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Influence of insecticide treatments against the European corn borer (Lepidoptera: Crambidae) on the Incidence of Fusarium ear rot

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Summary: Maize production is often threatened by numerous phytophagous insect species and diseases, among which the European corn borer (ECB) *Ostrinia nubilalis* and ear rot caused by *Fusarium* species stand out. The damage to maize ears caused by the European corn borer larvae can represent access points for *Fusarium* spores, increasing the probability of infection. The main objective of this study was to evaluate the efficacy of insecticide treatment against the European corn borer and its subsequent impact on reducing the incidence of ear rot caused by *Fusarium* species. Additionally, the study sought to assess the prevalence of *Fusarium* species throughout the four-year experimental period. The study was performed from 2013 to 2016 and included a treatment and a control, with four replications. Each experimental year, a combination of chlorantraniliprole and lambda-cyhalothrin at a recommended dosage was applied as an insecticide treatment, while in the control treatment no insecticides were applied. The obtained results indicated that the insecticide treatment significantly reduced the number of ECB larvae on maize ears in all years of the study, except in 2015. The efficacy of the insecticide treatment in reducing the total number of damaged ears was significant only in 2016, while in other years, the efficacy varied. The insecticide treatment had a significant effect on the disease severity index and significantly reduced ear rot only in 2013. During the four-year study, *F. verticillioides* was the most commonly identified fungal species on maize, except in 2014, when *F. graminearum* was dominant. The insecticide treatment had no effect on the species diversity within the *Fusarium* genus.
Keywords: *Ostrinia nubilalis*, European corn borer control, ear rot, *Fusarium* spp., insecticide treatment, chlorantraniliprole, lambda-cyhalothrin, maize

Introduction

Maize is among the most extensively cultivated crops globally and its production is often constrained by several pest insect species and diseases, among which the European corn borer (ECB), *Ostrinia nubilalis* (Hübner, 1796), (Lepidoptera: Crambidae) and Fusarium ear rot are the most significant (Mencarelli et al., 2013; Gromadzka et al. 2019). The larvae of the European corn borer cause significant damage to maize plants by boring into the stalk and ear, leading to severe mechanical injuries to the plant, directly affecting plant growth and damaging kernels, which ultimately leads to significant yield reductions. (Keszthelyi, 2010). In addition to this, the damage caused on kernels can also often lead to secondary fungal infections.

Fusarium species are particularly prevalent in northern, temperate regions, especially Central and Southeastern Europe. The genus *Fusarium* is the leading cause of ear rot in the field, as well as during storage. Infections in the field are most commonly detected visually by observing the appearance of pinkish-white mycelium on maize ears. During the storage of infected maize kernels, the infection continues to spread. *Fusarium* infections in maize can lead to smaller kernel size, reduced protein content, and adverse effects on germination, resulting in lowered yield and feed quality (Sueliman et al., 2013). *Fusarium* molds are known to produce several mycotoxins including fumonisins (FUM), zearalenone (ZEN) and deoxynivalenol (DON). These compounds can cause a whole range of health disorders, negatively affecting the biochemical processes inside the cell, especially the metabolism of carbohydrates, lipids, DNA, RNA, and protein synthesis (Pestka and Smolinski, 2005; Zinedine et al., 2007). An additional issue is the fact that mycotoxins are resilient and cannot be eliminated during food processing (Pinotti et al., 2016). The presence of these contaminants underscores the importance of addressing *Fusarium* infections in maize to ensure both kernel quality and the safety of the food supply chain.

Among the most common *Fusarium* species on maize are *F. graminearum* and *F. verticillioides* (Thompson et al., 2018; Logrieco et al., 2002), but also other species such as *F. proliferatum* (Munkvold, 2003), *F. subglutinans*, and *F. temperatum* (Stepien et al., 2019; Scauflaire et al., 2011) are frequently reported. In recent years a connection between ECB ear damage and *Fusarium* infections has been observed (Blandino et al., 2010; Scarpino et al., 2015). Kernels damaged by ECB larvae can represent entry points for plant pathogens, including *Fusarium* spores. Studies conducted by various authors indicate that chemical control of ECB (using deltamethrin, lambda-cyhalothrin, chlorpyrifos+cypermethrin, indoxacarb and alpha-cypermethrin) during the growing season affects the level of FUM in maize. (Alma et al., 2005; Saladini et al., 2008; Blandino et al., 2009). The main goal of this study was to research the relationship between ECB damage and Fusarium ear rot occurrence, and it can be divided into three steps: 1) Evaluate the efficacy of insecticide treatment against the European corn borer, 2) Assess its subsequent impact on the reduction of ear rot incidence caused by *Fusarium* species, and 3) Evaluate the prevalence of *Fusarium* species between insecticide-treated and untreated plots over a four-year experimental timeframe.

