fungi (Szeitzné Szabó, 2009). In traditional tillage, crop residues are incorporated into the soil by plowing the entire surface. Conservation tillage aims to maximize soil cover with root and crop residues of the previous crop, using minimal passes. In strip-till, cultivation occurs only in strips covering about 25–35% of the soil surface, using specialized equipment to manage residue-covered stubble; planting takes place in these tilled strips. Only a small portion of residues enters the soil, with most remaining on the surface for soil cover. No-till involves direct seeding after harvest without soil disturbance (Husti, 2015).

In recent years, various tillage practices have been introduced in many parts of the world, including Hungary, that enable higher maize yields with lower inputs while reducing or halting soil degradation processes. These practices also take the biological life of the soil into account protecting and enriching it (Serna-Saldivar, 2019). Various tillage systems are used in maize cultivation, and these have different effects on the soil's physical parameters and moisture content, which can influence the yield level (Simić et

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al., 2009). One such system is conventional tillage, which relies on the use of the plow for primary tillage. It is characterized by multiple operations to achieve a seedbed suitable for planting, with the soil surface fully tilled. Since it leaves no plant residues on the surface, the soil is left unprotected, increasing the risk of moisture loss and soil erosion. However, its advantage is effective weed control and the incorporation of plant residues, which helps reduce the buildup of pathogens harmful to the crop. As an alternative to the traditional plow-based tillage system, no-till methods have also become widespread. The long-term application of these practices can enhance soil organic matter content, improve soil structure, and lead to better soil structure compared to intensively cultivated soils (Rasmussen and Rohde, 1988). The intensity of tillage and its incorporation effects can be characterized by the ratio of plant residues on the soil surface shortly after planting. Based on this, tillage systems can be categorized as follows: systems with less than 15% residue cover are referred to as conventional tillage. Systems with residue cover between 15% and 30% fall into the category of reduced tillage, while those with more than 30% residue cover are classified as conservation, soil-protecting, or biological tillage (CTIC, 2009).

The agricultural practices aimed at reducing mycotoxin contamination in crops are based on the principles of plant disease epidemiology. The main goal is to alter the cultivation environment to prevent infection by pathogenic fungi. The tactics employed to combat plant pathogens include tillage practices, fertilization methods, crop rotation, plant density, timing of sowing, and irrigation. Additionally, modifying grain storage practices can help reduce the likelihood of mycotoxin development after harvest (Munkvold, 2003b). An important agronomic tool in disease management may be hybrid selection. Research by Chibuogwu et al. (2024) has shown that hybrid selection, rather than fungicides, will be crucial in combating diseases caused by *Fusarium graminearum* and the accumulation of mycotoxins.

Most toxic fungal species survive in plant residues, and the management of surface residues through tillage or crop rotation has been investigated as a strategy for reducing mycotoxins. Numerous studies have focused on *Fusarium graminearum* and its associated head blight in wheat. One study showed that crop rotation significantly affects deoxynivalenol (DON) concentration, which was more than twice as high when wheat followed corn in the rotation (Schaasfma et al., 2001). Another investigation found that head blight and DON content in wheat decreased when plant residues were incorporated through tillage (Dill-Macky and Jones, 2000). In the case of corn, several studies reported that tillage practices did not influence *Fusarium* infection levels (Flett and Wehner, 1991; Flett and McLaren, 1998). A more recent study also reached a similar conclusion, noting no significant difference in average fumonisin B1 (FB1) and fumonisin B2 (FB2) mycotoxin contamination between corn grown under conventional tillage and no-till practices, with nitrogen fertilization having no effect (Marocco et al., 2009). These findings are partly consistent with a previous study where conventional tillage and no-till practices did not significantly affect fumonisin contamination; however, they contradict another conclusion from the same research group that nitrogen fertilization significantly increased fumonisin content in the grains (Marocco et al., 2008). Vári and Pepó (2011) also found a correlation between the level of fertilization and ear rot infection, while plant density showed no relationship with disease spread. In Austria, the incidence of corn ear rot and DON contamination were examined using tillage at depths of 17 cm, 24 cm, and 30 cm, as well as loosening at the same depths and a 10 cm deep soil cutter, with no effect of tillage on the outcomes (Steinkellner et al., 2002). The most significant environmental factors influencing the *Fusarium* species responsible for maize ear rot and stalk rot are moisture and temperature. For *Fusarium graminearum* and *F. culmorum*, cooler temperatures combined with high precipitation create favorable conditions for infection. Conversely, infections by *Fusarium verticillioides*, *Fusarium subglutinans* or *Fusarium proliferatum* are more likely to occur under higher temperatures and drier conditions (Bottalico, 1998; Munkvold, 2003a). A study conducted in Germany that examined the impact of environmental conditions and agronomic practices on the occurrence of *Fusarium* species causing stalk and ear diseases in corn, yielded similar findings. The growing seasons of 2016 and 2017 were characterized by moderate average temperatures of 18.8 °C and substantial July rainfall of 110 mm. During these years, *F. graminearum* and *F. culmorum* were predominant, with over 70% of the analyzed ears and 80% of the examined stalks showing signs of infection from these species. In 2018, the average temperature rose to 20.6 °C, and July precipitation dropped significantly to 40 mm, leading to *F. verticillioides* colonizing nearly 40% of the sampled ears (Pfordt et al., 2020).

