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Original article

Influence of *Fusarium* spp. Inoculation Dose Simulating Pathogen Pressure on Grain Production Parameters of *Zea mays* L.

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Abstract

Several fungi species from genus *Fusarium* can infect maize crop and cause seedling blight, root rot, stalk rot, cob and ear rot. Maize yield and grain quality are negatively affected by the disease, while mycotoxins accumulating in grains represent a serious threat to human and animal health. In this research two maize hybrids were subject to artificial infection in field with *Fusarium* spp. suspension at three incremental dose levels simulating increasing pathogen pressure. Results indicated that earlier maturing hybrid ('Turda 248' – FAO 300 maturity group) following artificial infection displayed lower infection index of ears (up to 15.33%) while the later-maturing hybrid ('Turda 332' – FAO 380 maturity group) displayed higher infection levels of up to 30.87%. Uninoculated controls presented infection index of ears below 2% for both hybrids. For the hybrid 'Turda 248' the highest pathogen pressure (inoculation dose 12 mL/ear) caused 23.10% yield decrease relative to control, while same dose for the hybrid 'Turda 332' caused 21.20% yield decrease relative to control. Once with increased inoculation dose the grain quality parameters displayed changes. The observed trend was an increasing protein content and decreasing starch content once with increased pathogen pressure. Identification of resistant genotypes and study of the climatic factors that undergo disease development remain important research approaches for efficient control of the pathogen populations in agroecosystems.

Keywords: Kernel, Mycelia, Infection, Ear, Grain.

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INTRODUCTION

Maize is among the most cultivated cereals. It used as food, as animal feed as well as for obtaining various industrial products. Once with Romania integration into European Union (EU) the maize cultivation registered an expansion. Today Romania is one of the most important maize growers within EU and having excellent export potential (Ghețe et al., 2020).

Maize crop can be invaded by pathogens at any stage from germination to harvest. The disease can significantly reduce the quantity and alter the quality of the maize grain production (Șopterean et al., 2014). *Fusarium* is a fungi genus that represents one of the most important disease agents of maize crops. Several *Fusarium* species can infect maize plants causing seedling blight, root rot, stalk rot, cob and ear rot (Warham et al., 1996; Munkvold et al., 1997; Watson, 2007). Among these are: *F. crookwellense* Burgess, Nelson & Toussoun, *F. culmorum* (W.G. Sm.) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe, *F. poae* (Peck) Wollenw, *F. tricinctum* (Corda) Sacc., *F. verticillioides* (Sacc.) Nirenberg, *F. anthophilum* (A. Braun) Wollenweber (Warham et al., 1996; Fandohan et al., 2003). These fungi are widespread in soils. The infection routes are the following:

- Fungi from infected seeds are transmitted to seedlings

- Conidia from water splashed on plants or carried by the wind might end up on the silk and then reach to the kernels

- Infection propagating from kernels to cob can travel further through stalk

- Infection spreading through root and stalk can travel systemically and reach the cob

- Fungi propagules entering through injury sites and often being carried by vectors such as insects can infect the plants (Munkvold et al., 1997; Fandohan et al., 2003).

The main natural sources of infection with *Fusarium* for maize crop are illustrated in Figure 1. These are represented by pathogen reserves surviving on plant residues in soil as well as infected seeds that are used for starting the crop and which will eventually infect the plants and later affect the yield parameters (Watson, 2007).

Research on initial stages of infection with *Fusarium* of intact ears, revealed that the pathogen by accessing intact ears through silk channel can enter the healthy kernels through the opening at the point of junction of carpels. This strategy allows the kernel infection by kernel rot pathogens such as *Fusarium verticilloides* that otherwise are unable to form appressoria as penetration structures and cross natural structural barriers. The fungus then spreads and after entering the pericarp can fill the mesocarp cells. This spreading precedes the macroscopic symptoms (Duncan and Howard, 2010). Macroscopically, cob rot caused by *Fusarium* is characterized by a typical fungal mycelia growth that usually is whitish in color and covering the entire cob, being limited to some parts of the cob or affecting only individual kernels. These might display white streaking lines, but asymptomatic infection is also possible (Watson, 2007). Following kernel infection by *Fusarium*, poisonous metabolites produced by fungi can accumulate into maize grains representing a health danger for consumers. Alteration of grain quality following infection results in reduced nutritional value, changes in color and mycotoxins presence (Fandohan et al., 2003).