Material and methods

Field trial design

The trial was conducted in the period from 2013 to 2016 on the experimental fields of the Institute of Field and Vegetable Crops, Novi Sad, Serbia at the following localities: 45°19'26.10"N; 19°51'5.00"E in 2013, 45°20'10.66"N; 19°51'28.09"E in 2014, 45°20'1.67"N; 19°50'56.23"E in 2015 and 45°19'38.73"N; 19°49'28.48"E in 2016. Maize hybrid NS 6030 was sown at the beginning of May, with 75cm spacing between rows and 22cm spacing between plants, and standard agricultural practices were applied. The experiment included a plot treated with insecticide and a control plot, with each plot measuring 10 meters in length and 4.5 meters in width, repeated in four replicates. A combination of chlorantraniliprole and lambdacyhalothrin (Ampligo 150ZC, 200 ml ha-1), a specifically designed formulation for the control of lepidopteran pests, was applied as an insecticide treatment each year at the peak flight of the second ECB generation, at the beginning of August. The insecticide was applied using a backpack sprayer (200 kPa, 4-6 km h-1) with an elevated boom designed to spray over fully grown maize plants, simulating a standard high-clearance self-propelled sprayer.

European corn borer incidence

The ECB incidence was measured as the number of larvae found on maize ears and the number of damaged ears. 20 maize ears per treatment per plot were inspected for ECB larvae and ECB damage. The assessment of damage to maize ears was conducted only in instances where no larvae were present on the ears. This approach ensured that any observed damage from larvae feeding could be statistically analyzed separately, allowing for a more accurate evaluation of the impact of ECB larvae on maize ears and the effects of the insecticide treatment. The total number of larvae on ears (CE), and the mean number of larvae per treatment (MC) was calculated, as well as the total number of damaged ears (DE) and the mean number of damaged ears (MD). The efficacy of the insecticide treatments, based on the number of surviving larvae (E%CE) and the amount of damage (E%DE), was determined according to Abbott (1925). A two-sample t-test was conducted to compare the means of the control and treatment groups for each variable. The t-test was employed to assess if any significant differences between the groups could be detected (at a significance level of $p \leq 0.05$).

Disease estimation in the field

Field disease assessment of Fusarium ear rot involved the evaluation of a total of 160 plants in each treatment, at the stage 99 on the BBCH scale. Within each treatment replication, 40 ears from inner rows were visually inspected using the seven-class disease severity scale established by Reid et al. (1996): 1 - 0%, 2 – 1-3%, 3 - 4-10%, 4 – 11-25%, 5 – 26-50%, 6 – 51-75%, and 7 – 76-100% of kernels exhibiting discoloration, mycelia growth, and rot symptoms. Disease severity index (DSI) in the field was determined using the Towsend-Heuberger formula: $DSI = \sum [(n \times c)/N \times C] \times 100$, where "n" represents the number of ears per class, "c" is the number of classes, "N" is the total number of ears assessed per plot, and "C" is the number corresponding to the highest class.

Picture 1. Seven-class disease severity scale established by Reid et al. (1996) from 0-6 (from left to right). Photo © Filip Franeta

Fusarium spp. diversity analyses

For the analysis of *Fusarium* spp. diversity, the following method was used: ten kernels were selected from areas near damage and infestation sites on each ear, totalling 100 kernels per replication (400 kernels per treatment). The chosen kernels underwent surface sterilization using a 1% sodium hypochlorite solution for 1 minute, followed by three rinses with sterile distilled water. Subsequently, the kernels were dried and positioned on a 2% water agar (WA) medium with five kernels per Petri dish. After a 7-day incubation period at room temperature (25±2°C) and under ambient light, microscopic examinations were conducted. The presence of different fungal species was documented based on the criteria outlined by Watanabe (2010). Colonies belonging to the genus Fusarium were separated based on macroscopic morphological characteristics such as the colour of the formed mycelium. Mycelia of *Fusarium* species emerging from the maize seeds were subsequently transferred to potato dextrose agar (PDA) and water agar (WA), supplemented with streptomycin (50 mg L-1) to establish pure cultures. *Fusarium* species were identified using both macroscopic features such as the form and coloration of mycelium, as well as microscopic features like the presence or absence of macro and microconidia, and chlamydospores, along with their respective forms, according to Leslie and Summerell (2006). The diversity of Fusarium species was assessed based on their incidence, with the results expressed as percentages. The incidence of Fusarium species (I) was calculated as: I (%) = [Number of one species colonies which occurred in kernel sample/Total number of all fungal colonies registered in one kernel sample] x 100.

