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Original scientific paper

Fungal biodiversity on maize kernels in an insecticide evaluation trial

Sonja Tančić Živanov*, Mirjana Lalošević, Radivoje Jevtić, Filip Franeta, Željko Milovac, Dušan Stanisavljević and Božana Purar

Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21 000 Novi Sad, Serbia
**Corresponding author: sonja.tancic@ifvcns.ns.ac.rs*

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SUMMARY

The European corn borer (ECB) *Ostrinia nubilalis* and Fusarium ear rot *Fusarium* spp. pose a continuous threat to maize production worldwide. There are several reports indicating that ECB damage to maize ears promotes Fusarium ear rot infection. The aim of this study was to monitor the influence of different insecticide treatments (a.i. chlorantraniliprole, indoxacarb, and chlorantraniliprole+lambda-cyhalothrin) on the ECB and fungal diversity on maize kernels in the field in a four-year trial (2013-2016).

A total of 16 different fungal genera were isolated from maize kernels, and *Fusarium* species were confirmed to be the dominant pathogens, present in all treatments, throughout the four years of experiments. The incidence of *Aspergillus* spp. and *Penicillium* spp. was established to be low. Apart from *Fusarium* species, the most frequent genera were: *Aspergillus* spp., *Mortierella* spp., *Mucor* spp., *Penicillium* spp., *Acremonium* spp. and *Rhizopus* spp.

Treatments with chlorantraniliprole and chlorantraniliprole+lambda-cyhalothrin showed higher efficacy, though not statistically significant, compared to indoxacarb, in reducing the number of ECB larvae and damage they cause. However, no direct effect on the number of isolated fungal genera has been observed in any of the three insecticide treatments.

Keywords: plant pathogenic fungi; maize; biodiversity; European corn borer; insecticides

INTRODUCTION

Maize (*Zea mays* L.) is arguably the most widely grown crop in the world. It is mainly used for animal feed (82%), while only 4% of the global production is destined for human consumption (ISTAT, 2018). In Serbia, maize is cultivated on approximately 1.000.000 ha with an annual production of 4.000.000 t, which makes this plant the most important crop in Serbia (FAOSTAT, 2018).

The European corn borer (ECB) - *Ostrinia nubilalis* and Fusarium ear rot (*Fusarium* spp.) are two major yield reducing factors in maize production. The maize silk-channel is the most common pathway for ear rot infection. However, *Fusarium* species can also infect the ear through kernel damage created by insects or birds.

It has been well documented that second generation ECB larvae can enhance fungal infections (Mencarelli et al., 2013; Blandino et al., 2015). It has also been reported that the ECB promotes *Fusarium verticillioides* infection of maize kernels in temperate areas (Blandino et al., 2010).

The most common *Fusarium* species on maize kernels in temperate regions is *F. verticillioides* along with two accompanying species of the *Liseola* section - *F. proliferatum* and *F. subglutinans* (Logrieco et al., 2002; Tóth et al., 2012). Under cooler climatic conditions with heavy rainfall during the growing season, *F. graminearum* occurs more frequently (Reid et al., 1999). A recent study showed that *F. graminearum* (1.08%), *F. subglutinans* (8%) and *F. verticillioides* (25.75%) were present in freshly harvested maize kernels in Serbia in 2014 (Krnjaja et al., 2015).

Fusarium species are capable of producing a vast number of mycotoxins (fumonisins, fusaric acid, fusaproliferin, beauvericin, moniliformin, deoxynivalenol, zearalenone, diacetoxyscirpenol, etc.), which are able to cause serious problems to both animal and human health. This makes them one of the most important maize pathogens worldwide (Leslie & Summerell, 2006; Blandino et al., 2015). In recent years, fumonisins, deoxynivalenol, zearalenone and diacetoxyscirpenol have been detected on maize kernels in Serbia (Krnjaja et al., 2012; Tančić, 2009; Jajić et al., 2007; 2008). Considering the fact that maize is consumed both as unprocessed and processed food or feed, while mycotoxin contamination starts in the field, food quality and safety should be improved through agronomic prevention strategies.

Several factors can facilitate *Fusarium* infection of maize ears, and injuries caused by insect feeding create suitable infection sites for the fungus (Munkvold & Desjardins, 1997). There are reports which indicate that ECB damage to maize ears is the most responsible factor for Fusarium ear rot infection caused by several *Fusarium* species (Munkvold et al., 1997; Alma et al., 2005). It has also been documented that insecticide treatments against the ECB can also have a positive effect on maize production by decreasing the incidence of Fusarium ear rot development (Blandino et al., 2009). There is, however, much less evidence of the effects of ECB damage on the occurrence of fungi from other genera.

