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# MYCOFLORA OF COMMERCIAL MAIZE SEED IN 2010

ABSTRACT: 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 on the end use of the grain. Various fungi cause ear and kernel rots. 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 belonging to different hybrids. Seed health testing was done using filter paper and nutritive media (PDA) method.

Fungi from genera *Fusarium*, *Penicillium*, *Aspergillus* and *Alternaria* were isolated from tested corn seed by both methods. Two species from the genus *Fusarium* were found in the tested corn samples *F. graminearum* and *F. monilirome.* Tested hybrids that belonged to different FAO maturity groups showed differences in susceptibility to ear and kernel rot.

KEY WORDS: corn seed, mycoflora, *Fusarium* spp., 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, along with the quality and feed value of grain. Toxins produced by the fungi in corn can also have serious implications on the end use of the grain.

Various fungi can 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. (P a y n e, 1999). These fungi can also cause stalk rot. Other fungi that can cause ear and kernel rots include species of

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*Penicillium*, *Diplodia, Aspergillus*, *Nigrospora, Botryosphaeria, Cladosporium, Rhizoctonia,* and *Rhizopus*.

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

## MATERIAL AND METHOD

### *Corn seed samples*

Hybrid corn seeds of different FAO maturity groups were used PR37N01 (FAO 370), PR36K67 (FAO 530) and PR34N43 (FAO 690). Hybrid corn seeds were treated with insecticides: Gaucho 600 FS (a. m. imidacloprid 600 g/l), Cruiser 350 FS (a. m. thiamethoxam 350g/l) and Force zea 280 FS (a.m. thiamethoxam 200 g/l + tefluthrin 80 g/l). Prior to the insecticide treatment and during seed processing, seeds were treated with fungicide Maxim XL 035 FS (a. m. fludioxonil 25 g/l). Seeds were sown in the field at Sombor locality. Harvest was carried out on 15$^{th}$ October, 2010. From each treatment 25 ears were taken in 4 replicates. After harvest, the seed moisture content was measured by Dickey Jones apparatus. 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. One hundred seeds were taken from each sample and the health test was done in four replicates. For seed health test on PDA, 5 seeds in 4 replicates were used. Seeds were sterilized in 1% NaOCl and then incubated for seven days on filter paper and PDA at 25°C. The seed health was determined based on percentage of fungi present in the seeds. *Fusarium* spp. were isolated on carnation leaf medium (CLA) (F i s h e r et al.,1982) and identification of the isolates was performed according to N e l s o n et al. (1983), and Burgess (1994).

## RESULTS AND DISCUSSION

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

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

The moisture content in tested seed samples ranged from 18.17-20.43% (Table 1). Such high percent of moisture in seed was caused by extremely wet weather in the harvest period. These moisture values are significantly higher than maximum permitted for corn seed, which is 14% (T h e O f f i c i a l G a z e t t e, 1987).

Tab. 1 – Seed moisture content in tested corn seed samples

| Hybrid+insecticide | Seed moisture content |
|---|---|
| PR 34N43+Cruiser | 20.43 |
| PR 34N43+ (Maxim) control | 20.25 |
| PR 34N43+Gaucho | 20.13 |
| PR 36K67+Cruiser | 19.85 |
| PR 34N43+ Force zea | 19.8 |
| PR 37N01+Gaucho | 19.05 |
| PR 36K67+Force zea | 19.03 |
| PR 36K67+Gaucho | 18.98 |
| PR 36K67+ (Maxim) control | 18.33 |
| PR 37N01+Force zea | 18.25 |
| PR 37N01+Cruiser | 18.33 |
| PR 37N01+ (Maxim) control | 18.17 |

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. Fungi from genus *Aspergillus* were noticed in 4 samples (3 samples are hybrid PR 34 N43). *Alternaria* was observed in low percent only in two samples. Appereance of species from *Fusarium* genus 4 days after seed incubation was from 0.5-4.75%. Seven days after incubation on filter paper, number of seeds infected with *Fusarium* increased in some samples but it still ranged from 0.75-4.75% (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.

Seeds treated only with fungicide and seeds treated with both fungicide and insecticide showed no significant difference in the number of infected seeds.

The highest number of infected seeds was noticed in the hybrid PR 36 K 67, followed by PR 37 N 01, while the lowest infection was observed in the hybrid PR 34 N 43.

