https://doi.org/10.17221/80/2022-PSE

Harvesting and phytosanitary parameters with particular regard to mycotoxin content of maize as a function of different seasonal, fertilisation and hybrid effect

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Citation: Keszthelyi S., Kadlicskó S., Pásztor Gy., Takács A., Szolcsányi É., Pál-Fám F., Lukács H., Pónya Zs., Hoffmann R., Rudolf K., Sipos T., Piszker É., Treitz M., Mesterházy Á., Somfalvi-Tóth K., Jócsák I., Kazinczi G. (2022): Harvesting and phytosanitary parameters with particular regard to mycotoxin content of maize as a function of different seasonal, fertilisation and hybrid effect. Plant Soil Environ., 68: 262–271.

Abstract: The aim of our three consecutive years (2017–2019) field trial was to obtain information as to the effect of weather conditions of the actual year as well as to assess the impact of some technological parameters such as fertilisation, the choice on the hybrid type on the yield parameters, phytosanitary conditions and mycotoxin contamination of maize. According to our results, the climatic characteristics of the years, the examined hybrid characters (FAO 310 and 490) and the fact of N-fertilisation had significant effects on yield parameters and grain moisture content. The additional N-supply did not affect the development or severity of stem rot in any of the hybrid effects. In this respect, the year effect appeared to be the decisive factor since much higher stem rot values were recorded in the plots of the longer growing season hybrids. Among the mycotoxins examined, only zearalenone and fumonisin found in the harvest were significantly influenced by the effect of the year, the length of the growing season as well as nutrient replenishment. It can be stated that the applied technological parameters have a major effect on the expression of this toxin load in maize. Dry maize stocks that have lost their water in the vegetation are predisposing factors for toxin accumulation. N-content of soil and that of plants can play a different role in mycotoxin accumulation in maize plants.

Keywords: field crop analysis; harvesting data; *Zea mays* L.; phytopathological symptoms; environmental condition; ear mould diseases

Achieving high quantitative and qualitative parameters of maize harvest is a global interest of mankind because this cultivated plant provides the bulk of forage and energy for both animal husbandry and industry (Hossain 2020). Successful maize production is mainly determined by the correct application of cultivation inputs that provide sustainable agricultural production. These inputs, *inter alia*, are the proper choice of the cultivars/hybrids adapted to the environmental conditions of the given site, as well as ideal plant density, soil tillage, fertilisation, weed, insect and disease control, harvesting, mar-

Supported by the European Union, the European Social Fund and the European Regional Development Fund, Projects No. EFOP-3.6.3.-VEKOP-16-2017-00008, EFOP-3.6.3-VEKOP-16-2017-00005, and GINOP-2.2.1-15-2016-00021.

https://doi.org/10.17221/80/2022-PSE

keting, and financial activities (Aminu et al. 2015, Mwalupaso et al. 2019).

Field trials are very important in maize research. In particular, breeders conduct different types of field trials in the process of developing new varieties hence attaining useful information *inter alia* in connection to tolerance, nutrient recovery, and developmental features of new varieties and hybrids (Leggett et al. 2015, Hirte et al. 2018). Knowing these characters, they can play an important role in the calculation of the expected yield and can ensure acquiring more specific data about the phytosanitary conditions of the mature crop plant (Lone et al. 2018). An especially significant factor in this latter case is the mycotoxin content of yield because of the marketability of the produced maize is fundamentally jeopardised by these toxic secondary metabolites (Oldenburg and Ellner 2005, Oldenburg et al. 2017). Several human and animal disorders, and diseases can be traced back to the occurrence of mycotoxin producing microfungi, such as *Fusarium* spp. and *Aspergillus* spp. (Szabó et al. 2016).