Results

European corn borer incidence

The effects of the insecticide treatment against ECB larvae and their damage on maize ears compared with the control throughout all years of the trial are presented in Tble 1. The total number of recorded larvae on ears was the highest in 2013 (62,25), while the lowest was in 2015 (3,00). The total number of recorded damaged ears was also highest in 2013 (18,00), while the lowest was in 2014 with only 0,75. The values from 2013 indicate an extremely high ECB pressure on maize and maize ears, particularly when compared with the following three years. The t-test revealed a significant difference between the control and treatment groups ($p \leq 0.05$) for the total number of larvae on ears (CE) in all years except in 2015. These results suggest that the insecticide treatment had a noticeable effect on lowering the number of larvae on ears, except in 2015, when the ECP pressure was already very low. On the other hand, the effect of the treatment on the total number of damaged ears (DE) was statistically significant only in 2016, when none of the examined ears had any damage. The efficacy of the treatment, when compared to the control and expressed according to Abbott, was very high in all years of the trial, and even in 2013 (85.54%) when the insect pressure was the highest. On the other hand, the efficacy of the treatment in comparison with the control for the total number of damaged ears was variable, ranging from only 33,33% in 2014 to 100% in 2016 (Table 1).

Table 1. The total number of larvae on ears (CE), and the mean number of larvae per treatment (MC), the total number of damaged ears (DE) and the mean number of damaged ears (MD) in the control treatment (K) and the insecticide treatment (T).

Year	Treatment	CE	MC	DE	MD	E%CE	E%DE
2013	K	62.25	3.11	18.00	0.9		
	T	9.00	0.45	7.00	0.6	85.54	61.11
	P	0.000*	0.000*	0.412	0.412		
2014	K	8.50	0.41	0.75	0.04		
	T	0.75	0.04	0.50	0.03	90.91	33.33
	P	0.002*	0.002*	0.321	0.321		
2015	K	3.00	0.16	5.00	0.25		
	T	0.25	0.01	2.00	0.11	92.31	55.00
	P	0.056	0.056	0.113	0.113		
2016	K	11.50	0.58	4.75	0.24		
	T	1.25	0.05	0.00	0.00	91.3	100
	P	0.000*	0.000*	0.001*	0.001*		

E%CE represents the efficacy of the treatment compared with the control, expressed as a percentage, calculated according to Abbott, for the number of larvae on ears, E%DE represents the efficacy of the treatment compared with the control for the total number of damaged ears. * Significant if $p \leq 0.05$ or not significant if $p > 0.05$.

Table 2. Analysis of variance (ANOVA) of maize ear rot during four years of experiment

Effect	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F	p
Intercept	6989.068	1	6989.068	244.3677	0.000000
Treatment	137.158	1	137.158	4.7956	0.038488
Year	1196.761	3	398.920	13.9480	0.000018

* Significant if p ≤ 0.05

Pre-harvest disease rating

Characteristic symptoms of ear rot were observed in both treated and control plants and a pre-harvest disease rating was conducted. Significant differences (p≤0.05) were noted among treatments across four years of the experiment Table 2. During 2013 and 2014 the highest DSI was recorded, 20.21 and 24.38 %, while in 2015 and 2016 this value was significantly lower and was 11.27 and 10.83 %, respectively. Regarding the treatments, a statistically significant difference was observed between the control and treatment groups in 2013. The chemical treatment showed the most significant effect on reducing ear rot compared to the control, with DSI values ranging from 20.21 for the control to 13.44 % for the treatment (Figure 1).