Tab. 2 – Occurrence of fungi in tested corn seed (filter-paper method)

| Occurrence of fungi in tested corn seed (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HYBRID | *Fusarium* sp. | | *Penicilium* sp. | | *Aspergillus* sp. | | *Alternaria* sp. | |
| Number of days after incubation | 4th day | 7th day | 4th day | 7th day | 4th day | 7th day | 4th day | 7th day |
| PR 36 K 67 Control | 4 | 5 | 0.75 | 1.25 | – | – | 0.25 | 0.25 |
| PR 36 K 67 Gaucho | 4.75 | 4.75 | 2 | 3.5 | – | – | – | – |
| PR 36 K 67 Cruiser | 3 | 4.5 | 3.75 | 7.75 | – | – | – | – |
| PR 36 K 67 Force zea | 3 | 3.5 | 1.25 | 2 | – | – | – | – |
| PR 34 N 43 Control | 2.75 | 3.75 | 3.75 | 5.5 | 0.25 | 0.25 | – | – |
| PR 34 N 43 Gaucho | 1.75 | 4.5 | 1.5 | 4 | 0.75 | 1.25 | – | – |
| PR 34 N 43 Cruiser | 0.5 | 0.75 | 2.5 | 4 | – | – | 0.25 | 0.25 |
| PR 34 N 43 Force zea | 1 | 3.5 | 2.25 | 4 | 0.75 | 0.75 | – | – |
| PR 37 N 01 Control | 2 | 3.25 | 2.75 | 2.75 | – | – | – | – |
| PR 37 N 01 Gaucho | 2.75 | 3.25 | 2 | 2 | 0.25 | 0.25 | – | – |
| PR 37 N 01 Cruiser | 2 | 2.25 | 0.5 | 0.5 | – | – | – | – |
| PR 37 N 01 Force zea | 1.75 | 2.25 | 1.25 | 1.25 | – | – | – | – |

Fungi that developed in the tested corn seed on filter paper developed in the nutritive media as well. Seed infection was the highest with *Fusarium* species. The lowest number of infected seeds was noticed in the hybrid PR 34 N 43 (5.6), then in the hybrid PR 36 K 67 (7.2), while the highest number of infected seeds was in hybrid PR 37 N 01 (9.7). Number of infected seeds was mostly uniform within a hybrid, except in the case of hybrid PR 37 N 01, where significantly higher number of infected seeds was observed in the control (Table 3). Such a number of infected seeds indicates that the tested hybrids are, to a certain extent, susceptible to *Fusarium* species.

Two species from the genus *Fusarium* were found in the tested corn samples, *F. graminearum* and *F. moniliforme* (Figure 1). These two species are probably the most widespread causes of ear rot disease in maize. According to numerous authors, the causal agent of maize ear and kernel rot is caused by *Gibberella zeae* (*Fusarium graminearum*). *Fusarium verticilioides* syn. *Fusarium moniliforme*, *F. proliferatum* and *F. subglutinans*.

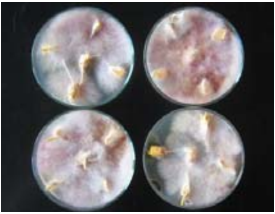

Fig. 1 – Occurrence of *F. graminearum* and *F. moniliforme* in tested corn seed on PDA

Tab. 3 – Occurrence of fungi in tested corn seed (PDA)

| Occurrence of fungi in tested corn seed (%) | | | | | | |
|---|---|---|---|---|---|---|
| HYBRID | *Fusarium* sp. (total) | F. *grami-nearum* | F. *monili-forme* | *Penicil-ium* sp. | *Aspergilus* sp. | *Alternaria* sp. |
| PR 36 K 67 Control | 6.25 | 1.25 | 5.00 | 2.50 | - | - |
| PR 36 K 67 Gaucho | 6.25 | 0 | 6.25 | 1.25 | 1.25 | 1.25 |
| PR 36 K 67 Cruiser | 7.50 | 3.75 | 3.75 | 6.25 | - | 0.75 |
| PR 36 K 67 Force zea | 8.75 | 6.25 | 2.5 | 3.75 | - | - |
| PR 34 N 43 Kontrola | 2.50 | 2.5 | 0 | 16.25 | - | 0.75 |
| PR 34 N 43 Gaucho | 6.25 | 1.25 | 5.00 | 1.25 | - | 1 |
| PR 34 N 43 Cruiser | 5.00 | 2.5 | 2.50 | 0 | 1.25 | 0.25 |
| PR 34 N 43 Force zea | 8.75 | 3.75 | 5.00 | 5.00 | -- | 0.5 |
| PR 37 N 01 Kontrola | 21.25 | 12.50 | 8.75 | 1.25 | - | 0.25 |
| PR 37 N 01 Gaucho | 2.50 | 0 | 2.50 | 3.75 | 1.25 | 0.75 |
| PR 37 N 01 Cruiser | 11.25 | 6.25 | 5.00 | 1.25 | - | 0.25 |
| PR 37 N 01 Force zea | 3.75 | 2.5 | 1.25 | 13.75 | - | 0.25 |

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, the entire ear. If infection is not so severe the streaks or white lines run across the kernels and are most likely to appear 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 have pinkish to reddish color.

