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EFFECTS OF THE LOW-LEVEL WESTERN CORN ROOTWORM EGG INFESTATION ON MAIZE PLANTS IN THE FIELD

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SUMMARY

From the economic perspective, the western corn rootworm (WCR), Diabrotica virgifera sp. *virgifera (Col., Chrysomelidae), poses the gravest threat to the field maize production in Serbia. The purpose of this study is to examine the impact of WCR larvae on the morphology of maize characters during a low-level artificial egg infestation. A field experiment involving the Serbian cultivar 'NS-640' was carried out in Bečej, Vojvodina,* Serbia, *in 2016. In the experimental field, a total of 96 maize plants were selected, marked and arranged in 48 pairs. Each pair consisted of an infested plant (WCR eggs injected in the root zone) and an uninfested plant (distillate water injected in the root zone). The number of leaves, height and stem diameter of the plants observed were recorded. Root damage and root weight were measured and evaluated at the end of the trail. For the purpose of statistical analysis, the non-parametric Kruskal-Wallis one-way analysis of variance and a correlation matrix were used. The statistical analysis performed indicate a highly significant difference in the number of leaves and plant height between the infested and uninfested maize plants examined in July (the third field observation). During the last field inspection, significant differences were recorded only between the stem diameters of the infested and uninfested maize plants. A negative correlation was found to exist between the root damage and root weight of the plant pairs. There were positive correlations between the stem diameter, plant height and number of leaves of the infested plants, whereas positive correlations were found between the root damage and plant height of the uninfested plants.*

Keywords: *WCR, infestation, maize, root system, height, leaves, stem diameter*

Abbreviations: WCR **-** western corn rootworm

INTRODUCTION

In addition to wheat and rice, maize provides at least 30% of the global food supply (FAOSTAT, 2010). As estimated by Rosegrant et al. (2009), the demand for maize production in the developing world will double. Apart from being an important food crop for human consumption, the demand for maize as livestock feed is increasing rapidly (Bekele et al., 2011). The agricultural production in Serbia has undergone qualitative changes, ultimately affecting its productivity (Stevanović et al., 2012). The maize production in the country is of paramount importance to human consumption, cattle production and the processing industry (Zubović et al., 2018). In the field, maize is exposed to a large number of pests, the presence of which contributes to yield and stem quality losses (Oerke, 2006). One of the most destructive maize pests is the western corn rootworm (WCR), *Diabrotica virgifera* sp. *virgifera* Le Conte (Col., Chrysomelidae), which causes yield losses by inflicting severe damage to the root, stem architecture and leaf surface (Bača, 1993; Ciobanu et al., 2009). It is an oligophagous, univoltine and the most economically important maize pest native to America (Bermond et al., 2012). Since the end of the 20th century, WCR has become the most economically important maize pest in Europe (Mahmoud et al., 2016). The first identification of WCR plant damage in maize fields in Europe was in Serbia, near the Belgrade airport, in 1992 (Bača, 1993). The WCR larval attack on the root system is the most important maize field damage (Ciobanu et al., 2009; Wesseler & Fall, 2010). A larval attack on the maize root system (nodal and lateral roots) can cause the inability of maize to uptake water and nutrients, leading to plant logging (Dun et al., 2010). Plant logging, called "goose neck", is the primary manner of WCR presence in the field (Chiang, 1973; Wesseler & Fall, 2010). Under a severe larval attack, lodging can reduce yields by 11–34% (Estes et al., 2015; Pereira et al., 2015). Maize has the ability to decrease plant lodging and increase the root system (Riedell and Evenson, 1993; Gray & Steffey, 1998), as well as to decrease yield losses under favourable climatic conditions (Zubović et al., 2018). Compared to larvae, WCR adults cause less damage feeding on young leaves and maize silk (Raspudić et al., 2013). The purpose of this research is to examine the impact of WCR larvae on the root weight, number of leaves, plant height and stem diameter of maize plants in the field during a low-level artificial egg infestation.

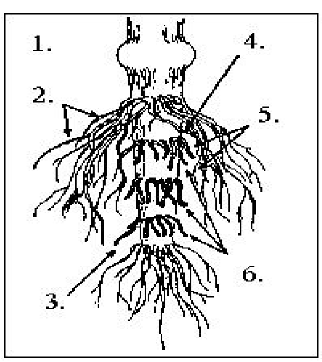
MATERIAL AND METHODS

The field experiment was carried out in Bečej, the Province of Vojvodina, Northern Serbia, in 2016. The field selected for the experiment exhibited a low level of natural WCR infestation. The experiment was set up on 17th May and it lasted until 30th September. The Serbian cultivar 'NS-640' was sown in the experimental field. During the experiment, a total of 96 maize plants were selected, labelled and arranged into pairs. There was a spacing of 1 m between the labelled plants, with 7 plants on average. In each pair, one plant was artificially infested in the root zone with 4 mL of WCR eggs in the 0.125% agar suspension (D plants). One mL of the suspension contained 136 WCR eggs. A total of 48 maize plants were infested with 544 eggs per plant i.e. 26,122 eggs per year in the experimental field. The other plant in the pair was the control plant (C) whose root zone was injected with 4 mL of distilled water.