Fusarium species diversity

The occurrence of Fusarium species in maize kernels varied across the four-year study (Figure 2). Specifically, seven distinct *Fusarium* species were detected: *F. verticillioides*, *F. graminearum*, *F. proliferatum*, *F. subglutinans*, *F. incarnatum*, *F. solani*, and *F. semitectum*. *Fusarium verticillioides* consistently dominated in 2013, 2015, and 2016, while *Fusarium graminearum* was most prevalent in 2014. The data indicates that insecticide application did not significantly influence the diversity of Fusarium species, as the same seven species were detected in both control and treated plots. However, slight variations in abundance were observed, particularly in 2016, where *F. proliferatum* and *F. subglutinans* showed an increase in the treated plots. The yearly fluctuations suggest that environmental conditions, more than insecticide treatment, had an influence on the fungal diversity. *F. verticillioides* was the dominant species over three years of experiment, so we can presume that there is a greater risk of fumonisin production, while *F. graminearum*, which produces deoxynivalenol, was dominant only in 2014. These findings highlight the persistent presence of Fusarium species in maize and the need for further investigation into the environmental factors influencing their prevalence.

Discussion

Characteristic symptoms of ear rot were observed in the field with the highest DSI recorded in 2014. Interestingly, the insecticide application against ECB did not significantly reduce ear rot, except in 2013, when the highest number of ECB larvae was recorded on maize ears. In this year, a significant reduction in ECB larvae on maize ears was observed, however, this reduction did not correspond to a decrease in ear damage. Saladini et al. (2008) reported contrasting results, noting a 44.1% reduction in ear damage as a result of the insecticide treatment. Additionally, Blandino et al. (2022) confirmed that the best formulation for managing fungal ear rot is the proper application of insecticides to control ECB damage, mycotoxins and it also led to a significant increase in grain yield. Our experimental findings corroborate those of Eckard et al. (2011), who similarly identified *F. verticillioides* and *F. graminearum* as the predominant species isolated from maize samples. Additionally, our results align with Krnjaja et al. (2015), where the authors reported *F. graminearum* (1.08%), *F. subglutinans* (8%), and *F. verticillioides* (25.75%) in maize in Serbia during 2014.

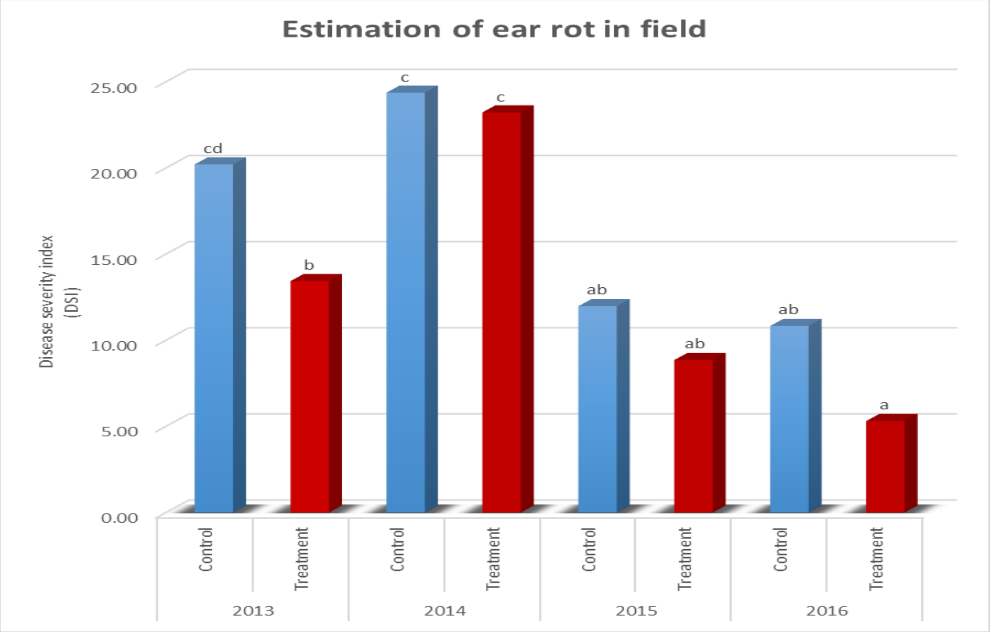

Figure 1. Disease severity index (DSI) during four years of examination in control and treated plots. *Means marked with different letters are significantly different from each other according to LSD test at p≤0.05