The primary objective of this study was to evaluate the effects of different tillage systems and maize hybrids on *Fusarium* infection and associated mycotoxin contamination (DON and fumonisins) under real field conditions in Hungary. Given the increasing relevance of sustainable tillage practices and the associated plant health challenges, our goal was to determine how conventional plowing, reduced tillage, conservation tillage, and strip tillage influence fungal colonization and toxin accumulation in maize kernels.

Furthermore, by including hybrids of different maturity groups, we aimed to assess hybrid-specific sensitivity to *Fusarium* infection and mycotoxin contamination, thereby providing practical guidance for hybrid selection in environments prone to such risks. The results also serve to support growers in selecting appropriate tillage practices and hybrids to mitigate mycotoxin exposure and related yield or quality losses.

By integrating classical agronomic field experimentation with fungal identification and mycotoxin quantification, this research provides a comprehensive evaluation of the interactions between

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soil management practices, hybrid genetics, and fungal contamination. Thus, the study aims to bridge the gap between plant pathology, agronomy, and food safety, contributing valuable insights to sustainable crop production systems.

MATERIALS AND METHODS

Experimental site

The investigations were conducted in 2020 and 2021 at the KITE Co. experimental station located on the outskirts of Nádudvar (47°25'41" N, 21°12'3" E, 85 m) in a moderately warm and dry agricultural zone. The experimental area was situated in intensively cultivated agricultural land typical of the Hajdúság mesoregion. The site had a good cultural status, with a medium-textured meadow chernozem soil. Its texture was classified as K_A48 (clay loam), with an average pH value of 7.1 (neutral) and a humus content of 3.8% (good). The original AL-extractable P_2O_5 content of the soil was 286 mg kg^{-1} (very good) and the AL-extractable K_2O content was 499 mg kg^{-1} (very good) in air-dry soil.

Presentation of the hybrids used in the experiment and the tillage systems

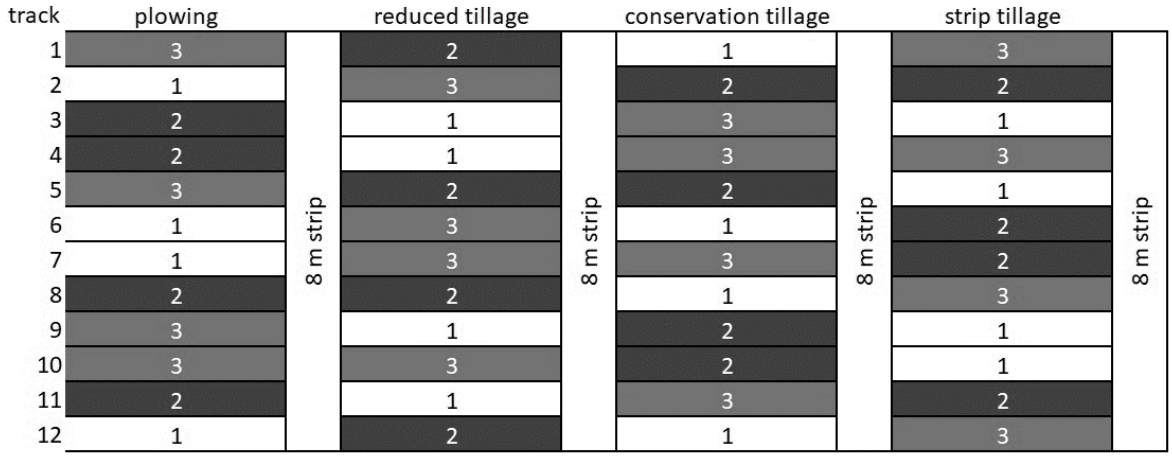
The experiments were conducted using four different tillage methods: plowing, reduced tillage, soil-conserving tillage, and strip tillage, along with three hybrids of different maturity groups: FAO 380 (Loupiac), FAO 420 (Fornad), and FAO 490 (Armagnac) (KITE Zrt., Hungary). The experimental plots were arranged in four blocks corresponding to the tillage methods, with the hybrids randomly assigned in four replications to the established mesoplots (*Figure 1*). The plot size was 4.572 m × 120 m (~550 m²).