During the growing season, the field experiment was regularly inspected, on a weekly basis, for five months, totalling 14 inspections (starting from the last week of May until the end of September). The plant inspection involved the measurements of plant height, stem diameter and number of leaves. Plant leaves were counted and plant heights were recorded during each field inspection. For the purpose of statistical analysis, the data obtained during the 3rd (the inspection with no registered desiccation of oldest leaves) and 8th observation (recording the same number of leaves until the end of vegetation) were used. The stem diameter of the plants observed was measured during the last inspection, and the experiment ended on 30th September. Stem diameter measurements were performed in the field using a ± 0.05 mm precision Caliper, Pro-Max 67 IP Sylvac System USA.

During the last field observation, the root inspection entailed excavating the marked plants and removing soil from the roots (by manual shaking). Thereafter, the roots were packed, transported to the lab and rinsed with water. Maize roots were dried under room climatic conditions for 7 days. Root damage was rated from 1 to 6 (Fig. 1), according to the scale recommended by Ostlie and Notzel (1987), and then dry roots weight was measured using a technical balance (Kern EW 1500-2 M, Germany).

Differences in root damage, root weight, plant height, stem diameter and number of leaves between the D and C plants were analysed using the non-parametric Kruskal-Wallis one-way analysis of variance. Correlations between root damage, root weight, number of leaves, plant height and plant diameter of the D and C plants were determined by basic statistics (correlation matrix) at the significance level $p<0.05$.



Root rating scale (Ostlie and Notzel, 1987)
1 – No feeding damage
2 – Visible feeding scars present
3 – At least one root chewed to within 1.1/2 inches of plant
4 – One entire node of roots destroyed
5 – Two nodes destroyed
6 – Three or more nodes destroyed

Figure 1. Root damage scale (Ostlie and Notzel, 1987)

RESULTS

According to the data obtained in 2016, only three D plants, i.e. 6.25%, exhibited healthy root systems (Rate 1) or no registered root damage. Root injuries rated as level 2 were recorded in seven D plants, i.e. 14.58%. The largest number of D plants, i.e. 12 (25%), was rated 3. Rate 4 was registered in 7 D plants, i.e. 14.58%, and Rate 5 in eight plants, i.e. 16.6%. The most severe and observable damage, Rate 6, was recorded in 10 D plants, i.e. 20.83% (Fig. 2). Of the total number (48) of the C plants, only three C plants, i.e. 6.25%, were rated as level 1, exhibiting a healthy root system. Root injuries rated as 2 were recorded in three C plants, i.e. 6.25%. Rate 3 damage was registered in 13 C plants, i.e. 27.08%. Five C plants, i.e. 10.41%, were rated level 4, whereas 13 C plants, i.e. 27.08%, were rated level 5 (Fig. 2).

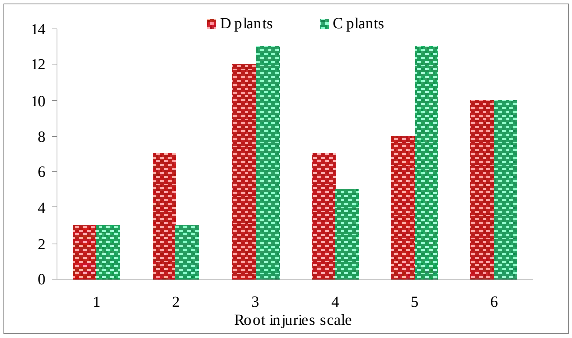


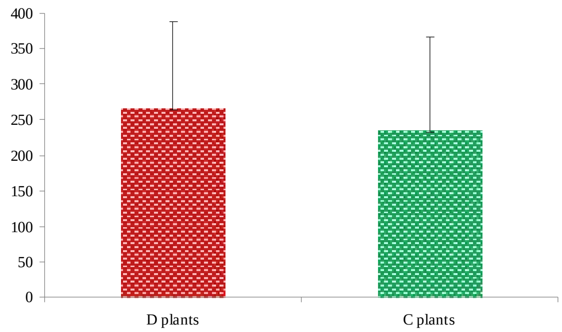
Figure 2. Number of plants rated using different scale numbers (Ostlie and Notzel 1987) according to the root damage of infested (D) and uninfested (C) plants during 2016

According to the Ostlie and Notzel (1987) root scale used in this study, 93.75% of the total number of plants (96 i.e. 48 D and 48 C plants) examined in 2016 were diagnosed with different root injuries caused by WCR larvae.
The statistical analysis performed in 2016, using the non-parametric Kruskal-Wallis test, showed no significant difference in the root damage between the D and C plants (Tab. 1).