The aim of this study was to monitor the influence of different insecticide treatments (a.i. chlorantraniliprole, indoxacarb, and chlorantraniliprole+lambda-cyhalothrin) on the ECB and fungal diversity on maize kernels in the field in a four-year trial (2013-2016).

MATERIAL AND METHODS

Field trial design

The experiment was carried out on a commercial maize dent hybrid (NS 6030, NS Seme, FAO group 600) on an experimental site at Rimski Šančevi near Novi Sad, Serbia, from 2013 to 2016. Three insecticide treatments were compared to an untreated control: T1 - chlorantraniliprole (100 ml ha^{-1}); T2 - indoxacarb (250 ml ha^{-1}); and T3 - chlorantraniliprole +lambda cyhalothrin (200 ml ha^{-1}). The experiment setup consisted of a completely randomized block design in four replicates. Each plot consisted of four rows of maize, separated from other plots by one untreated row on each side. The length of each plot was 10 m, with a spacing of 2 m between blocks.

ECB flight activity was monitored using a light trap (model RO Agrobečej, Serbia), which was active from 20:00 to 7:00 h every day during the maize growing season. The sampled specimens were removed and counted on a daily basis. The insecticides were applied during peak flight of the ECB, which usually occurred in the first week of August, using a backpack sprayer unit with a high clearance attachment with 6 nozzle booms (model 315-HCB-4) from Bellspray Inc, USA. The working height of the sprayer was manually adjustable (0.6-4.2 m) and the spray volume was 400 l ha^{-1} at a pressure of 200 kPa with an operation speed of 4-6 km h^{-1}.

Assessments of insecticide effectiveness consisted of counting the number of surviving larvae and the number of injuries on 80 ears per treatment.

Analyses of mycobiota biodiversity

Ten ears with fungal disease symptoms were randomly collected from each plot (totaling 40 ears per treatment) for biodiversity assessment. Ten kernels per ear were chosen from infected area. In total, 400 kernels per treatment were analyzed for fungal species diversity. The kernels were surface sterilized in 1% sodium hypochlorite solution for 1 minute, rinsed three times with sterile distilled water, dried and placed on 2% water agar (WA) medium amended with streptomycin (50 mg L^{-1}). After 7 days of incubation at room temperature (25±2°C) in daylight, the kernels were examined and morphological identification of different species was performed according to Watanabe (2010).

Data analysis

The incidence of species (I) was calculated as: I (%) = [Number of one species colonies which occurred in a kernel sample/total number of all fungal colonies found in one kernel sample] × 100.

The insecticide efficacy was calculated using Abbott's formula: (1-nt/nc) × 100 where: n= number of larvae, t= treatment, c= control (Abbott, 1925).

The effects of treatments on the average number of fungal species, number of ECB larvae and number of injuries *were determined using an analysis* of variance (*ANOVA*) and Tuckey's test. The analyses were performed in Minitab 17 (trail version).

RESULTS AND DISCUSSION

Insecticide efficacy against ECB larvae and the damage they caused were evaluated in a four-year field trial. In 2015, there were no statistically significant differences in the efficacy of insecticides, compared to control treatment (Abbott values, Table 1). This may be attributed to unfavorable weather conditions which resulted in low abundance of ECB larvae on maize kernels in all treated and control plots.

However, the years 2013, 2014 and 2016 were more favorable for pest development and the number of ECB larvae varied from 36-249 in 2013, 3-33 in 2014 and 5-46 in 2016 (Table 1). During those years, significant differences were found in the efficacy of all insecticide treatments compared to the control, and chlorantraniliprole and chlorantraniliprole+lambda-cyhalothrin (T1 and T3) showed the highest efficacy (Table 1). Nevertheless, there were no statistically significant differences in the efficacies of different treatments.

Furthermore, the lowest fungal diversity was found in chlorantraniliprole (T1) treatments over all four years of the trial, while the highest diversity of up to eight genera was observed in indoxacarb (T2) treatments. Chlorantraniliprole+lambda-cyhalothrin treatments (T3) resulted in fewer fungal genera than in control plots in all years except 2013 (Table 1). Lower fungal diversity in treatments T1 and T3, compared to T2, can be attributed to the higher efficacy of those insecticides, which was confirmed by a lower number of ear injuries in those treatments in all four years of the trial (Table 1). This is consistent with data reported by Blandino et al. (2009), Saladini et al. (2008), and Scarpino et al. (2015), who showed the importance of ECB larvae as vectors of *Fusarium* species in Italy. However, based on average species counts, there were no statistically significant differences in fungal diversity among the treatments (Table 1).