Under field conditions, the plant protection of maize is determined by the control of pests and weeds. However, the increasing damage of toxin-producing fungi (*Fusarium*, *Aspergillus* and *Penicillium* species) in recent years may also necessitate the chemical control of phytopathogenic fungi. *Fusarium* species are responsible for *Fusarium* stem and ear rots, one of the most important fungal diseases of maize. Aggressiveness tests proved that isolates of *Fusarium graminearum*, *F. culmorum* and *F. avenaceum* are the most aggressive to cereals among *Fusarium* species in Hungary. Cereals can also be infected with *Penicillium*, *Aspergillus* which may produce mycotoxins during mostly storage (Varga et al. 2004). Parasitic fungal wound pathogens can induce secondary infections *via* insects which can damage corn ears. Therefore, insecticide treatments timed to the flight peak of these pests can significantly reduce the infection caused by these microfungi (Mesterházy 2018).

Symptoms of toxin-producing fungi are variable (death of the young seedlings, stem rot), but the most serious one is the mould on maize ears. The economic damage caused by fungi is manifested not so much in crop losses but rather indirectly in the production of toxins that are dangerous to both human and animal health. Reducing the toxin contamination of feed is a fundamental element, as the contaminated crop can only be rendered suitable for feed-use with significant expenditure (antibiotics, toxin binders). Therefore, the growing health concerns have forced decision-makers to set maximum tolerable levels for each mycotoxin in feedlots through different regulations (Mesterházy et al. 2012).

Out of the species of the *Fusarium* genus 19 can be found in Europe (Logrieco et al. 2002). Identification of the genus is simple, but species identification is more complicated and generally requires expensive molecular investigations. *F. graminearum* and *F. verticillioides* are the most important ones in Hungary, the latter one, especially under dry seasons.

Additionally, other species, such as *F. culmorum* and *F. solani* may also be important to the basal part of the stem causing stem rot (Mesterházy and Vojtovics 1977). In wet conditions, the presence of more *Fusarium* species can be also detected (Mesterházy et al. 2012). In the case of natural infections, the occurrence and toxin production of other *Fusarium* species and fungi belonging to other genera (*Aspergillus*, *Penicillium*) are common even on the same corn ear (Mesterházy et al. 2012). In some years, the damaging effect of fungi causing stem rot (*Fusarium* spp., *Macrophomina phaseolina* etc.) can be also significant (Keszthelyi and Pónya 2019).

Some scientific proofs confirm that the in-crop colonisation of microfungi producing mycotoxins producing microfungi can be fundamentally determined by fertilisation practices. It is a known fact that these necrotrophic parasites and DON contamination may be directly correlated with an increase in the dose of nitrogen fertilisation (Heier et al. 2005). This phenomenon could be traced back to the physiological conditions of crop plants and the changing of the crop canopy structure (Bernhoft et al. 2012). Besides, rather controversial physiological reactions to nitrogen dosing were observed in maize, where the mycotoxin concentration was manifested wide range as a function of the different fertilisation practices (Blandino et al. 2008a). Moreover, also the mobilisation of micronutrients can strongly influence plant development, as well as the resistance abilities related to the abiotic and biotic stressors, which depend on the presence or lack of these nutrients (Hajiboland 2012), and all of these can enhance plants to symptoms triggered by magnesium deficiencies.

Naturally, besides the fertilisation, the production potential and health condition of maize are determined by hybrid, agro-ecological and climatic conditions as well as by the growing technology

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(Ewees et al. 2008, Delibaltova et al. 2009). From this point of view, the effect of different hybrids on the qualitative and quantitative parameters is not yet sufficiently clear. The interaction between maize hybrids and nitrogen fertilisation levels, grain and biological yields could not be confirmed by several studies (Sharifi and Taghizadeh 2009, Akmal et al. 2010). Thus, the consequences of hybrid-dependent cultivation technology parameters on mycotoxin content are also controversial. According to Blandino et al. (2008b), the plant density significantly influenced the percentage of kernels infected by *Fusarium* species and the fungal ear rot severity. In contrast, the plant density did not influence the type of mycotoxin found in the kernel, which only depended on the climatic conditions during the season and their influence on the infection and the development of different fungal genera and species.

The objective of our field trial research conducted throughout consecutive years was to obtain information about the effect of weather conditions of the examined year as well as about some technological parameters, e.g. fertilisation, the hybrid effect on the yield parameters, phytosanitary conditions and mycotoxin contamination of maize samples.