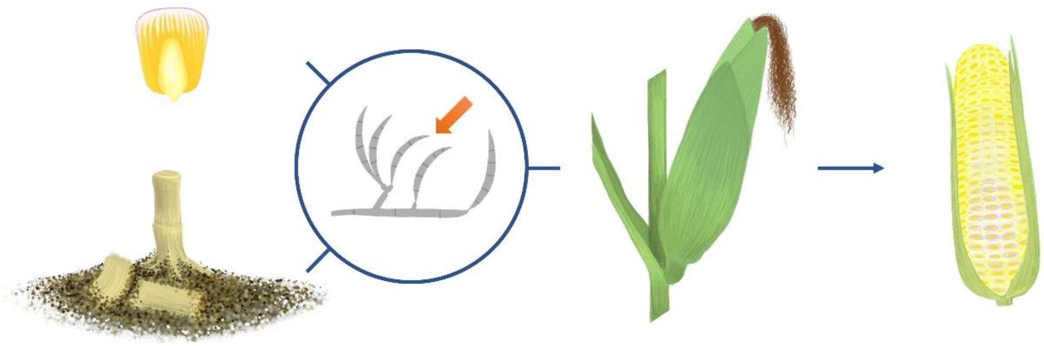


Figure 1. Main natural infection sources with Fusarium sp. of maize crop; arrow indicates fungi

Source: original

Maize crop in field can experience conditions more or less favourable to the development of the disease, while the natural abundance of pathogens can also range. Thus, the response of maize under increased pathogen pressure can provide information on the potential levels of disease and yield loss of given maize genotypes. These provide an important characterization of genotypes with immediate and practical implications.

Aim of this research was to study the behavior of two maize hybrids of different maturity groups under a gradient of pathogen pressure simulated through artificial inoculation with *Fusarium* spp. suspension of ears in field conditions. The objectives of the study were:

- Identification of the relationship between pathogen pressure and disease occurrence

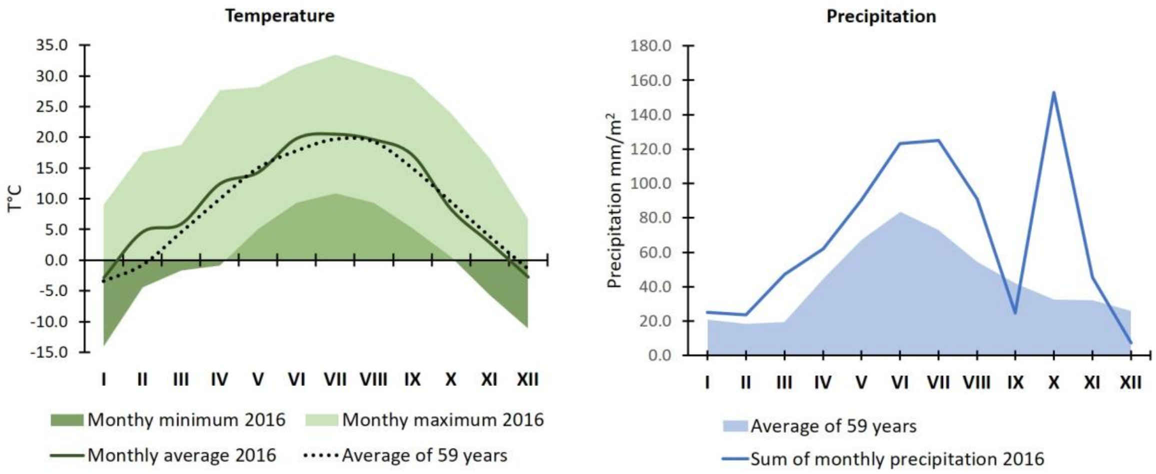
- Identification of incremental pathogen pressure influence on productivity parameters.

MATERIALS and METHODS

Location and Climatic Conditions

The study was conducted at Research and Development Station (ARDS) from Turda, Romania, during the year 2016. Turda is situated in Transylvanian Plain. The climate is temperate continental, with four seasons. The climatic conditions during experimental year are presented in Figure 2.