The tillage methods applied were carried out as follows: for plowing, the soil was plowed in the fall with a plow, followed by spring tillage using a Rabe Sturmvogel (Rabe, Germany) implement (at a depth of 3 cm) before sowing. For reduced tillage, a disc ripper (Disk Ripper 2720, John Deere) was used in the fall, and a Rabe Sturmvogel was applied in the spring. For soil-conserving tillage, a Rabe Digger with straight blades (Rabe, Germany) was used in the fall, followed by a Rabe Sturmvogel in the spring. Strip tillage involved using a strip-tiller (Environmental Tillage Systems, USA) in the fall and then a Rabe Sturmvogel in the spring.

Experimental design

In each treatment, four replicates of the hybrids were sown in a randomized arrangement, as shown in *Figure 1*. The area of each plot is 4.572 m × 120 m.

Figure 1. **Design of the micro-plot experiments**



Legend: Gray: Fornad; FAO 420; White: Armagnac; FAO 490; Black: Loupiac; FAO 380

Cultivation technology:

The preceding crop on the site was corn in 2019. After harvesting the preceding crop, fertilizers were applied in the fall of 2019 and 2020, including 32 kg of N, 96 kg of P_2O_5, and 96 kg of K_2O. During sowing, 20 l ha^{-1} of 10:40 NP starter fertilizer was placed in the seed furrows using a seed drill. To control corn rootworm larvae, Force 1.5 G (15 g kg^{-1} teflutrin) soil insecticide was also applied to the seed furrows at a dose of 15 kg ha^{-1} in both years. A pre-emergent herbicide was sprayed in both years at a dose of 0.44 l ha^{-1} of Adengo (150 g l^{-1} cyprosulfamide, 225 g l^{-1} isoxaflutole, 90 g l^{-1} thiencarbazone-methyl). The top dressing was done in one pass using a cultivator (Orthman 1tRIPr, a six-row cultivator with wings), applying a liquid Urea Ammonium Nitrate (UAN-30) solution with a specific active ingredient content of 117 kg. The hybrids were sown at a density of 75,000 plants ha^{-1} at a depth of 5 cm. The sowing dates were April 20, 2020, and April 12, 2021.

Method of investigating endofusariosis

The internal Fusarium infection of the samples was examined at the Institute of Plant Protection, Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen. The corn

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kernels were soaked in distilled water containing Neomagnol tablets and Tween 20 surfactant for 5 minutes, and then rinsed in sterile water before placing them on the medium. From each sample, after external disinfection, 20 corn kernels were placed in large glass Petri dishes filled with PDA medium. Prior to cultivation, streptomycin sulfate antibiotic (100 mg l^{-1}) was added to the cooled medium. In some samples, species from the *Mucoraceae* family, which grew more intensively than *Fusarium* spp., proliferated, potentially hindering the production of pure cultures. To eliminate this, pentachloronitrobenzene (PCNB) (5 g l^{-1}) was dissolved in the medium, which inhibits the growth of *Mucor* species but has no effect on toxin-producing fungi (Papavizas, 1967). After placing the kernels on the medium, the Petri dishes were sealed with Parafilm and incubated in a laboratory thermostat cabinet for 5 days at 25 °C. Following incubation, *Fusarium* and other fungi colony counts were determined for each sample.

Method of molecular genetic identification

Pure cultures were prepared from various colony morphologies of Fusarium and other fungi that developed from corn kernels. A square approximately 0.5 cm x 0.5 cm in size was cut from the selected colonies and placed with the hyphae-covered side onto small plastic Petri dishes filled with potato dextrose agar (PDA) medium (Biolab, Hungary). These dishes were then incubated for 8 days at 25 °C. DNA was extracted from the pure cultures using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. After extraction, the concentration of the DNA samples was measured by a NanoDrop 2000/2000c spectrophotometer (Thermo Scientific, MA, USA).

Molecular identification of the fungal isolates was carried out by sequencing the product of the ITS1-ITS4 primer pair (Integrated DNA Technologies, Belgium), targeting the conserved ribosomal internal transcribed spacer (ITS) region of the genomic DNA. The primer sequences were as follows: ITS1 5' TCC GTA GGT GAA CCT GCG G 3' and ITS4 5' TCC TCC GCT TAT TGA TAT GC 3' (White et al., 1990). The volume of the PCR reaction was 20 µl, consisting of 11 µl DreamTaq Green Master Mix (Thermo Fisher Scientific, Germany), 1 µl ITS1 primer and 1 µl ITS4 primer (5 pM each), 6 µl distilled water and 2 µl genomic DNA (10 pM). PCR reactions were performed in a T100 thermocycler (Bio-Rad, Germany) with the following steps: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 55 °C for 45 sec, and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

PCR products were visualized on a 1% native SeaKem LE agarose gel (Lonza, Switzerland) stained with GelRed dye (Biotium, USA). This was followed by purification of the ITS fragments using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. Sanger sequencing of the purified fragments was performed by Microsynth (Wien, Austria), and the sequences were submitted to a BLAST search in the GenBank database (Sayers et al., 2022).