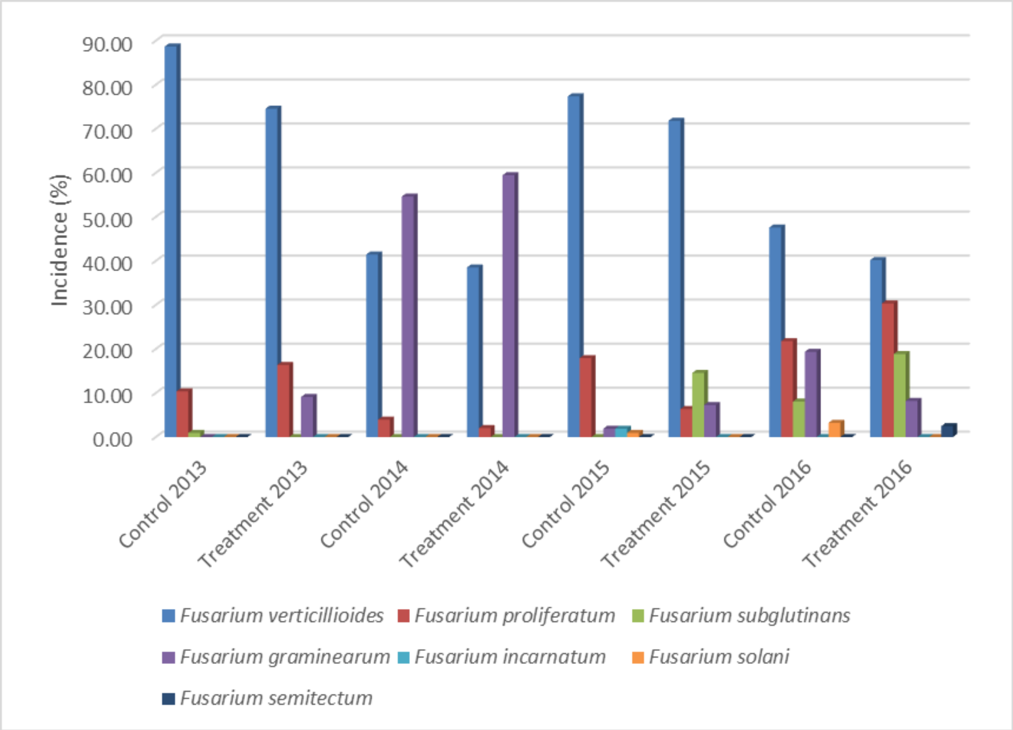

Figure 2. Incidence of *Fusarium* species obtained from harvest samples during four years of examination

In Germany, a group of authors found that *F. verticillioides, F. graminearum* and *F. proliferatum* were the most frequently isolated species from maize samples during 2006. Additionally, the same authors indicated that *F. verticillioides* and *F. proliferatum* were more prevalent in increasingly arid conditions with elevated temperatures, whereas *F. subglutinans* thrived in cooler and more humid environments (Goertz et al., 2010). The prevalence of *F. verticillioides* is closely linked to its various pathways of entry into maize, facilitated in part by insects that feed on maize plants (de la Torre-

Hernández et al., 2014). This could be correlated with results of this study where the dominance of *F. verticillioides* was recorded in 2013, 2015, and 2016, along with the highest recorded number of larvae and damaged ears in 2013, suggesting a strong correlation between insect activity and fungal prevalence. In addition, the total number of recorded damaged ears was also the highest in 2013, while the lowest was in 2014 and in that year *F. graminearum* was the most prevalent species. Additionally, *F. verticillioides* possesses the capability to neutralize bioactive substances found in maize that may account for its prevalence over other species from the same genus. (Bacon et al., 2008).

Environmental changes as well as the widespread nature of *Fusarium* species are increasingly affecting their composition in maize, impacting both the amount and type of mycotoxins (Bryła et al., 2022). Over the past decade, there has been an increase in the prevalence of certain Fusarium species in maize kernels across several countries, likely linked to climate change and evolving agricultural practices. Results from Castañares et al. (2019) are in line with this study, their results showed that during the 2015, 2016 and 2017 harvest season *F. verticillioides* was the most frequently isolated species from maize followed by *F. subglutinans*, *F. graminearum* species complex, *F. proliferatum*, and *F. cerealis*.