By comparing the monthly temperatures registered in the experimental year with the multiannual average values, it was determined that 2016 had higher temperatures in the months of February-April, June and September. On average, the year 2016 had higher temperatures than the multiannual average of the previous 59 years (Figure 2a). Regarding the precipitations registered during 2016, in comparison with multiannual average values for 59 years, it was determined that some months were drier (September) but most of them were rainier. Overall, the experimental year had with 303.6 mm/m^2 more rainfall than multiannual average (Figure 2b).



(a) (b)

Figure 2. Climatic conditions during experimental year (2016): (a) temperature, (b) precipitation.

Source: ARDS Turda weather station situated at long. 23°47', lat. 46°35' altitude 427 m

Biologic Material and Artificial Infection

The maize genotypes used for this study were represented by two hybrids created at ARDS Turda: 'Turda 248' and 'Turda 332'. These are both simple hybrids. First one is from group FAO 300, while the second one is from FAO group 380 (https://scdaturda.ro).

The plants were artificially infected with a suspension of *Fusarium* spp. that has prevalence in the disease of maize ear and stalk in conditions from Transylvania, Romania. For obtaining *Fusarium* spp. inoculant, maize seeds were placed in Linhardt trays for incubation. Infected seeds that developed *Fusarium* spp. mycelia confirmed under microscope were grinded and then mixed into 1 L of distilled water. After stirring, the suspension mix was strained. The inoculation of maize ears was performed in the morning during 8-10 a.m. by injecting the suspension of *Fusarium* spp. inoculant using Socorex syringe in the upper part of the maize ear in two points. The inoculation was performed in blister stage after pollination when the silk turned brown. The procedure followed a standard methodology (Ceapoiu and Negulescu, 1983; Reid and Hamilton, 1996). In order to simulate an increasing pathogen pressure, three suspension doses were tested: 4 mL/ear, 8 mL/ear and 12 mL/ear.

Determinations at Harvest

At harvest was determined the infection index of the maize ears by applying the formula:

$$infection\ index = \frac{(infection\ incidence \times infection\ severity)}{100}$$

Infection incidence refers to the frequency (%) of the maize ears infected, that is expressed as relative number of maize ears showing signs of infection divided by the total number of ears analyzed, applying the formula:

$$infection\ incidence = \frac{number\ of\ infected\ ears}{total\ number\ of\ ears\ analysed} \times 100$$

The infection severity expresses the intensity of the infection on ears, established based on the scoring following visual evaluation, by applying the formula:

$$disease\ severity = \frac{\Sigma\ (i \times f)}{n}$$

where: i – represents the score according to the evaluation scale that defines the infection extensiveness (ear infected 5%, 10%, 30%, 75%), f – number of ears with the respective score, n – total number of ears infected (Puia, 2005; ISTIS, 2009).

Yield was determined following manual harvest and weighing, followed by correction at STAS humidity of 15.5%. Qualitative parameters of the yield (starch and protein) were determined using NIR Tango spectrometer.

Data was processed and analyzed using Microsoft Excel and Polifact statistical software. The test applied was Fisher's Least Significance Difference test (LSD).

RESULTS and DISCUSSION

In the Figure 3 is presented the relationship existing between maize ear infection index and grain yield. Once with incremental inoculation dose that caused increased maize ear infection, the yields decreased progressively in value from 4.5 t/ha to 3.5 t/ha. At lowest inoculation dose tested (4 mL) although the infection index was significantly higher than control the yield decrease was not significant. Only starting with inoculation doses of 8 mL and respectively 12 mL the yields decreased significantly compared to control. This suggests that there was a threshold up to which the plants were coping with the pathogen pressure and as a result the yields were not significantly affected.

When analyzing separately the influence of pathogen pressure on the two maize hybrids tested, can be observed that maize ear infection index for non-inoculated plants of both hybrids was below 2%, and increased progressively once with incremental inoculation dose. For hybrid 'Turda 248' the ear infection index was distinctly significantly higher than control at all three dose levels, but infection remained below 20% even under highest pathogen pressure. For the hybrid ‘Turda 332’, the inoculation dose of 8 mL and 12 mL caused significantly higher infection index of maize with values exceeding 20% and respectively 30% (Figure 4). Thus, infection reached higher levels in the later-maturing hybrid (‘Turda 332’) than earlier-maturing hybrid (‘Turda 248’).