Method for measuring Fumonisin B1, B2, B3, B4, and DON content

The fumonisin B1, B2, B3, B4, and DON content in corn samples was measured using the HPLC-ESI-MS method. During sample preparation, corn samples were milled with a Perten laboratory mill (type 3310, Perkin Elmer Inc., Waltham, MA, USA) and subsequently measured (5 g) into 50 ml centrifuge tubes, and finally, 20 ml extraction solvents ($MeCN/H_2O$ 60/40, v/v) were pipetted in. The content of the centrifuge tubes was shaken, ultrasonicated for 10 minutes in an ultrasonic bath (Bandelin, Berlin, GER), then extracted for 2 hours with an overhead shaker (Cole-Parmer, Vernon Hills, IL, USA), followed by another 10 min ultrasonication, and finally centrifuged (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 10,000 RPM for 10 min. The supernatants were pipetted into a well plate and injected directly (4 µl). Stock solution for calibration was freshly prepared from vials containing 100 µg of each toxin (DON, FB1, FB2, FB3, FB4; Fumizol Ltd., Szeged, HU). This stock solution was dissolved with 1 ml of bank sample which did not contain mycotoxins (based on the sample preparation described above). The calibration points diluted from the stock solution with blank sample solvent were as follows: 50; 10; 5; 2.5; 1.25; 0.625; 0.3125; 0.1563; 0.1; 0.05; 0.02 ng/4 µl.

Samples were analyzed with an Agilent 1100 high-performance liquid chromatograph (HPLC; Santa Clara, CA, USA) connected to an Agilent 1946D mass spectrometer (MS) equipped with an electrospray ion source (ESI). The HPLC system consisted of the following modules: a vacuum degasser, a binary pump, a well plate sampler, and a column thermostat set to 40 °C. The gradient program started with 0% B and increased to 48.6% in 4 min, then increased to 79% in 2 min, and further increased to 100% in 0.2 min, held it for 2 min and returned to the initial value in 2.3 min and held it for 3 min. During the analysis, the flow rate changed as follows: started with 0.8 ml min^{-1}, held it for 6.6 min, increased to 2.0 ml min^{-1} in 0.4 min held it for 1.4 min then returned to 0.8 ml min^{-1} in 1.4 min. Fully labelled 13C isotope deoxynivalenol and fumonisin B1 internal standards (Romer Labs Ltd, Getzersdorf, AT) were added to each sample (1 µl; 5 ng $µl^{-1}$) from the same vial by using an injector program. The mass spectrometer was set to the following ESI parameters: nebulizer gas (N2) pressure, 50 psi; drying gas (N2) flow rate, 12 l min^{-1} and temperature, 350 °C; capillary high voltage, 3000 V. MS data was acquired in positive ion SIM (selective ion monitoring) mode: 2–4 min: m/z 297 and 312; 4–7 min: m/z 690, 706 and 722. The results were evaluated by isotope-labelled internal standard calibration. Limit of detection (LOD) and limit of

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quantification (LOQ) values were established as the lowest detectable and quantifiable concentration level of calibration samples with the suitable signal to noise ratio (3:1 for LOD and 10:1 for LOQ). LOD and LOQ of the components were as follow: FB1-FB4 (20, 50 µg kg^{-1}), DON (312.5, 156.3 µg kg^{-1})

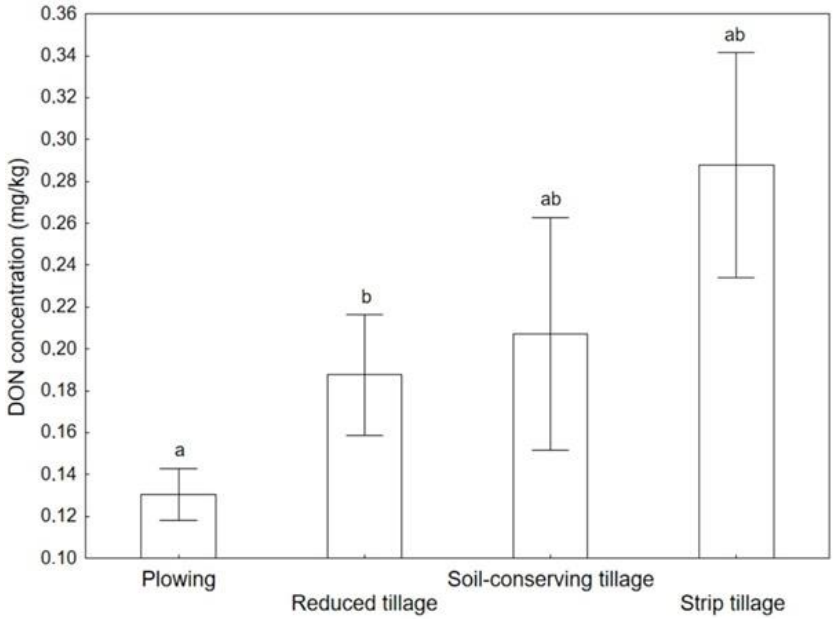
Statistical Analysis

Data sorting was performed using MS Excel 2016 and Statsoft Statistica 10 software, while Statsoft Statistica 10 was used for statistical analysis and displaying results. Our measured data did not show a normal distribution, so non-parametric testing, specifically the Kruskal-Wallis test, was applied and confirmed by pairwise comparisons with Mann-Whitney U tests.