The most important toxigenic fungi occurring in moderate climatic zones of North America and Europe are *Fusarium* fungi (K o s et al., 2003).

Zearalenone, deoxynivalenol (DON) and fumonisins are more prevalent mycotoxins that occur in grain (S c h a a f s m a 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 the hybrid susceptibility in the field and also health testing of seed lots. Maize seed monitoring from 2009 harvest (65 samples) in Serbia showed that two samples were contaminated with zerealenone above the established maximum level adopted by European Commission for unprocessed maize (J a j i ć et al., 2010 ).

*Penicillium* rot on corn seed is usually evident as discrete tufts or clumps of a blue-green or gray-green mold of individual kernels. The fungi appearance is more common in broken kernels. *Penicillium* appears in the form of small, discrete colonies of mold growth with a dusty or powdery appearance. (C i m m y t, 2011). The attack of fungi from genus *Penicillium* in tested corn seeds ranged from 1.25-16.25%. Only one sample did not show the presence of these fungi. Number of infected seeds varied between hybrids, as well as within the hybrid (Table 3).

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

## CONCLUSION

One of the main factors influencing kernel health is the amount of rainfall, especially in the harvesting period. Susceptibility of hybrids to ear and kernel rots is also very important factor in the 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 the grain up to 15% of moisture as quickly as possible, and to monitor the grain on a regular basis throughout its storage life in order to ensure that the moisture and temperature are maintained at correct levels. If the infection potential is present the optimal storage condition can prevent severe seed contamination.

## REFERENCES


A l m a š i, R., B a č a, F., B o š n j a k o v i ć, A., Č a m p r a g, D., D r i n i ć, G., I v a n o v i ć, D., L e v i ć, J., M a r i ć, A., M a r k o v i ć, M., P e n č i ć, V., S e k u l i ć, R., S t e f a n o v i ć, L., Š i n ž a r, B., V i d e n o v i ć, Ž. (2002): *Bolesti, štetočine i korovi kukuruza i njihovo suzbijanje*. Beograd-Zemun: Institut za kukuruz 'Zemun polje, 695

B u r g e s s, L., S u m m e r e l l, B., B u l l o c k, S., G o t t, K., B a c k h o u s e 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 for preserving cultures of Fusarium species*. Phytopathology, 72: 151–153.
Jajić, I., Jurić, V., Glamočić, D., Krstović S. (2010): *Occurrence of deoxynivalenol 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.
The Official Gazette of SFRY No. 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. 44–47.
Republic Hydrometeorological Service of Serbia (2010): http://www.hidmet.gov.rs/ciril/meteorologija/agrometeorologija.php
Schaafsma, 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.

## МИКРОФЛОРА МЕРКАНТИЛНОГ СЕМЕНА КУКУРУЗА У 2010. ГОДИНИ

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Резиме

Плеснивост клипа и семена кукуруза могу довести до смањења приноса и утицати на квалитет и вредност зрна. Токсини које продукују гљиве могу имати значајан утицај на крајње коришћење зрна. Различите гљиве могу проузроковати трулеж клипа и зрна. Гљиве из рода *Fusarium* су најзначајнији проузроковачи трулежи клипа и семена кукуруза.

Циљ овог рада је био да се испита здравствено стање меркантилног семена различитих хибрида који припадају различитим FAO групама зрења. Здравствено стање семена је утврђено методом филтер папира и на хранљивој подлози.

Са семена кукуруза применом обе методе изоловане су гљиве из родова *Fusarium*, *Penicillium*, *Aspergillus* и *Alternaria*. На семену су идентификоване две врсте *Fusariuma – F. graminearum* и *F. moniliforme*. Испитивани хибриди који припадају различитим FAO групама зрења показали су различит ниво осетљивости према гљивама проузроковачима трулежи семена.

Укупна количина падавина за локалитет Сомбор током вегетације је била 744 mm што је скоро двоструко више у односу на вишегодишњи просек за Србију који износи 415. Овакви метеоролошки услови су утицали на значајнију појаву гљива проузроковача трулежи клипа и зрна кукуруза.