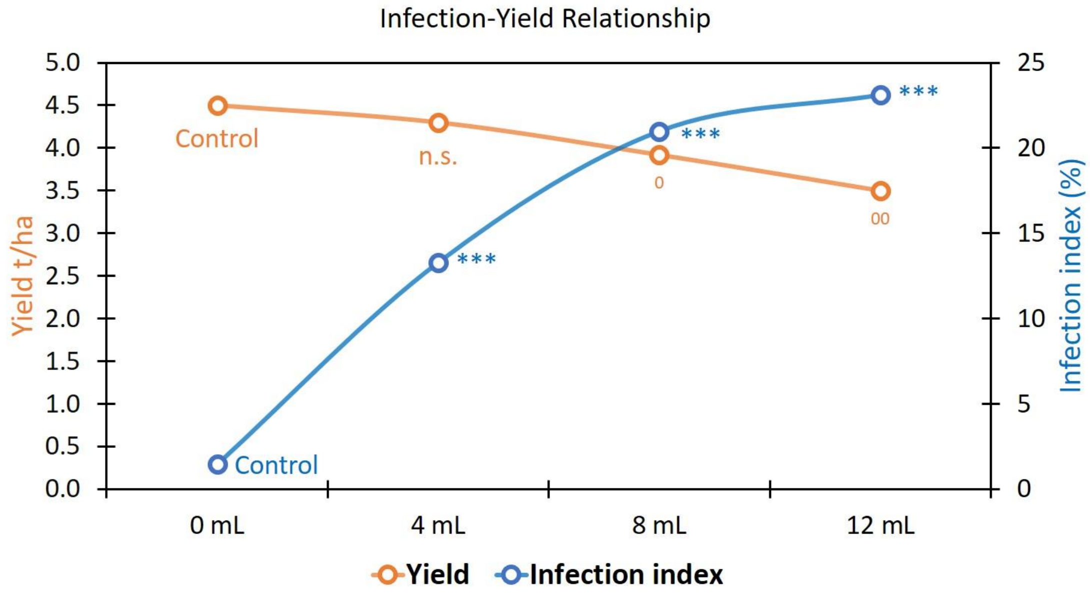


Figure 3. Relationship between grain yield and *Fusarium* infection index of maize ears; infection index (LSD *p* 5% = 5.36, *p* 1% = 7.52, *p* 0.1% = 10.62), yield (LSD *p* 5% = 0.47, *p* 1% = 0.66, *p* 0.1% = 0.93).

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Once with increasing inoculation dose simulating increasing pathogen pressure, the grain yield registered a decrease for both hybrids. For the hybrid ‘Turda 248’ the highest pathogen pressure (inoculation dose 12 mL/ear) caused 23.10% yield decrease relative to control, while same dose for hybrid ‘Turda 332’ caused 21.20% yield decrease relative to control. For both hybrids this decrease at this dose level was distinctly significantly lower than control. For neither of the two hybrids tested the low pathogen pressure (dose of 4 mL/ear) induced statistically significant yield loses, yet the infection index at this dose level was significant. Interestingly, although the hybrid 'Turda 332' registered higher infection index values with highly significant increase of infection index at dose level of 8 mL/ear, the yield was not significantly affected, while at same inoculation dose level the hybrid 'Turda 248' although had lower infection index values, the yield decrease was significant (Table 1).