Sixteen fungal genera in total were detected in maize seed samples during the four-year trial (Figure 1). *Fusarium* species were confirmed as the dominant pathogens of maize kernels in all four years and they were present in all treatments. The incidence of *Fusarium* species on maize kernels was almost constant with little variation throughout the trial: 82.99% (2013), 83.55% (2014), 85.02% (2015) and 85.01% (2016). In total, 11 *Fusarium* species were identified on maize kernels: *F. equiseti, F. graminearum, F. oxysporum, F. proliferatum, F. pseudograminearum, F. semitectum, F. solani, F. sporotrichioides, F. subglutinans, F. verticillioides*, and one unidentified *Fusarium* species. The number of *Fusarium* species was more or less constant and varied from 8 to 10 depending on trial year (data not shown). As expected based on earlier research in Serbia, the dominant species were: *F. verticillioides, F. graminearum* and *F. proliferatum*. In general, the most common *Fusarium* species that have been isolated from maize kernels are: *F. graminearum, F. verticillioides* and *F. subglutinans*, but also *F. culmorum, F. proliferatum* and *F. equiseti,* depending on geographic location (Cotten & Munkvold, 1998; Velluti et al., 2000; Torres et al., 2001). In Serbia, those species were detected on maize kernels in a 1994-1996 study by Lević et al. (1997), and *F. verticillioides* was the most abundant species in those samples (63%), followed by *F. subglutinans* (50.6%), *F. graminearum* (12.2%), *F. proliferatum* (9.6%) and *F. oxysporum* (5.8%). *Fusarium* species which have been sporadically found in maize kernel samples in Serbia are: *F. solani, F. equiseti, F. sporotrichioides*, *F. chlamydosporum, F. crookwellense* and *F. semitectum* with incidences of less than 3 % during the period from 1994 to 1996 (Lević et al., 1997), and *F. equiseti, F. poae, F. polyphialidicum, F. solani* and *F. sporotrichioides* in the period from 2004 to 2007 (Lević et al., 2009). This is consistent with the results of this present study, in which *F. equiseti, F. oxysporum, F. semitectum* and *F. sporotrichioides* were sporadically found in maize kernel samples.

Apart from the genus *Fusarium*, species from another 15 genera were also isolated from infected maize kernels over the period from 2013 to 2016: *Acremoniella* sp., *Acremonium* spp., *Alternaria* spp., *Aspergillus* spp., *Botrytis* spp., *Geotrichum* spp., *Gliocladium* spp., *Hyalodendron* spp., *Mortierella* spp., *Mucor* spp., *Paecilomyces* spp., *Penicillium* spp., *Rhizopus* spp., *Staphylotrichum* spp. and *Trichoderma* spp. (Figure 1). Species from the genera *Mucor* (9%) and *Aspergillus* (4.27%) were the most common in 2013 (excluding *Fusarium*), while the genus *Mortierella* was the most common in 2014, 2015 and 2016 with average incidence frequencies of 9.3%, 5.37% and 4.85%, respectively. Species found in all four trial years in at least one treatment were: *Fusarium* spp., *Gliocladium* spp. and *Mucor* spp. The genera *Acremonium* and *Aspergillus* were found in all years except 2016, *Rhizopus* in all except 2014, while *Mortierella* was present in each year except 2013. Species from the genera *Alternaria* and *Paecilomyces* were found in 2013 and 2014, *Penicillium* in 2013 and 2015, and *Hyalodendron* in 2013 and 2016. Species of the genera *Acremoniella, Botrytis, Geotrichum, Staphylotrichum* and *Trichoderma* were detected in one trial year only. The species commonly found together with *Fusarium* spp. as accompanying species in the examined maize kernel samples were: *Penicillium* spp., *Aspergillus* spp., *Mortierella* spp., *Mucor* spp., *Rhizopus* spp., and *Gliocladium* spp. with incidences of up to 7.33%, 2.45%, 16.09%, 21.33%, 12.50% and 6.54%, respectively (Figure 1). These accompanying species are common mycobiota on maize kernels in Serbia and worldwide and already confirmed in some previous studies (Krnjaja et al., 2004; Tančić, 2009; Mendoza et al., 2017; Xing et al., 2018; Tóth et al., 2012). The lower incidence of *Aspergillus* and *Penicillium* species in the present study is consistent with the findings of Rodríguez Páez et al. (2011), who also detected *Penicilium* spp. and *Aspergillus* spp. in maize kernels at low percentages - 0.5% and 0.25%, respectively. This apparently unusual finding could be partly attributed to the high incidence of competitive *Fusarium* species found during all trial years. Lević et al (2013), on the other hand, observed an uncommonly high frequency and incidence of *Aspergillus* species on maize kernels in 2012. This fact emphasizes the complexity of the factors influencing fungal infections (weather conditions – insect occurrence – fungal development). The same authors suggest that high incidences of *Aspergillus* species could be attributed to extremely stressful agrometeorological conditions, i.e. high temperatures and severe drought, which occurred in 2012, while the ECB occurrence and damage were listed as a secondary factor.