RESULTS AND DISCUSSION

The DON levels measured in 2020 did not follow a normal distribution. The results are illustrated in *Figure 2* using letters for visualization of significant differences ($p<0.05$). The lowest DON concentrations were measured in samples derived from plowing-based tillage, which were statistically significantly lower than the toxin levels in samples from strip tillage. The DON levels in samples from reduced tillage and strip tillage did not show statistically significant differences either from each other or from the results of conservation tillage. Although reduced tillage resulted in slightly higher contamination than loosening, strip tillage resulted in the highest level of contamination. No statistically significant differences were observed in DON concentrations among the hybrids.

Figure 2. **Extent of DON toxin contamination by tillage methods in 2020**



Whiskers show ±SE and compact letter display shows pairwise comparisons based on Mann-Whitney U-tests

The effect of hybrids and tillage methods on fumonisin content in 2021

In 2021, DON contamination levels were not noteworthy; however, fumonisin content was measurable and therefore analyzed. In that year, we examined the levels of B-group fumonisins in relation to hybrids and tillage methods. First, the sensitivity of the hybrids was compared while keeping the tillage methods constant, and the results are presented in *Figure 3*. Regarding fumonisin B_1, the contamination levels of Loupiac and Armagnac were statistically significantly lower than those of Fornad, with no statistically significant difference between Loupiac and Armagnac. For fumonisin B_2, similar to B_1, the toxin levels of Loupiac and Armagnac were the lowest, showing no statistically significant difference from each other, while Fornad exhibited statistically significantly higher levels compared to the other two hybrids.

In the case of fumonisin B_3, the toxin content of samples collected from the Armagnac hybrid was statistically significantly lower than that of Fornad. Loupiac displayed the highest toxin content, but due to high data variability, it did not statistically differ from the other two hybrids in the experiment. For fumonisin B4, Armagnac showed statistically significantly lower toxin levels compared to Fornad. When comparing Loupiac with Armagnac and Fornad, no statistically significant differences were observed.

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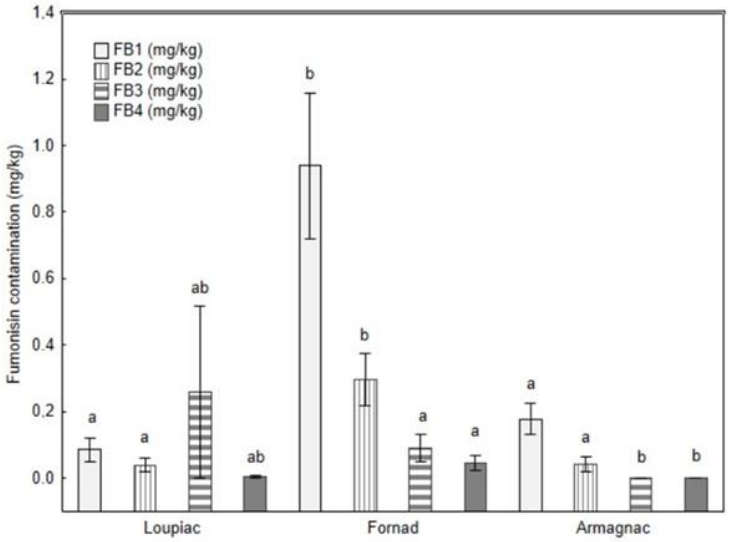
Figure 3. **FUM concentration in hybrid comparison in 2021**



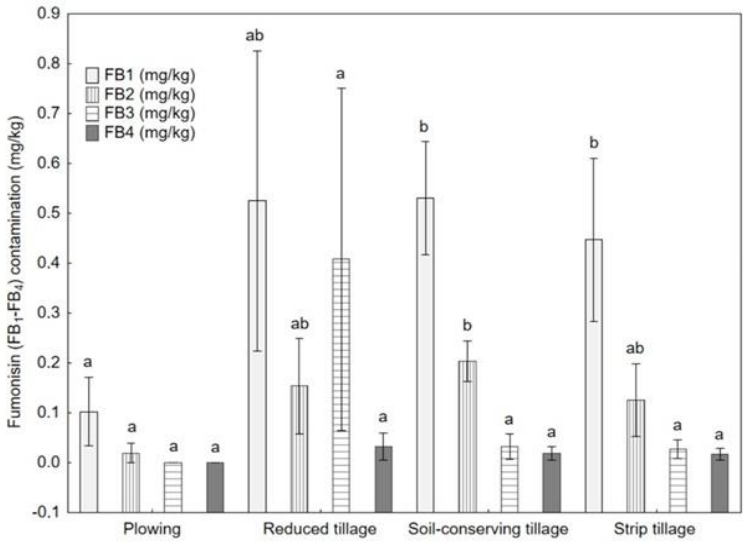
Whiskers show ±SE and compact letter display shows pairwise comparisons based on Mann-Whitney U-tests