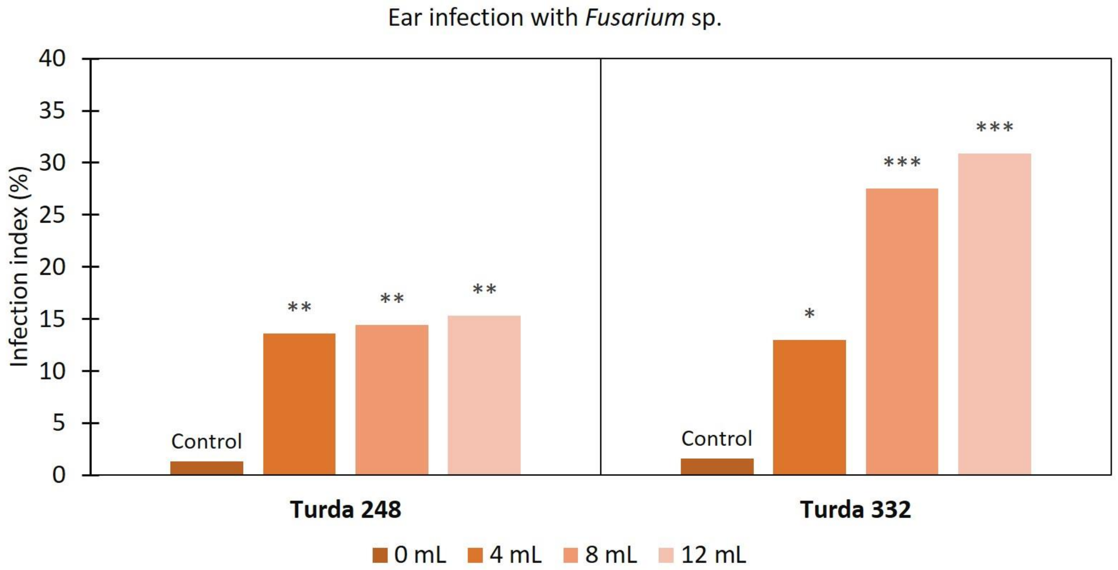


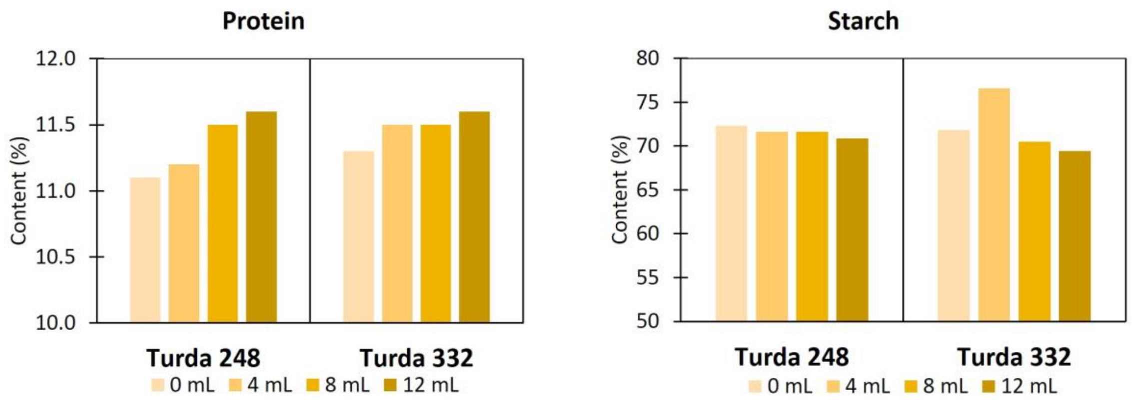
Figure 4. Ear infection index of maize following inoculation with incremental *Fusarium* spp. dose; Turda 248 (LSD *p* 5% = 7.08, *p* 1% = 10.72, *p* 0.1% = 17.23), Turda 332 (LSD *p* 5% = 9.74, *p* 1% = 14.75, *p* 0.1% = 23.70).

Following incremental inoculation dose with *Fusarium* spp., the grain quality parameters displayed changes. The trend observed was an increasing protein content and decreasing starch content once with increased pathogen pressure.

For the maize hybrid 'Turda 248' the protein increased from 11.10% registered by grains from ears that were not inoculated up to 11.60% following dose of 12 mL/ear *Fusarium* spp. inoculation dose. The starch decreased from 72.30% registered by control plants to 70.90% under 12 mL/ear inoculation. In the case of the hybrid 'Turda 332' there was also an increased protein content in grains but at 4 mL/ear and 8 mL/ear the protein content remained the same (11.50%). Starch content showed a decreasing trend as well under increased pathogen pressure like the other hybrid except for 4 mL/ear inoculation dose when was registered a high starch content (Figure 5).