In conclusion, the results of this study showed that a total of 16 fungal genera were isolated from maize kernels, while *Fusarium* species were confirmed as the dominant pathogens of maize kernels in all four years, and they were present in all treatments. The incidence of *Aspergillus* spp. and *Penicillium* spp. was found to be low. Apart from *Fusarium* species, the most commonly represented other genera were: *Aspergillus* spp., *Mortierella* spp., *Mucor* spp., *Penicillium* spp., *Acremonium* spp. and *Rhizopus* spp.

Treatments with chlorantraniliprole and chlorantraniliprole+lambda-cyhalothrin showed higher efficacy, though not statistically significant compared to indoxacarb, in reducing the number of ECB larvae and their resulting damage. However, no direct effect on the number of isolated fungal genera was observed in any of the three insecticide treatments. The relationship between insect feeding-caused damage and fungal occurrence indicates that reduction in fungal development, and consequently in mycotoxin production, is a complex problem which requires further research and an integrated pest management approach.

Table 1. Treatment effects on fungal diversity and ECB larval survival in maize ears

Treatment	Species No.	Species average*	Larvae No.*	Injuries No.*	Abbott (%)	Species No.	Species average*	Larvae No.*	Injuries No.*	Abbott (%)
		2013					2014			
Control	6	2.5 a	249 a	72 a	-	6	2.5 a	33 a	3 a	-
T1	5	2.2 a	49 b	28 a	80.32	3	2.5 a	0 b	0 a	100.0
T2	8	3.1 a	97 b	56 a	61.04	7	3.4 a	9 b	2 a	75.76
T3	6	2.5 a	36 b	48 a	85.54	4	2.4 a	3 b	2 a	90.91
P-Value		0.268	0.000	0.312			0.076	0.002	0.426	
		2015					2016			
Control	7	2.8 a	13 a	20 a	-	5	2.7 a	46 a	19 a	-
T1	4	2.5 a	0 a	9 a	100.0	4	3.2 a	5 b	2 b	89.13
T2	5	2.0 a	2 a	8 a	84.62	5	2.8 a	13 b	2 b	71.74
T3	4	2.2 a	1 a	9 a	92.31	4	2.9 a	5 b	0 b	91.30
P-Value		0.420	0.056	0.153			0.899	0.000	0.001	