Among the treatments applied, plowing resulted in the lowest toxin levels for the fumonisins analyzed. The fumonisin B1 levels in corn treated with loosening and strip tillage were statistically significantly higher than those in samples from plowing-based tillage, but no statistically significant differences were observed between loosening and strip tillage. The fumonisin B_1 content in reduced tillage was more than five times higher than that of plowing; however, due to the high variability in the data, this difference was not statistically significant when compared to plowing or the other two tillage methods (*Figure 4*).

For fumonisin B_2, plowing achieved significantly lower toxin levels than loosening-based tillage. Reduced and strip tillage did not show statistically significant differences either from each other or from the other two tillage methods. The fumonisin content of all samples in the experiment remained below the regulatory limit for unprocessed corn, which is 4000 µg/kg for the combined levels of FB_1 and FB_2 (*Figure 4*).

In the case of fumonisin B_3, the contamination levels in corn treated with reduced tillage were several times higher than those in the other three tillage methods; however, no statistically significant differences were observed among them. For fumonisin B_4, all tillage methods resulted in low toxin levels, with no statistically significant differences detected between the treatments (*Figure 4*).

Figure 4. **FUM concentration in tillage system comparison in 2021**



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Results of internal *Fusarium* infection from 2020 and 2021

Results from 2020

The analysis of samples collected in 2020 revealed no detectable correlation between the levels of internal *Fusarium* infection and DON content. Regarding susceptibility to internal *Fusarium* infection, no statistically significant differences were observed among the hybrids included in the experiment. The Fornad hybrid exhibited the lowest infection rate, while the Loupiac and Armagnac hybrids showed similarly higher levels of infection (*Figure 5*).

Among the tillage methods, the treatment based on plowing resulted in the lowest internal *Fusarium* infection rate. Slightly higher but similar levels of infection were observed in samples treated with loosening and strip tillage. The highest infection rate was recorded in samples subjected to reduced tillage. However, no statistically significant differences were found between the tillage methods concerning internal *Fusarium* infection (*Figure 5*).