Table 1. Influence of *Fusarium* spp. incremental inoculation dose on maize yield

No.	Hybrid	Suspension dose	Yield t/ha	Diff. %	Diff.	Significance
1	Turda 248	non-inoculated	4.55	100.0	0.00	Control
2		4 mL/ear	4.21	92.6	-0.34	-
3		8 mL/ear	3.73	81.9	-0.82	^{0}
4		12 mL/ear	3.50	76.9	-1.05	^{00}
5	Turda 332	non-inoculated	4.44	100.0	0.00	Control
6		4 mL/ear	4.39	98.7	-0.06	-
7		8 mL/ear	4.11	92.4	-0.34	-
8		12 mL/ear	3.50	78.8	-0.94	^{00}
LSD (*p* 5%)					0.67	
LSD (*p* 1%)					0.93	
LSD (*p* 0.1%)					1.32	



(a) (b)

Figure 5. Influence of *Fusarium* spp. inoculation dose on: (a) protein and (b) starch content of the maize grains of two hybrids

Results of this study are in consensus with previous reports. Thus, a previous study conducted during 2011-2012 in conditions of Transylvanian Plain on eight maize hybrids, indicated that artificial infection with *Fusarium* spp. also reduced the yields, in that case with 0.3-1.4 t/ha and caused grains to have lower starch content (Șopterean et al., 2014). It has been asserted that late-maturing maize genotypes that present a slow loss of grain moisture content are highly susceptible to *Fusarium*, as well as those with thinner pericarp, those that are tight-husked, those with up-right cobs or with higher propensity towards kernel splitting (Fandohan et al., 2003). One of these aspects was also suggested by the results of this research, since the later maturing hybrid displayed higher infection values compared to the earlier maturing hybrid.

Screening of 14 maize hybrids inoculated in field conditions in Poland during the span of several consecutive years revealed that inoculated ears presented *Fusarium* damaged kernels ranging from less than 1% up to 60%. It was also identified that the year of inoculation exercised a significant influence over the infection development and accumulation of fumonisins (Pascale and Visconti, 1997), highlighting the importance of environmental factors and particularly climatic ones on the evolution and dynamics of the disease. Maize ears collected between 2016-2018 from 58 locations across Germany were tested for *Fusarium* presence. Most common species infecting the maize ears were *F. graminearum*, *F. verticillioides* and *F. temperatum* while the precipitation and temperature during month of flowering was determined to be of uttermost importance for establishment of the pathogen. Other species identified on maize ears but with lower prevalence were *F. poae*, *F. cerealis*, *F. proliferatum*, *F. tricinctum*, *F. avenaceum*, *F. culmorum*, *F. subglutinans*, *F. equiseti* and *F. sporotrichioides* (Pfordt et al., 2020).

A field experiment that conducted silk inoculation of maize with *F. graminearum* and *F. verticillioides*, managed to identify a potential negative interaction between the two pathogens, while wet conditions favored the growth of *F. graminearum* more than the growth of the other species (Stewart et al., 2002). Another experiment on the effect of inoculation of maize with *Fusarium* has indicated that the relationship existing between disease severity, yield and mycotoxin accumulation in grains was explained by linear models (Presello et al., 2008), fact that indicate to the strong relationship existing between these parameters. Similarly, in the current study a relationship was also observed between yield and infection index of maize ears.

Considering the current trend of maize cultivation in Romania and worldwide, there are good prospects for expansion of the crops. However, high quality grains can be obtained from healthy crops where disease is effectively mitigated and pathogen control is achieved through sustainable means. Thus, more efforts have to be directed for the identification of genotypes displaying resistance to the disease that might be used in breeding programs. Also, elucidation of the role played by environmental factors on the dynamic of a pathogen population for a given geographical area, could be just as important in strategically addressing pathogen management in agroecosystems locally.

CONCLUSION

Fusarium spp. are fungi ubiquitous to agroecosystems that can infect maize crop at any stage during development, causing decreased quantity and quality of the yield.

This study tested pathogen pressure simulated through artificial infection in field conditions for two maize hybrids of different maturity groups.

Results indicated that hybrid ‘Turda 248’ (from FAO 300 maturity group) following artificial infection displayed infection index of ears between 13.60-15.33% while the hybrid ‘Turda 332’ (from FAO 380 maturity group) displayed infection index of ears between 12.97-30.87%. Higher infection levels reached by the later-maturing hybrid compared to earlier maturing one is in consensus with sources from literature that indicate a higher susceptibility to *Fusarium* of the genotypes with late-maturity. Uninoculated control presented infection index of ears below 2% for both hybrids.

Highest pathogen pressure tested (suspension dose 12 mL/ear) caused 23.10% yield decrease relative to control for the hybrid 'Turda 248', while same dose for hybrid 'Turda 332' caused 21.20% yield decrease relative to control.

The trend observed for grain quality parameters was an increasing protein content and decreasing starch content once with increased pathogen pressure.

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