MATERIAL AND METHODS

The set-up of the field experiments. Field experiments have been carried out for three consecutive years in order to examine the effect of different nutrient supplies, growing seasons of hybrids, and the climatic characteristics of the given vegetation cycle on the harvesting and phytosanitary parameters (between 2017–2019). The plots of investigations were located on arable fields in Iregszemcse (Tolna county, Hungary; GPS coordinates: WGS: X: 46.693509, Y: 18.173268). In these experimental areas, maize was cultivated for consecutive years *via* monoculture.

Fertilised and non-fertilised treatments of two maize hybrids were examined in these three years' field experiments. The seed stocks belonged to either an FAO 310 hybrid-derived, early-ripening maturity group or a medium-ripening group represented by an FAO 490 hybrid. N-content fertilisations applying 120 kg N/ha were effectuated right before the sowing in half of the plots set up for examining the hybrids. Each trial was set up as a randomised complete block design in four replicates. Plant densities were set according to local recommendations for high-yield production: 71 000 plants/ha (in early ripening maturity maize), and 64 000 plants/ha (in medium ripening maturity maize). All seeds were sown regularly at the end of April as the established optimum local planting dates. The active ingredients of the uniformly applied fungicide for the seed treatment were: 25 g/L fludioxonil and 9.7 g/L metalaxyl-M. Table 1 contains the field and cultivation data of the experiment. Insecticide treatments were done at the flight peak of *Ostrinia nubilalis* (Hübner).

Determination of harvest and yield parameters. Harvesting was carried out with a parcel harvester (Sampo, Pori, Finland). All trials were harvested at the optimum harvesting time during September or October depending on the individual maturity of hybrids. At the end of the vegetation cycle, the grain moisture (with the Dickey-John fast moisture meter, Auburn, USA), 1 000-grain weight and hectolitre weight per plot was determined. Seeds were harvested when the grain moisture content in the examined hybrids was approximate: 18–20%. Grain yield was adjusted to 14% moisture content (t/ha). The main abiotic data (daily temperature and precipitation) were supplied by the outsourced iMETOS 3.3 meteorological mobile station, which directly communicated to the FieldClimate© platform for the evaluation of the effect of climatic parameters on the yield values of the given year.

Field phytopathological bonitation and determination of mycotoxin content. Field phytopathological bonitation (evaluation of ear mould and stem rot) was performed in the field before harvest. To evaluate ear mould, 10–10 cobs were removed and used from each plot prior to harvest. The infestation degree was expressed as a percentage and applied to the entire ear area. During the field bonitation, the fungi damaging the ear (*Fusarium* spp., *Aspergillus* spp., *Penicillium* spp.) were determined only to the level of genera, no species-level determination was performed. When it was not possible to separate the fungal genera based on symptoms, identification was performed under laboratory conditions, after incubation in a humid chamber, by light microscope examination of the propagation and overwintering formulas developed on the ears.

To assess stem rot, the cross-section of the basal part of the stem was examined on a bonitation scale of 0–5 (Christensen and Wilcoxson 1966); 0 value – hybrid with green stalk, intact intestinal tissue; 1 – minimal intestinal tissue damage (rather, only discolouration indicating the presence of infection); 2 – 20% of intestinal tissue is missing; 3 – 21–50% of

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Table 1. Soil, agrotechnics, cultivation and evaluation parameters of the experimental area

	2017	2018	2019
Type of soil	calcareous chernozem		
Physical parameters of the soil	Arany-type soil cohesion index (K_A): 36; pH_{KCl}: 7.32; soil organic carbon content: 2.36%; carbonated lime: 8.07 Wt%; water soluble total salt content: < 0.02 Wt%; 87.9 mg P/kg; 201.42 mg K/kg; 3.74 mg N/kg		
Applied fertiliser	lime-ammonium nitrate: 27% N; 5% Ca; 3% Mg		
Dose of fertiliser	120 kg N/ha		
Dates of fertilisation	05. 03.	02. 05.	28. 04.
Dates of sowing	05. 04.	14. 05.	29. 04..
Name of hybrids and their FAO number	DKC 4014 (FAO 310), P 0216 (FAO 490)		
Plant densities	FAO 310: 71 000 plants/ha, FAO 490: 64 000 plants/ha		
Size of plots	3 × 9 m = 27 m^2		
Trade name and dose of the applied herbicide	Laudis 2 L/ha		
Active ingredients of herbicides	44 g/L tembotrione + 22 g/L isoxadifen-ethyl		
Dates of herbicide treatment	30. 05	04. 06.	19. 06.
Dates of field bonitation of phytopathological symptoms	04. 11.	18. 10.	13. 11.
Dates of harvest	10. 11.	20. 10.	18. 11.
Dates of toxin analytical works	05. 12.	04. 12.	04. 12.