Our study confirmed that *F. verticillioides* is particularly suited for secondary infections, likely due to its morphology and its capacity for high proliferation of large quantities of microconidia, which are easily dispersed. In the years 2013, 2015, and 2016, when *F. verticillioides* was identified as the dominant species, the influence of ECB treatment on DSI was recorded. The most significant statistical differences on DSI between the ECB treated and untreated plots were recorded in 2013, the same year that exhibited the highest pest ECB pressure and the highest CE and DE differences. Additionally, the number of secondary infections decreased as the incidence of ECB also declined. On the contrary, in 2014, climatic conditions characterized by high rainfall and lower temperatures during the generative stages of maize (Graph 2) were more favourable for the development and predominance of *F. graminearum*. In contrast, during the other three years of the experiment, the lack of precipitation in July and August and very high temperatures (Graphs 1, 3, and 4) did not support the occurrence of *F. graminearum*. In 2014, the DSI in ECB-treated plants did not decrease despite the low incidence of ECB. This indicates that the DSI mainly reflected the primary infections recorded in the field during that year.

Conclusions

The results of this four-year study highlight the complexity of protecting maize from the European corn borer and preventing ear rot caused by *Fusarium* species. An insecticide treatment based on the combination of chlorantraniliprole and lambda-cyhalothrin generally proved to be successful in reducing the number of ECB larvae on maize ears. However, its efficacy in decreasing the total number of damaged ears and in lowering the disease severity associated with ear rot varied across different years. The diversity of *Fusarium* species remained unchanged in both the treated and control plots, indicating that chemical control of ECB has a limited effect on fungal populations responsible for ear rot. Furthermore, environmental factors such as rainfall and temperature significantly influenced *Fusarium* infections. Overall, the results indicate that while *Fusarium* species are consistently present in maize, *F. verticillioides* was the dominant species across the three years of the experiment, which were characterized by drought and high temperatures during the generative stages of maize. This suggests an increased risk of fumonisin production under such climatic conditions. Conversely, *F. graminearum*, which produces deoxynivalenol, was dominant in 2014, when climatic conditions were marked by high rainfall and lower temperatures during the generative stages of maize. Also, the indication that insecticide treatments do not have a significant indirect effect on maize ear rot in specific weather conditions, emphasizes the need for other crop protection measures, such as resistant hybrids, monitoring of environmental conditions, crop rotation and implementation of irrigation strategies.

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Uticaj insekticidnog tretmana protiv kukuruznog plamenca (*Ostrinia nubilalis*: Lepidoptera, Crambidae) na učestalost pojave fuzarioze klipa

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Sažetak: Proizvodnja kukuruza često je ograničena brojnim fitofagnim vrstama insekata i bolestima, među kojima treba izdvojiti kukuruznog plamenca (*Ostrinia nubilalis*) i trulež klipa uzrokovana vrstama iz roda *Fusarium*. Oštećenja klipa kukuruza koja izazivaju larve kukuruznog plamenca mogu predstavljati mesta za ulazak spora vrsta iz roda *Fusarium*, čime se povećava verovatnoća infekcije. Cilj ovog istraživanja bio je procena uticaja insekticidnog tretmana namenjenog suzbijanju kukuruznog plamenca na smanjenje učestalosti fuzarijozne truleži klipa. Istraživanje je sprovedeno od 2013. do 2016. godine i obuhvatalo je tretman i kontrolu, u četiri ponavljanja. Svake eksperimentalne godine primenjivana je kombinacija hlorantraniliprola i lambda-cihalotrina u dozi od 200 ml ha^{-1} kao tretman insekticidom, dok se u kontroli nisu primenjivali insekticidi. Takođe, izvršena je analiza prisustva vrsta iz roda *Fusarium* na klipu kukuruza tokom četvorogodišnjeg eksperimentalnog perioda. Dobijeni rezultati su pokazali da je tretman insekticidom značajno smanjio brojnost gusenica na klipovima kukuruza u svim godinama istraživanja, osim u 2015. Efikasnost tretmana na smanjenje ukupnog broja oštećenih klipova bila je značajna samo u 2016. godini, dok se u drugim godinama efikasnost razlikovala. Tretman insekticidom imao je značajan uticaj na smanjenje učestalosti truleži klipa samo u 2013. godini. Tokom četvorogodišnjih istraživanja, *F. verticillioides* je ustanovljen kao dominantni patogen na kukuruzu, osim u 2014. godini kada je *F. graminearum* bio dominantan. Tretman insekticidom nije imao uticaja na raznolikost vrsta unutar roda *Fusarium*.

Ključne reči: *Ostrinia nubilalis*, suzbijanje kukuruznog plamenca, trulež klipa, *Fusarium* spp., insekticidni tretman, hlorantraniliprol, lambda-cihalotrin, kukuruz

Additional information:

Climate diagrams after Walter and Lieth (1960) on the locality Rimski šančevi for the period 2013-2016