

## MYCOFLORA OF MAIZE SEED

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**Abstract:** Corn is the one of the most important crops in Serbia. Corn is grown on 1.200.000 hectares. It is susceptible to a number of ear and kernel rots, some of which are widely distributed and can cause significant damage in humid areas. Ear and kernel rots can reduce yield, quality and feed value of grain. Toxins produced by the fungi in corn can also have serious implications to the end use of the grain. Fungi belonging to the genus *Fusarium* are the most significant fungi which can cause corn ear and kernel rots. The aim of this paper is to test health of mercantile maize seed which belong to 3 different hybrids (PR 34N43, PR 36K67 and PR 37N01). Seed health testing was done using filter paper and nutritive media (PDA) method. The weather during vegetation period (April-October) was very variable. Total rainfall amount for Sombor for that period in Sombor area was 744 mm. Fungi from genera *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria* were isolated from tested corn seed by both methods. No significant differences were found between tested hybrids in the level of seed contamination. Two species from the genus *Fusarium* were found in the tested corn samples *F. graminearum* and *F. moniliforme*. These species are some of the most common on corn in Serbia. Tested hybrids which belong to different FAO maturity groups showed differences in susceptibility to ear and kernel rot. Although the observed percent of *Fusarium* species was from 5.6-9.7% there is no significant difference between yield of tested hybrids. But if the infection potential is present, during storage condition (if the conditions in the storage are not optimal) severe seed contamination could appear. So, the next step is monitoring of mycotoxins production of isolated species, in the first place the two *Fusarium* species and *Penicillium* and *Aspergillus*.

**Key word:** mycoflora, corn seed, *Fusarium* spp., yield, meteorological conditions,

### INTRODUCTION

Corn is susceptible to a number of ear and kernel rots, some of which are widely distributed. These rots can cause considerable damage in humid areas, especially when rainfall is above normal from silking to harvest. The prevalence of rots can be increased by insect and bird damage to ear and stalk. Ear and kernel rots can reduce yield, plus the quality and feed value of grain. Toxins produced by the fungi in corn can also have serious implications to the end use of the grain.

The various fungi that cause ear and kernel rots. Fungi belonging to the genus *Fusarium* are capable of causing ear rots. For *Fusarium* ear rot, the species that cause infection include *F. verticillioides*, *F. proliferatum* and *F. subglutinans*. Rot caused by *Gibberella zea* (asexual state *F. graminearum*) is often called *Gibberella* or red ear rot (PAYNE, 1999). These fungi can also cause stalk rot. Other fungi can cause ear and kernel rots including species of *Penicillium*, *Diplodia*, *Aspergillus*, *Nigrospora*, *Botryosphaeria*, *Cladosporium*, *Rhizoctonia*, and *Rhizopus*.

The aim of this paper is to test health of mercantile maize seed which belong to different hybrids from different maturity groups.

### MATERIAL AND METHODS

#### Corn seed samples

Hybrid corn seed of different FAO maturity groups were used PR37N01 (FAO 370),

PR36K67 (FAO 530) and PR34N43 (FAO 690). Hybrid corn seeds were treated with fungicide Maxim XL 035 FS (a. m. fludioxonil 25 g/l). Seeds were sown in the field at the Sombor locality (Vojvodina province, North part of Serbia). Harvest was done on 15th October, 2010. For the yield estimation 100 ears in 4 replicates from each hybrid were measured and calculated per hectare. Seed sub samples were made and seed health testing was performed.

#### Seed health testing

Seed health testing was done using filter paper and nutritive media (PDA) method. From each sample for seed health test on filter paper 100 seed in four replicates were used. For seed health test on PDA 20 seeds in 4 replicates were used. Seeds were sterilized in 1% NaOCl and than incubated for seven days on filter paper and PDA at 25°C. The seed health was determinate basing on percentage of fungi present in seeds. *Fusarium* spp. were isolated on carnation leaf medium (CLA) Fisher et al (1982) and identification of the isolates was performed according to NELSON et al (1983), and BURGESS (1994).

Data were analyzed by ANOVA and Duncan test using software Statistica 10.

#### RESULTS AND DISSCUSION

Total rainfall amount for Sombor during vegetation period (April-October) was 744 mm. This amount is almost double related to multi annual average in Serbia which is 415 mm. Average temperature is at the level of multi-annual average for that period. Rainfall amounts recorded in Sombor for May and June were three times higher than multi annual average, while in September the amount of rainfall was doubled (108 mm) related to multi annual average. In July the amount of rainfall was at the level of 76% for this month. At the Sombor locality during September air temperature was lower for 1°C related to multiannual average and it was 16°C. October was characterized with colder weather (temperature was for 2-3°C lower than multiannual average) (RHMZ, 2010). The weather during vegetation period was very variable and there are significant differences between amounts of rainfall.