intestinal tissue is missing; 4 – 50–75% of intestinal tissue is missing; 5 – 75–100% of intestinal tissue is missing (Mesterházy 1979).

Additionally, toxin tests were also carried out on samples taken from the crop harvested per plot. The content of deoxynivalenol (DON), zearalenone (F-2 toxin) and all fumonisin in the harvested maize crop was performed by the Laboratory of Physiology and Biochemistry (ÉBL) of the MATE (the Hungarian University of Agriculture and Life Sciences) Food Science Laboratory Network (test method identifiers: DON: ÉBL_EM-05: 2015; ÉBL_EM-03: 2015). Total fumonisin content was determined by rapid methods, enzyme-linked immunosorbent assay (ELISA) as described in the Romer Labs Total fumonisin kit.

Statistical analysis. Statistical analysis of different treatments such as year, growing season, and different nutrient replenishment was applied to evaluate their effects on the general health condition, toxin content, yield and grain moisture of maize kernels. The results were analysed by two-way ANOVA combined with the Tukey test using SPSS for Windows 11.0 software (Chicago, USA). Besides, the relationship between the extent of ear mould infection and different toxin content was analysed by correlation analysis by means of Excel 2016 software (Redmond, USA).

RESULTS AND DISCUSSION

Evaluation of the prevailing weather conditions of the experimental years. Figure 1 shows the Walter-Lieth climate diagram based on calibrated and verified meteorological data measured at the Iregszemcse meteorological station. Yields from the same treatment show statistically significant differences between years ($P < 0.001$), which can be explained by the annual fluctuations of weather characteristics.

During the three experimental years, there has not been observed such prolonged and extreme heatwave that would have negatively influenced the crop production of maize. Thus, in contrast to many years in the past decade, no production losses due to summer drought were detected in Iregszemcse (Hungary). If we examine the characteristics of the weather of each year, the 2019 one is considered to be the most ideal for the physiological development of maize. In this year, the initial development of the plant was adequately supported by higher than average rainfall recorded in late spring, mainly in May. In addition, the dry period coupled with ideal heat conditions during the ripening/dissimilation, stage after tasselling facilitated the water release from

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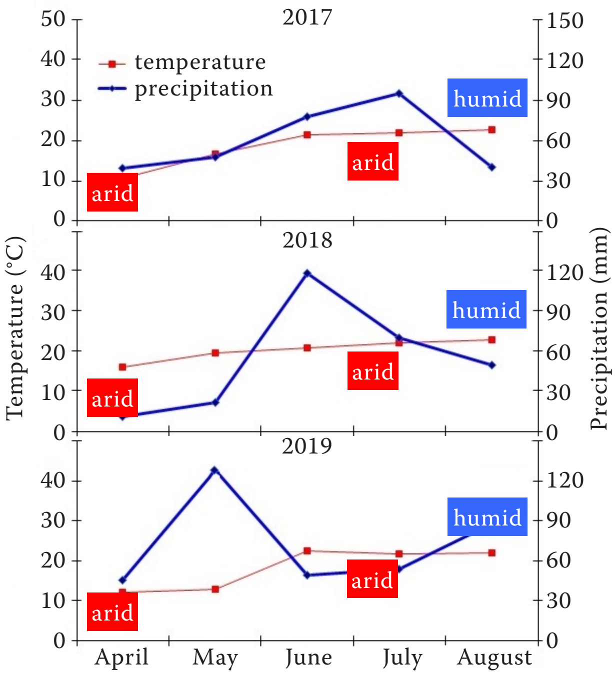