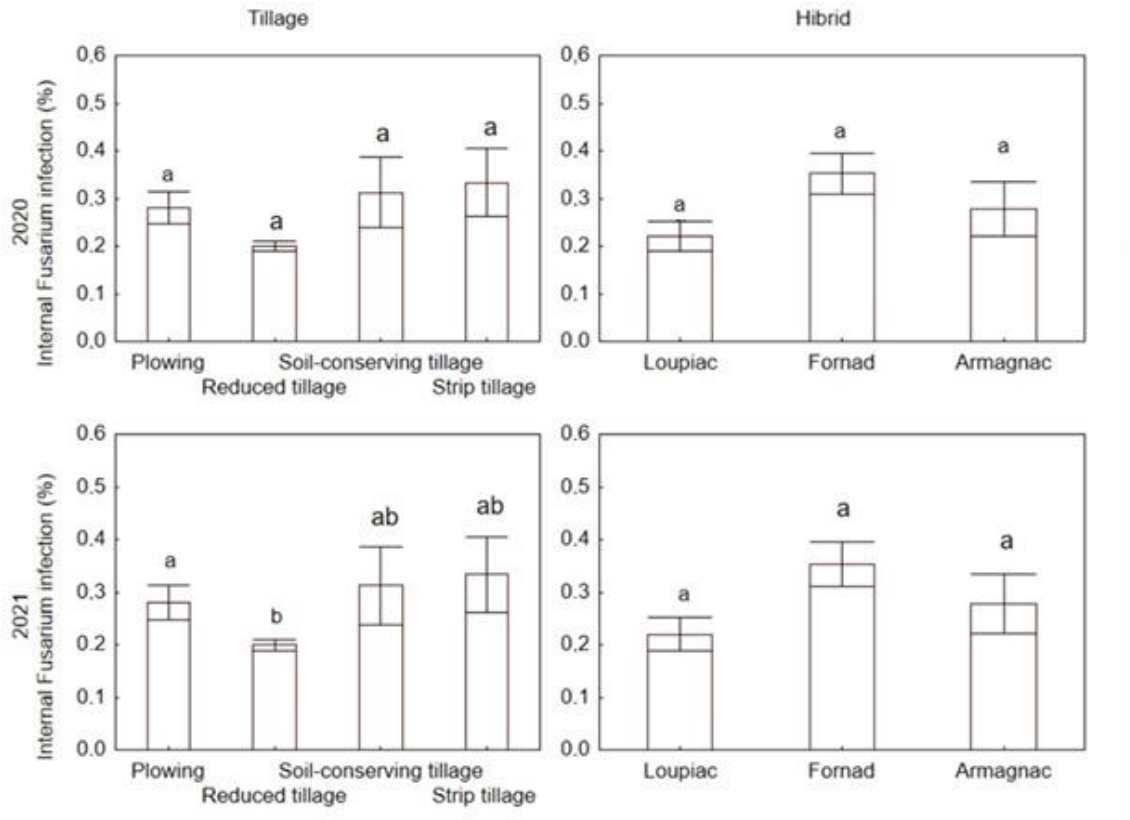
Results from 2021

In 2021, the samples were contaminated with fumonisin toxins; however, no correlation was found between the toxin levels and the rates of internal Fusarium infection. Regarding the infection rates, the Fornad hybrid had the highest level of internal Fusarium infection, followed by the Armagnac hybrid with a lower rate, and the Loupiac hybrid, which showed the lowest infection rate. Nevertheless, the Kruskal-Wallis test did not indicate statistically significant differences in hybrid susceptibility (*Figure 5*).

When evaluating the impact of tillage methods in 2021, the reduced tillage treatment resulted in the lowest infection rate, which was statistically significantly lower than the infection rate associated with plowing. The results of loosening, strip tillage, and plowing did not differ statistically significantly from one another.

This analysis highlights the complex interaction between tillage practices, hybrid susceptibility, and *Fusarium* infection, warranting further investigation under varying environmental conditions (*Figure 5*).

Figure 5. **Endofusariosis rates from different tillage practice and hybrids in 2020 and 2021**



Whiskers show ±SE and compact letter display shows pairwise comparisons based on Mann-Whitney U-tests.

Identification of the fungal isolates by PCR

Fungal isolates of corn kernels were identified by sequencing of their ITS region. As *Table 1* shows, numerous isolates needed subsequent sequencing by additional primers, because ITS fragments gave ambiguous results, multiple species names or genus names only.

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Table 1. **Fungal isolates identified by ITS PCR fragment sequencing**

Isolate	Identified species
LA11	*Fusarium oxysporum*
LA12	*Aspergillus clavatus*
LA13	*Aspergillus flavus, A. oryzae*
LA14	*Aspergillus clavatus, A. terreus, A. amstelodami*
LA21	*Aspergillus clavatus*
LA22	*Aspergillus clavatus*
LA23	*Fusarium sp.*
LA24	*Aspergillus clavatus, A. terreus, A. niger*
LA25	*Aspergillus clavatus, A. terreus, A. amstelodami*
LA26	*Fusarium sp.*
LA31	*Aspergillus flavus, A. oryzae, A. austwickii*
LA32	*Aspergillus terreus*
LA4	*Aspergillus niger*
LF2	*Fusarium sp.*
LF41	*Aspergillus flavus, A. oryzae, A. austwickii*
LF42	*Fusarium sp.*
LL11	*Fusarium sp.*
LL12	*Talaromyces pinophilus, T. flavus*
LL13	*Talaromyces purpureogenus*
LL14	*Talaromyces purpureogenus, T. funiculosus*
LL2	*Fusarium sp.*
LL3	*Talaromyces pinophilus, T. flavus*
LR2	*Aspergillus flavus*
LR31	*Fusarium oxysporum, F. verticillioides*
LR32	*Fusarium sp.*
LR33	*Fusarium sp.*
RA31	*Fusarium oxysporum, F. verticillioides*
RA32	*Fusarium sp.*
RF31	*Fusarium verticillioides*
RF4	*Fusarium sp.*
RL42	*Chaetomium elatum*
SA4	*Talaromyces pinophilus*
SF3	*Talaromyces purpureogenus, T. funiculosus*
SZF2	*Mucor fragilis*
SZF3	*Fusarium sp.*
SZL1	*Fusarium sp.*
SZL2	*Aspergillus clavatus*

CONCLUSIONS

In Hungary, typically one-quarter of the arable land is used annually for corn cultivation, making it our most significant grain crop alongside wheat. Nowadays, traditional tillage based on plowing has been largely replaced by loosening and strip-tillage in many places. Despite the acknowledged advantages, a frequent counter-argument is that these methods can increase the occurrence of various plant protection issues. One of the major plant protection problems in corn is ear rot caused by toxin-producing fungi, particularly *Fusarium* species, which are notorious not only for causing direct yield losses but for quality loss due to the mycotoxins they produce.

In our research, we examined the relationships between three different corn hybrids and four different tillage methods–plowing, reduced loosening, soil-protective loosening, and strip-tillage–in terms of endophytic *Fusarium* contamination and DON and fumonisin mycotoxin contamination.