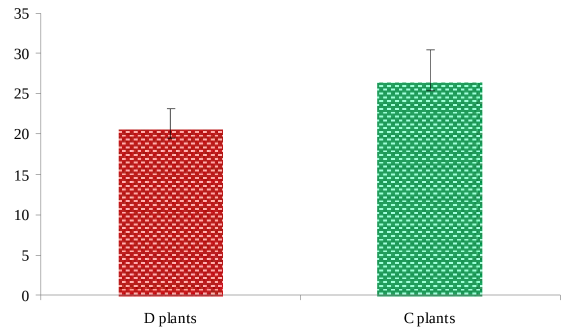
Table 1. Statistical analysis based on the root damage, root weight and stem diameter of the D and C plants

Year	Parameter	Mean values ± Sd		Sig.
		D plants	C plants	
2016	Root damage	3.851±1.574	4.106±1.507	0.421ns
	Root weight (g)	263.8 ±123.9	233.7 ±134.03	0.164ns
	Stem diameter (mm)	20.48±2.759	26.32±4.170	**0.001****

The lowest measured root weight during 2016 in the D and C plants was 93.12 g and 67.7 g, respectively. In the same year, the greatest measured root weight of D and C plants was 563.44 g and 546.03 g, respectively. The average root weights in 2016 of the D and C plants were 263.8 g and 233.7 g, respectively (Fig. 3). The statistical analysis performed in 2016, using the non-parametric Kruskal-Wallis test, showed no significant differences between the root weights of D and C plants (Tab. 1).
The stem diameter values recorded were in the range of 13.28–31.29 mm in the D plants and 19.32–35.26 mm in the C plants. The average values of the stem diameter for the D and C plants were 20.48 and 26.32 mm, respectively (Fig. 4). The statistical analysis performed revealed highly significant differences between the stem diameters of the D and C plants (Tab. 1).

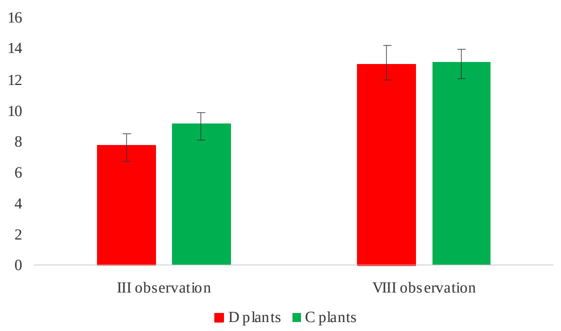


* Bars represent ±Sd value
Figure 3. The average root weight (g) of the infested (D) and uninfested (C) plants in 2016

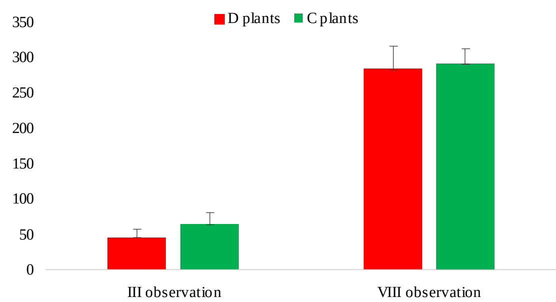


* Bars represent ±Sd value
Figure 4. The average stem diameter (mm) of the infested (D) and uninfested (C) plants in 2016

During the first and second filed observations, a different number of leaves were recorded relative to the desiccation of oldest leaves found on the plants.
Durind the third field observation, the number of leaves on the D plants ranged from 6 to 9, whereas that on C plants ranged from 7 to 10. The mean values of the leaf numbers on the D and C plants were 7.708 and 9.042, respectively. During the eighth and last field observation, the D plants exhibited 8 to 16 leaves, whereas the leaf number on the C plants ranged from 11 to 15 (Fig. 5). The mean values of the leaf number at the end of the trial on the D and C plants were 12.94 and 13.02, respectively.
The non-parametric Kruskal-Wallis test employed for statistical analysis in 2016 showed significant differences in the number of leaves between the D and C plants during the third field observation, and no significant difference during the last observation (Tab. 2).