Figure 1. Walter-Lieth climate diagram based on calibrated and verified meteorological data measured at Iregszemcse during the three experimental years (2017–2019). Temperature/precipitation data were visualised using the Middle-European standard of 1:3 rate between *y*-axes (temperature/precipitation)

corn. Most of the spring and summer vegetation periods of the previous two years could be described with humid conditions (which was especially pronounced in 2017 when they had an adverse effect on plant development). By the end of summer of both years, the weather conditions were turned to be arid. Owing to this weather pattern, similarly to that of 2019, the physiological development of the vegetation (determined by the prevailing weather conditions) was undisturbed.

The effect of the hybrid and nutrient supply on the yield parameters of maize. Yield parameters (yield amount for a unit area, wet content of grain, seed kernel weight, hectolitre weight) showed significant yearly differences. Independent variables of the field multifactorial experiment (nutrient supply, the effect of hybrid, etc.) have a multiple effect on the different weight values and wet content of the grain. Figure 2 shows the crop yield of maize, during the experimental period of three years. The analysis of variance (ANOVA, $P < 0.001$) performed on the data obtained during the three experimental years confirmed the significant effects of the two hybrids (DKC 4014, P0216) on crop yield and grain wet content. Similarly, nutrient supply had also a significant effect on the yield parameters. These results are consistent with those of a number of field studies (e.g. Sinclair and Muchow 1995, Imran et al. 2015). The effect of fertilisation on grain wet content alone could not be statistically confirmed. Based on the literature, the effect of extra nitrogen added can contribute to the "extension" of the growing season, thus slowing down the release of water during the ripening processes (Farnhan 2001, Biswas and Ma 2016, Shrestha et al. 2018), which could even contribute to decreasing of the plant health condition through the triggered tissue dilution.

The 2017 year is an exception, which can be due to the special climatic characteristics already mentioned. It can be presumed that the big amount of precipitation during the growing season of 2017 did not assist the nutrient uptake of the P0216 (FAO 490) maize hybrids. Nevertheless, P0216 (FAO 490) generally provided 500–1 000 kg plus yield on average for a hectare during the experimental periods. The positive effect of fertilisation on the yield was proven in the case of both hybrids. This finding is

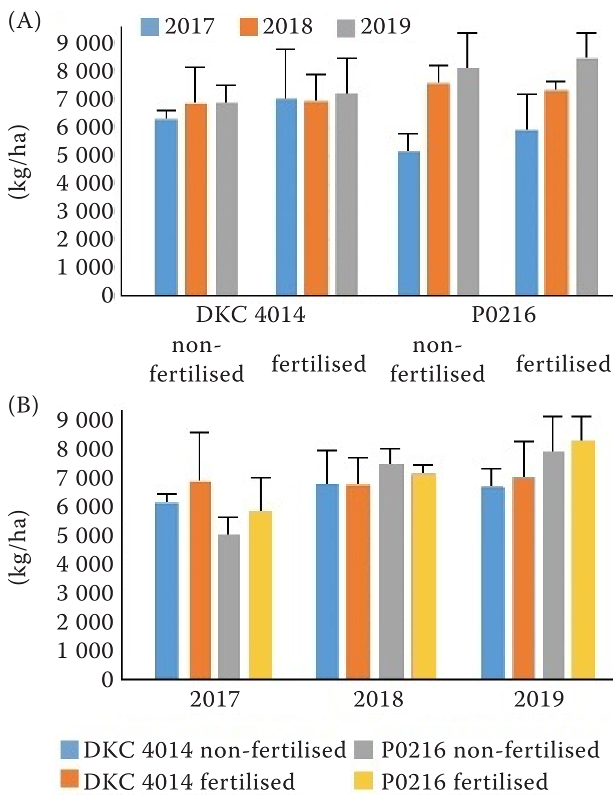

Figure 2. The crop yield (mean + standard error) of maize is due to the different treatments. (A) per treatments, and (B) per years

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especially valid for the year 2019, during which the environmental factors could be considered to be optimal for maize development. The positive correlation between vegetation time and yield was confirmed by several scientific studies (Farnhan 2001, Widdicombe and Thelen 2002, Shrestha et al. 2018).