*F.moniliforme* – disease development and spread are favored by dry warm weather. Cool wet weather within three weeks of silking favors development of red ear rot caused by *F. graminearum* (ALMAŠI et al., 2002). These authors also confirm there are clear differences in corn hybrid susceptibility to causal agents of ear rots.

The moisture content in tested seed samples ranged from 18.45-20.15% (table 1). Hybrid PR 34N43 has significant higher moisture content, than to other tested hybrids. Such high percent of moisture in seed was caused by extremely wet weather in the period of harvest. These values of moisture are significantly higher than maximum permitted for corn seed which is 14% (Official gazette, 1987).

Table 1.

Seed moisture content in tested corn seed samples	
Hybrid	Seed moisture content
PR 34N43	20.15a
PR 36K67	19.04 b
PR 37N01	18.45 b
p	0.002*

Fungi from genera *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria* were isolated from tested corn seed by filter paper method. Fungi from genera *Fusarium* and *Penicillium* were noticed in all tested seed samples. In four samples fungi from genus *Aspergillus* was not noticed in PR 36 K 67. *Alternaria* was observed in low percent in PR 36 K 67 and PR 34 N 43. Apperance of species from *Fusarium* genus 7 days after seed incubation was from 2,8-4,4%.

(table 2). Fungi were noticed on mechanically damaged seed, on seed with discoloration and on seed without symptoms. Infected seed had brittle kernel and its cavity was filled with mycelium.

The highest number of infected seeds noticed in hybrid PR 36 K 67, than PR 37 N 01, lowest infection was in hybrid PR 34 N 43.

Table 2

Occurrence of fungi in tested corn seed (filter-paper method)

Occurrence of fungi in tested corn seed (%)				
HYBRID	<i>Fusarium</i> sp.	<i>Penicillium</i> sp.	<i>Aspergillus</i> sp.	<i>Alternaria</i> sp.
PR 36 K 67	4,4	3,6	0	0,1
PR 34 N 43	3,1	4,4	0,6	0,1
PR 37 N 01	2,8	1,7	0,1	0
p	0,12ns	0,16ns	0,73ns	0,62ns

Fungi which were developed in tested corn seed in filter paper also were developed in nutritive media. Seed infection was the highest with *Fusarium* species. The lowest number of infected seeds noticed in hybrid PR 34 N 43 (5.6), than in hybrid PR 36 K 67 (7.2), while the highest number of infected seeds was in hybrid PR 37 N 01 (9.7). Such number of infected seeds indicates that tested hybrids have some susceptibility to *Fusarium* species, but significant difference was not observed between tested hybrids.

Two species from the genus *Fusarium* were found in the tested corn samples *F. graminearum* and *F. moniliforme*. These two species are the most widespread diseases of maize ears. According to many authors the causal agent of maize ear and kernel rot is caused by *Gibberella zeae* (*Fusarium graminearum*). *Fusarium verticillioides* syn. *Fusarium moniliforme*, *F. proliferatum* and *F. subglutinans*.

Table a. 3.

Occurrence of fungi in tested corn seed (PDA)

Occurrence of fungi in tested corn seed (%)				
HIBRID	<i>Fusarium</i> sp.	<i>Penicillium</i> sp.	<i>Aspergillus</i> sp.	<i>Alternaria</i> sp.
PR 36 K 67	7,8	3.4	0,3	0.5
PR 34 N 43	5,6	5.6	0.3	0.
PR 37 N 01	9,7	5	0.3	0.4
p	0.56	0.85	1,0ns	0,72ns

At harvest time there were a lot of cobs with visible *Fusarium* infection. *Fusarium* cob rot caused by *F. verticillioides*, *F. proliferatum* and *F. subglutinans* is characterized by pale orange mycelium covering either individual kernels or, in serious cases, covering the entire ear. If infection is not so severe the streaks or white lines run across the kernels and are most likely on some kernels on ear every year without being noticed. Seed infected with *Fusarium* species can be without visible symptoms (latent infection). *Gibberella* ear rot (caused by *Gibberella zeae*) usually begins as a reddish mold at the tip of the ear. Infected kernels had a pinkish to reddish color.