* Bars represent ±Sd value
Figure 5. The average number of leaves on the infested (D) and uninfested (C) plants in 2016



* Bars represent ±Sd value
Figure 6. The average plant height (cm) on the infested (D) and uninfested (C) plants in 2016

Table 2. Statistical analysis based on the number of leaves and plant heights of the D and C plants

Year	Parameter	Observation	Mean values ± Sd		Sig.
			D plants	C plants	
2016	Number of leaves	III	7.708±0.743	9.042±0.824	**0.001****
		VIII	12.94±1.227	13.02±0.956	0.711ns
	Plant height	III	45.23±10.94	63.56±17.28	**0.001****
		VIII	283.4±31.13	290.4±21.36	0.204ns

During the eight (21st July) and last field inspection (30th September), the maximum measured height of D plants was 310 cm and their minimum height was 130 cm. The maximum height of C plants was the same as in D plants, 310 cm, while their minimum height was 220 cm. The average height of D plants was 283.4 cm and that of C plants 290.4 cm (Fig. 6). During the third field observation, the maximum and minimum height of D plants was 75 cm and 25 cm, respectively. The maximum and minimum height of C plants in same period was 90 cm and 28 cm, respectively. The average height of D and C plants during the third observation was 45.23 cm and 63.56 cm,

respectively (Fig. 6). Performed statistical analysis showed significant difference in plant heights between D and C plants during the third observation, while no significant difference was shown during the last (eight) observation (Tab. 2).
The statistical analysis revealed negative correlation between root damage and root weight, and between root damage and number of leaves in D plants (Tab. 3), while positive correlation was determined between plant height and stem diameter and between stem diameter and number of leaves.

Table 3. A correlation matrix based on observed parameters in D and C plants

Variable	D plants					C plants				
	Root damage	Root weigh t	Plant height	Stem diameter	Number of leaves	Root damage	Root weigh t	Plant heigh t	Stem diameter	No. of leaves
Root damage	1.00	**-0.59**	-0.22	-0.28	**-0.31**	1.00	**-0.73**	**-0.36**	-0.17	-0.27
Root weight	**-0.59**	1.00	0.28	0.09	0.07	**-0.73**	1.00	**0.41**	0.06	0.28
Plant height	-0.22	0.28	1.00	**0.37**	**0.50**	**-0.36**	**0.41**	1.00	0.15	0.23
Stem diameter	-0.28	0.09	**0.37**	1.00	**0.35**	-0.17	0.06	0.15	1.00	0.09
No. of leaves	**-0.31**	0.07	**0.50**	**0.35**	1.00	-0.27	0.28	0.23	0.09	1.00

As shown by the data obtained for the C plants, negative correlations were found between the root damage and root weight, as well as between the root damage and plant height, whereas only the root weight and plant height were positively correlated (Tab. 3).

DISCUSSION

A comparison of the present results on root injury with those of Popović et al. (2017) and Tanasković et al. (2017) indicates that soil moisture, determined by rainfall and temperature, exerts a crucial impact on the development and harmfulness of WCR larvae as soil dwelling insects, which is consistent with the results of Cagáň et al. (2016). Furthermore, Grozea et al. (2009) reported that dry and warm weather generally increases the number of insects. These results corroborate the data obtained in our study which indicate that the 2016 growing season was favourable for maize production in contrast to the growing seasons of 2015 and 2017.
The data obtained on the maize plant height in 2016 show that environmental conditions play a very important role in the maize physiology, as well as in determining the level of root injury. The level of root injury recorded in 2016 was lower than that recorded in 2017 (Popović, 2017).
Stem diameter growth is directly connected to plant nutrition, and it reflects the level of root injury, i.e. the ability of maize plants to utilise water and nitrogen. Popović (2017) listed the average stem diameters of WCR infested and uninfested maize plants (29.4 mm and 30.43 mm, respectively), and found no statistically significant differences. However, the present results indicate that there is a highly significant difference between the average diameter values of infested (20.48 mm) and uninfested (26.32 mm) maize plants.
Popović (2017) made a comparison between 10 plants with highly damaged roots (Rate 6) and 23 plants registered in the same category, which suggested that the growing season of 2016 had less favourable conditions for the WCR larval development. This finding is completely consistent with the results obtained in Slovakia by Cagáň et al. (2016).

CONCLUSION

According to the statistical analysis performed (the non-parametric Kruskal-Wallis test), statistically significant differences were found to exist between the number of leaves and plant heights of the infested (D) and uninfested (C) maize plants ($p<0.001$) during the third field observation. During the last field observation, the results obtained showed highly significant differences only between the stem diameters of the D and C plants. Negative correlation between the root damage and root weight of the D and C maize plants were also found, whereas positive correlations were observed between the stem diameter, plant height and leaf number of the D plants. Moreover, a positive correlation was found between the root damage and plant height values of the C plants.

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