Effects of treatments on plant health and primary diseases (stem rot, ear mould). Table 2 shows the stem rot and ear mould values recorded as a function of each particular year and nutrient replenishment. The analysis of the effects on stem rot reveals that fertilisation and nutrient replenishment did not affect the development and severity of stem rot in any of the maize ripening groups.

On the other hand, it was shown that the year-effect is the decisive factor in this aspect as well. The stem rot values recorded during each study year differed significantly. The outstanding degree of stem rot in 2019 is particularly noteworthy. Furthermore, it can be mentioned that in the plots of the maize hybrid characterised with a longer growing season, exceptionally strong stem rot values were recorded in the last year of the study, which can be explained by the humid spring and summer season of the given year.

It has been experimentally proven (Oldenburg and Ellner 2005, Oldenburg et al. 2017) that stem rot is the predominant cause leading to yield losses, especially when the disease is occurring during premature growth stages, resulting in disturbances in the plant nutrient/water supply hence leading to drying out and lodging. Based on the work of Teich (1989) and Reid et al. (2001) it is clear that the extra nitrogen (N) supply enhances the chances of corn stem rot disease. Reid et al. (2011) confirmed that N supply at higher rates, such as 200 kg N/ha increased the chance of occurrence of *Fusarium* stem rot.

Results of mycotoxin content. To support phytosanitary results, Table 3 shows the effect of fertilisation, vegetation time and experimental years on the mycotoxin content of maize. The recorded mycotoxin contents did not exceed the toxicological upper limits declared by EC No. 1881/2006 for any of the samples. Based on the three-year database analysis the different influencing effects such as the nutrient supply, vegetation time, pesticide treatments and experimental years were also proven.

Fumonisin content of maize grains was significantly influenced by the experimental years (meteorological, climatological factors), the effect of the hybrids and the fertilisation. This was especially expressed in higher fumonisin content in the case of hybrid with longer vegetation period (FAO 490) on those plots where fertilisation was also applied. A strong correlation should be observed between fumonisin content and severity of ear mould infection only in the case of hybrid characterised with a shorter growing season (DKC 4014) when plots were not fertilised. The relationship between the variables of the two factors can be described with a positive polynomial function (Figure 3).

Our results show that the histological characteristics due to the longer vegetation time and good

Table 2. Average and other statistical values of stem rot (CW) and ear mould (%) calculated by two-way analysis of variance as a function of maize growing season, nutrient supply and year effect

		Mean			(*df*) *P*		
		2017	2018	2019	effect of the year (A)	effect of fertilisation (B)	interactions of two factors (A + B)
Stem rot							
DKC4014 (FAO310)	non-fertilised	3.97	0.55	9.59	(2) 0.003*	(1) 0.353	(2) 0.704
	fertilised	0.225	0.77	7.71			
P0216 (FAO490)	non-fertilised	1.009	0.83	26.703	(2) 0.001*	(1) 0.14	(2) 3.66×10^{-5}*
	fertilised	8.15	5.025	3.262			
Ear mould							
DKC4014 (FAO310)	non-fertilised	3.775	5.93	3.26	(2) 0.006*	(1) 0.212	(2) 0.866
	fertilised	4.509	6.306	4.421			
P0216 (FAO490)	non-fertilised	7.71	7.1	4.4	(2) 0.006*	(1) 0.059	(2) 0.316
	fertilised	13.42	8.775	5.15			

*indicate statistically significant correlations; *df* – degree of freedom ($P < 0.05$)

Table 3. The effect of fertilisation, vegetation time and experimental year on the mycotoxin content of maize (based on two-way ANOVA) as a function of toxicological upper limits declared by Commission Regulation (EC) No. 1881/2006