The most important toxigenic fungi occurring in the moderate climatic zones of North America and Europe are *Fusarium* fungi (KOS et al., 2003). Zearalenone, deoxynivalenol (DON) and fumonisins are more prevalent mycotoxins that occur in grain (Schaafsma et al., 1998). The presence of these mycotoxins can affect various animals so they must be monitored and managed. The first step in that process is monitoring of hybrid susceptibility in the field and also health testing of seed lots. Monitoring of maize seed from 2009 harvest (65 samples)

in Serbia showed that two samples was contaminated by zerealenone above the established maximum level adopted by European Commission for unprocessed maize (JAJIĆ et al, 2010 ).

*Penicillium* rot on corn seed was usually evident as discrete tufts or clumps of a blue-green or gray-green mold of individual kernels. The fungi appearance was more common in broken kernels. *Penicillium* appeared as small, discrete colonies of mold growth with a dusty or powdery appearance (CIMMYT, 2011). Number of infected seeds varied between hybrids and the attack of fungi from genus *Penicillium* in tested corn seeds ranged from 3.4-5.6% (Table 3).

Fungi from genus *Aspergillus* was observed in low percent (table 3). The reason for such low percent of infection may be in the fact that *Aspergillus* species is favored by high temperatures and dry conditions. *Aspergillus* ear rot is typically associated with drought stress.

Although the observed percent of *Fusarium* species was from 5.6-9.7% there is no significant difference between yield of tested hybrids (table 4).

Table 4.

Seed yield of tested genotypes	
Hybrid	Seed yield (t/ha)
PR 36K67	10,9
PR 34N43	10,8
PR 37N01	11,4
p	0,36ns

## CONCLUSIONS

One of the main factors that have influence on kernel health is amount of rainfall especially in harvesting period. Susceptibility of hybrids to ear and kernel rots is also very important factor in disease development. However, if ear and kernel rots developed in the field, it is important to harvest the field in a timely manner and to store the grain under the best possible conditions. It is very important to dry grain to 15% moisture as quickly as possible and monitor grain on a regular basis throughout storage life to insure moisture and temperature are maintained at correct levels. If the infection potential is present the optimal storage condition can prevent sever seed contamination. The next step is monitoring of mycotoxins production of isolated species.

## BIBLIOGRAPHY

- ALMAŠI, R., BAČA, F., BOŠNJAKOVIĆ, A., ČAMPRAK, D., DRINIĆ, G., IVANOVIĆ, D., LEVIĆ, J., MARIĆ, A., MARKOVIĆ, M., PENČIĆ, V., SEKULIĆ, R., STEFANOVIĆ, L., ŠINŽAR, B., VIDENOVIĆ, Ž. (2002) Bolesti, štetočine i korovi kukuruza i njihovo suzbijanje. Beograd- Zemun: Institut za kukuruz 'Zemun polje, 695
- BURGESS, L., SUMMERELL, B., BULLOCK, S., GOTT, K., BACKHOUSE D., (1994): Laboratory Manual for *Fusarium* Research. Third edition, Sydney.
- CIMMYT (2011): <http://maizedoctor.cimmyt.org/en/pests-and-diseases/241?task=view>
- FISHER, N., BURGESS, L., TOUSSOUN, T., NELSON, P. (1982): Carnation leaves as a substrate or preserving cultures of *Fusarium* species. *Phytopathology* 72: 151-153.
- JAJIĆ, I., JURIĆ, V., GLAMOČIĆ, D., KRSTOVIĆ S. (2010): Occurrence of deoxynivalenon and zearalenone in maize. *Contemporary Agriculture* 59 (3-4) 227-233.
- KOS, G., LOHNINGER H., KRASKA, R. (2003): Development of a method for the determination of *Fusarium* fungi on corn using mid-infrared spectroscopy with attenuated total reflection and chemometrics. *Analytical Chemistry*, 75:1211-1217.
- NELSON, P., TOUSSOUN, T., MARASAS, W.(1983): *Fusarium* species An Illustrated Manual for Identification. The Pennsylvania State University Press.
- OFFICIAL GAZETTE SFRJ BR. 47 (1987): Pravilnik o kvalitetu semena poljoprivrednog bilja
- PAYNE, G.A. (1999). Ear and Kernel Rots. In Donald G. White (ed), *Compendium of Corn Diseases*. St. Paul, Minnesota: The American Phytopathology Society. Pp. 44-47.

10. REPUBLIČKI HIDROMETEROLOŠKI ZAVOD SRBIJE (2010)  
<http://www.hidmet.gov.rs/ciril/meteorologija/agrometeorologija.php>
11. SCHAAF SMA, A.W., NICOL, R.W., SAVARD, M.E., SINHA, R.C., REID, L.M., ROTTINGHAUS G. 1998):  
Analysis of Fusarium toxins in maize and wheat using thin layer chromatography.  
*Mycopathologia*, 142:107-113, 1998.