*Different letters indicate significant differences according to Tukey's test

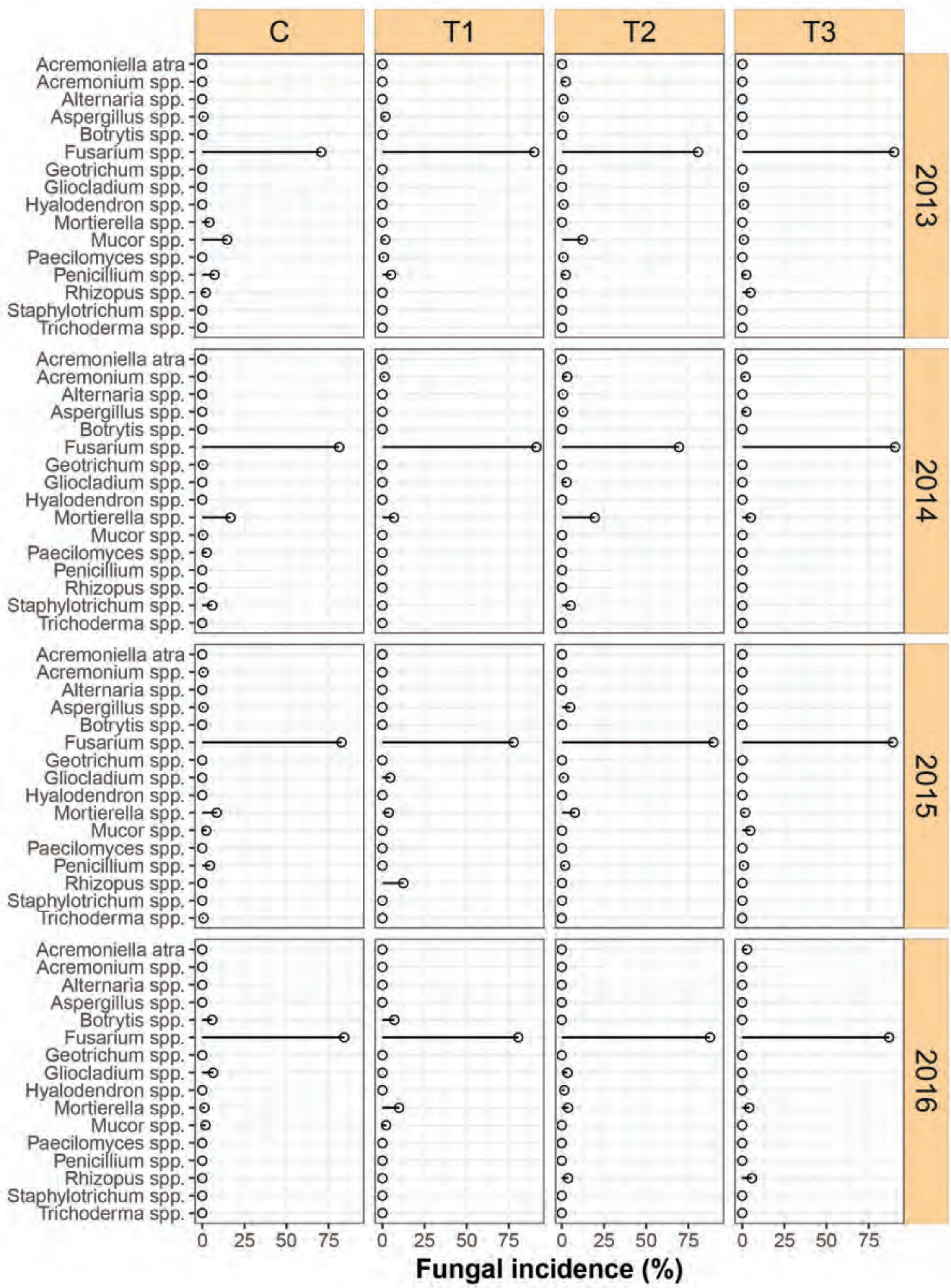

Figure 1. Fungal incidence detected on maize ears and kernels after different insecticide treatments from 2013 to 2016 (control – C, chlorantraniliprole – T1, indoxacarb - T2, chlorantraniliprole+lambda-cyhalothrin - T3)

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Biodiverzitet gljiva na zrnima kukuruza u ogledu za ispitivanje efikasnosti insekticida

REZIME

Kukuruzni plamenac (*Ostrinia nubilalis*) i fuzariozna trulež klipa (*Fusarium* spp.) pričinjavaju najveće štete u proizvodnji kukuruza. Postoji nekoliko istraživanja koja su potvrdila da, svojom ishranom, larve kukuruznog plamenca doprinose i razvoju fuzarioznih plesnivosti na oštećenjima zrna i klipa kukuruza. Stoga je glavni cilj ovih četvorogodišnjih istraživanja bio da se odredi efikasnost insekticidnih tretmana na smanjenje brojnosti larvi kukuruznog plamenca, kao i njihov uticaj na diverzitet gljiva na zrnima kukuruza. Ukupno 16 različitih rodova gljiva je identifikovano u uzorcima zrna kukuruza tokom četvorogodišnjeg perioda istraživanja. *Fusarium* vrste su registrovane u svim tretmanima tokom sve četiri godine istraživanja, i svojim procentualnim učešćem potvrdile da su najdominantniji patogeni zrna kukuruza. Pojava vrsta rodova *Aspergillus* i *Penicillium* u uzorcima zrna kukuruza je bila slaba. Vrste koje su često bile registrovane zajedno sa *Fusarium* spp. na zrnima kukuruza su bile: *Aspergillus* spp., *Mortierella* spp., *Mucor* spp., *Penicillium* spp., *Acremonium* spp. i *Rhizopus* spp.

Tretmani sa hlorantraniliprolom i hrorantraniliprol+lambda-cihalotrinom su pokazali veću efikasnost, ali ne i statistički značajnu, u odnosu na indoksakarb u smanjenju broja larvi kukuruznog plamenca. Takođe, i pored manjeg broja vrsta registrovanih u ova dva tretmana, nije uočen statistički značajan uticaj ni jednog ispitivanog tretmana na broj rodova gljiva prisutnih u uzorcima.

Ključne reči: patogene gljive; kukuruz; biodiverzitet; kukuruzni plamenac; insekticidi