		Mean			(*df*) *P*		
		2017	2018	2019	effect of year (A)	effect of fertilisation (B)	interactions (A + B)
Fumonisin (mg/kg) (toxicological upper limit: 60 mg/kg)							
DKC4014 (FAO310)	fertilised	1.1275	2.375	0.5875	(2) 0.0001*	(3) 0.0003*	(6) 0.396
	non-fertilised	2.18	1.46875	0.64375			
P0216 (FAO490)	fertilised	2.617	4.075	2.275			
	non-fertilised	2.257	3.812	1.525			
DON (mg/kg) (toxicological upper limit: 8 mg/kg)							
DKC4014 (FAO310)	fertilised	0.5	0.5	0.675	(2) 0.5105	(3) 0.5353	(6) 0.247
	non-fertilised	0.766	0.5	0.5			
P0216 (FAO490)	fertilised	0.5	0.5	0.5			
	non-fertilised	0.516	0.5	0.5			
Zearalenone (μg/kg) (toxicological upper limit: 350 μg/kg)							
DKC4014 (FAO310)	fertilised	25	26.72	44.012	(2) 3.63×10^{-5}*	(3) 2.88×10^{-8}*	(6) 0.0005*
	non-fertilised	31.5	21.875	26.05			
P0216 (FAO490)	fertilised	27	0	14.88			
	non-fertilised	25.625	0	3.556			
Aflatoxin (μg/kg) (toxicological upper limit: †)							
DKC4014 (FAO310)	fertilised	–	1.175	1.487	(1) 0.236	(3) 0.414	(3) 0.441
	non-fertilised	–	1.175	1.612			
P0216 (FAO490)	fertilised	–	1.275	9.937			
	non-fertilised	–	1.1	1.9			

*indicate statistically significant correlations; *df*: (P < 0.05), treated-untreated: treated with pesticides, or not treated with pesticides; † – as the aflatoxins are carcinogenic and genotoxic acceptable daily intake cannot be established

nutrient supply favour the fumonisin production of the observed phytopathogenic fungi.

In the case of DON toxin, the effect of the meteorological factors and technological parameters was not so pronounced as compared to that of the fumonisin content. Neither experimental years nor agrotechnical parameters influenced significantly the amount of DON toxin.

The concentration of zearalenone was greatly influenced by the climatological factors of the experimental year. Extreme high values were determined in 2019. In parallel, the effect of hybrids was also believed to be an important factor in the zearalenone content of maize. Surprisingly, much higher values were recorded in plots of hybrid with shorter growing seasons. Nutrient supply was also a significant factor influencing the zearalenone content. This finding was supported by the higher toxin concentration of the unfertilised plots. We have also shown that the interactions of the examined abiotic and human (growing technological) factors also have a statistically significant effect on the zearalenone content.

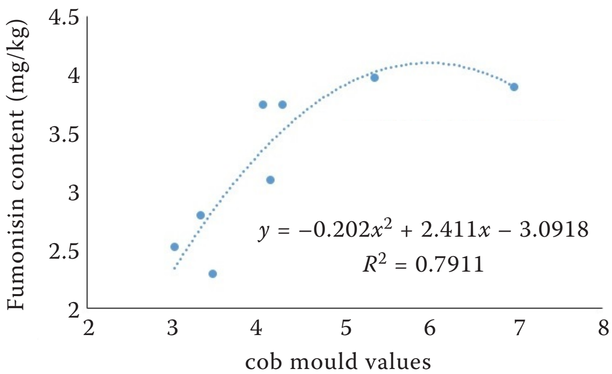

Figure 3. Correlation between the ear mould values and fumonisin content

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According to Atlin et al. (1983) work, in which covariance adjustment for mould level was performed, hybrids were found not to differ significantly toxin accumulation in either year. An effect due to the genetic nature of the hybrid was observed only for the accumulation of zearalenone. Therefore, it can be concluded that its accumulation was influenced by more technological parameters.

Aflatoxin content of maize was examined only in 2018 and 2019. Similar to DON content no statistical correlation was found between the aflatoxin content and the technological parameters/climatic factors examined.