In 2020, there was no significant fumonisin contamination in the samples; however, DON was present, so statistical analyses were performed on "vomitoxin." The lowest toxin levels were found in corn samples cultivated with plowing, which, according to statistical analysis, were significantly lower than in samples treated with strip-tillage. There was no statistically significant difference in sensitivity among the hybrids. Earlier studies have noted that conservation tillage, such as strip-tillage, can increase the fungal infection rate in maize, likely due to the residue left on the soil surface, which serves as a substrate for fungal growth (Munkvold and Desjardins, 1997; Wu et al., 2021). Our findings corroborate these observations, as strip-tillage was consistently associated with higher DON (2020) and fumonisin (2021) toxin levels compared to plowing. The protective effect of plowing on reducing toxin levels aligns with the literature, which attributes the effect to the burial of crop residues and the disruption of fungal propagation cycles (Wegulo et al., 2015).

In 2021, DON contamination was irrelevant, but fumonisin contamination was significant, with substantial differences in FB_1 and FB_2 levels regarding tillage methods. FB_1 and FB_2 contamination in plowed field samples was remarkably lower than in samples treated with the other three tillage methods. For FB_1, the contamination was statistically significantly lower than in samples treated with soil-protective loosening and strip-tillage; for FB2, it was lower than with soil-protective loosening. This year, statistically significant differences in sensitivity among the hybrids were also observed: the FAO 490 hybrid had lower levels of FB_1, FB_2, FB_3, and FB_4. In comparison, the FAO 380 hybrid had lower levels of FB_1 and FB_2 compared to the FAO 420 hybrid. The differential hybrid sensitivity observed in our 2021 results, particularly the lower FB1 and FB2 levels in the FAO 490 hybrid, is consistent with findings emphasizing genetic resistance in maize hybrids as a key factor in mitigating mycotoxin contamination (Lanubile et al., 2017). However, our two-year study did not observe such differences in DON sensitivity, which might suggest variability in environmental or methodological factors influencing toxin accumulation.

Throughout the experiment, even the samples with the highest mycotoxin content remained below the limit values. However, for farmers who prefer modern, loosening-based tillage methods and encounter challenges with DON and fumonisin levels in their corn, it may be worth considering the beneficial effects of plowing on these toxins and the relative resistance of the FAO 490 hybrid to fumonisin contamination.

Regarding endophytic *Fusarium* infection, no statistically significant differences in sensitivity were found among hybrids across the two years. However, in 2021, the contamination percentage was statistically significantly lower with reduced loosening compared to plowing. No correlation was observed between endophytic *Fusarium* infection rates and toxin levels

DOI: 10.34101/ACTAAGRAR/1/15272

during the experiment, which may be related to overall *Fusarium* contamination. Verifying this relationship would require additional studies using methods capable of detecting both internal and external occurrences of *Fusarium*. Studies on endophytic *Fusarium* contamination have shown mixed results regarding tillage practices. While conservation tillage methods often lead to higher external fungal loads, the internal contamination rates observed in our study were not significantly correlated with the tillage method, except for a decrease in contamination rates with reduced loosening in 2021. This partially aligns with the findings of Nicolaisen et al. (2018), which emphasize that *Fusarium* occurrence is influenced more by environmental conditions than by tillage alone.

We also assessed the internal infection rates of non-*Fusarium* fungi, with *Aspergillus* species being predominant among these. No statistically significant difference in sensitivity was found among the hybrids. Of the tillage methods, plowing consistently achieved the lowest contamination rates in both years. In 2020, contamination rates for plowing and strip-tillage were statistically significantly lower than for soil-protective loosening, while in 2021, rates were lower than for reduced loosening. Further studies to measure the aflatoxin content of the samples and compare these with internal non-*Fusarium* fungal contamination rates would be worthwhile. The prevalence of *Aspergillus* species in our study complements existing research highlighting their opportunistic growth under certain conditions (Cervini et al., 2020). Plowing's consistent association with the lowest non-fungal contamination rates supports earlier conclusions about its efficacy in reducing soil-borne pathogen loads (Fernandez et al., 2008).

We performed PCR analysis on a portion of the fungi infecting the kernels using the ITS1-ITS4 primers. The results confirmed that our morphological colony identification was accurate and that most internal non-*Fusarium* infections were caused by *Aspergillus* species. Nevertheless, identification of fungal species by the ITS1-ITS4 primer pair has its limits. Species-level resolution needs further PCR experiments, targeting primarily the elongation factor and beta-tubulin genes (Crous et al., 2021).

ACKNOWLEDGEMENTS

The authors are grateful to KITE Zrt. (Hungary) for providing the experimental site and technical equipment, and to Csaba Majoros, Viktor Nagy, Mihály Rásó, Imre Szűcs, and Ferenc Lovas for their technical contribution.

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