In summary, based on our studies, it can be stated that the applied technological parameters have a major effect on the expression of this toxin load in maize. Dry maize stocks that have lost their water in the vegetation are believed to be predisposing factors for toxin accumulation. N-content of soil and that of plants can play a different role in mycotoxin accumulation in maize plants. According to the results of Yi et al. (2001), the high mycotoxin content in soil is a predisposing factor for high mycotoxin concentration in plant organs, as opposed to the appropriate N supply of the plant. Nitrogen seems to play a critical role in forming a plant's immune system because its deficiency limits the plant's growth rate, whilst reducing the yield potential (Szulc et al. 2012). Overall appropriate mineral fertilisation – especially with regard to nitrogen – and sowing date may contribute to the reduction of plant infection by fungi of the *Fusarium* genus. Several researchers have claimed that using various types of fertilisers (urea, ammonium nitrate and calcium nitrate) causes a decrease in the degree of grain contamination with mould fungi; however, an excess of nitrogen in the soil may increase the infection of grains (Yi et al. 2001). The sowing date of the crop is believed also to be a very important factor to avoid competition between flowering time and spore dispersal period of fungi. Therefore, earlier dates for sowing maize in specific areas often result in a lower level of infection (Molnár et al. 2004).

Many fungal species can produce multiple toxins or the same toxin can be produced by multiple fungal species (Logrieco et al. 2002, Szécsi et al. 2010, Bartók et al. 2006, 2013). In the case of *Aspergillus* species, which had previously been considered to be storage pathogens, the field origin of the aflatoxins produced by them has been demonstrated recently (Guo et al. 2017). This was also confirmed by our results. This may also be a consequence of global warming tendencies, as the *Aspergillus* genus has a higher temperature requirement than that of *Fusarium* species.

Resistance breeding is believed to be the most efficient method against plant diseases. The reaction of maize hybrids to pathogens may greatly differ, nevertheless, an absolutely resistant hybrid to pathogens does not exist currently (Mesterházy et al. 2020). The expression of differences in susceptibility greatly depends on the year-effect. If environmental factors are favourable for the pathogens ("great infection pressure"), differences in susceptibility between hybrids can be well observed and evaluated, however, if conditions are not optimal for pathogen action, even a more susceptible hybrid may show an adequate level of resistance. Based on the results of Mesterházy (2018) it can be said that the resistance of maize to fungal pathogens occurring on the ear is generally not associated with the hybrid characters. During breeding, all fungal species must be counted individually and the degree of resistance must be tested separately for each fungal pathogen (Mesterházy et al. 2000, Szabó et al. 2018). This is all the more justified because the dominance of species damaging the ear may vary from year to year. If we follow this guideline in breeding and assessing the degree of resistance, there will be significant quality improvements in food and feed safety in the future (Szabó et al. 2018).

We could not find the correlation between the toxin content of maize and the intensity of the infection in all cases. High toxin levels can be measured in ears with little or no symptoms, so it is not possible to take a clear stand on resistance issues without toxin analysis (Mesterházy et al. 2000).

Plant breeding and phytopathological research show that in order to acquire reliable pieces of information about the level of resistance of a hybrid (or breeding line), it is not enough to examine the natural infection, but provocative tests and artificial infections are also necessary. Assessment of natural infection and symptomatic identification remain important, especially in field plant-protection experiments. However, these need to be examined over several years in order to measure the impact of changing environmental conditions from year to year (Mesterházy et al. 2000). Expression of disease symptoms are influenced not only by environmental factors, but genetic background and the level of resistance as well (Munkvold and White 2016).

https://doi.org/10.17221/80/2022-PSE

Under field conditions, it can be even more difficult to achieve accurate results, as the so-called stem rot is considered to be a "pseudo-resistance factor", as plants showing this symptom can be characterised with sudden and rapid water release and this can reduce the symptoms of ear mould by up to 50%. In epidemic years, high levels of infection definitely imply a lack of resistance, but the absence of symptoms does not necessarily hint at resistance, it can also be due to a simple disease avoidance of the plant (Mesterházy et al. 2000, Mesterházy 2018).

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Received: May 10, 2022
Accepted: June 7, 2022
Published online: June 15, 2022