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Maize Tolerance to Natural and Artificial Infestation with *Diabrotica virgifera virgifera* Eggs

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***Abstract*—** Western corn rootworm – WCR (*Diabrotica virgifera* sp.*virgifera,* Coleoptera, Chrysomelidae) is economically the most important pest of maize worldwide. WCR natural population is already very abundant on Serbian fields, and keeps increasing each year. Tolerance is recognized by larger root size and bigger root regrowth. Severe larval injuries cause lack of compensatory regrowth and lead to reduction of plant growth and yield. The aim of this research was to evaluate tolerance of commercial Serbian maize hybrid NS 640, under natural WCR infestation and under conditions of artificial infestation, and to obtain the information about its tolerance to WCR larval feeding in two consecutive years. Field experiments were conducted in 2015 and 2016, in Bečej (Vojvodina province, Serbia). In experimental field, 96 plants were selected, marked and arranged in 48 pairs. Each pair represented two plants. The first plant was artificially infested with 4 mL WCR egg suspension in agar (550 eggs plant^{-1}) in the root zone (D plant). The second plant represented control plant (C plant) with injection of 4 mL distilled water in root zone. The experimental field was inspected weekly. A hybrid tolerance was assessed based on root injury level and root mass. Root injury was rated using the Node-Injury Scale 1-6, during the last field inspection (September – October). Comparing the root injuries on D and C plants in 2015, more severe damages were recorded on D plants (12 plants - rate 5 and 17 plants - rate 6) compared to C plants (2 plants - rate 5 and 8 plants - rate 6). Also, the highest number of plants with healthy roots (rate 1), was registered in the control (25 plants), while only 4 D plants were rated as injury level 1. In 2016, root injuries caused by WCR larvae on D and C plants did not differ significantly. The reason is the difference in climatic conditions between the years. The 2015 was extremely dry and more suitable for WCR larval development and movement in the soil, compared to 2016. Thus, more severe damages appeared on artificially infested plants (D plants). Root mass was in strong correlation with the level of root injury, but did not differ significantly between D and C plants, in both years.

Keywords*—D. v. virgifera,* maize, root injury, tolerance.

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I. Introduction

WCR *Diabrotica virgifera* sp. *virgifera* Le Conte (Col., Chrysomelidae) is an oligophfaguos pest, originating from America [1]. The first identification of WCR in Europe was in Serbia, near the Belgrade airport, in the early 90`s [2]. From that point forward, the pest spread to almost every maize field in Europe [3] and according to [4], it can spread up to 100 km per year. It is a very dangerous pest because the larvae attack roots [5], [6] and adults feed on young leaves and maize silk. However, the damages from adults are less significant compared to those caused by larvae [7]. Due to larval damages of the root system, water and mineral nutrients uptake by maize plants is impeded [8], [9]. One of the main symptoms that indicate the presence of WCR larvae in the field is the so called "goose neck" [10]. It is a result of WCR larval feeding on the nodal and lateral roots [11], and it results in yield loss [12]. Development of root system, plant logging and the amount of secondary roots are the main indicators of maize tolerance to WCR [13]. Measure of higher tolerance of maize to WCR roots injury is, according to [14], larger and more developed root system and according to [15] decreased lodging. Damages caused by WCR larvae are highly dependent of soil moisture, soil type and larval abundance in soil [16]. Environmental conditions also have strong influence on the level of root damages [17].

II. Material and Methods

The field experiment was carried out in Bečej, Vojvodina province, Serbia, from May 30th to September 10th 2015, and from May 18th to October 18th in 2016, with cultivar NS-640. The filed chosen for the experiment represents a field with low natural WCR infestation.

Prior to the experiment set up, 96 maize plants were selected, labeled and arranged into pairs. The plants were sown in two rows with 1 m distance between labeled plants. Each pair consisted of one artificially infested plant (D plant) and a control plant (C plant). D plants were infested with 4 mL of WCR eggs in 0.125% agar suspension, by injecting the solution in the root zone. One mL of suspension contained 136 WCR eggs. For the control (C plants) treatment, 4 mL of distilled water was injected in the root zone.

The experiment was inspected on weekly bases, in both years. During each observation, the presence of "goose neck" symptoms (GN) was recorded. During the final field inspection, the damages of maize root causes by WCR larvae were evaluated. The root inspection was conducted as follows: All marked plants were excavated, the soil was removed from roots and the roots were rinsed. After the preparation, root damages were ranked from 1 to 6, according to scale [18]. Also, the root biomass was measured on a technical balance (Kern EW 1500-2 M).

The differences between damages on D and C plants, based on the root damage rate, were analyzed using non-parametric Mann-Whitney test (Z), for the confidence interval of 95%.

III. Results and Discussion

A. Root Damages in 2015

The obtained results for root damages in 2015 are presented in Fig. 1. The biggest number (25) and the highest percent (52.1%) of control plants (C plants) were with healthy root systems (rate 1). Slightly damaged roots (rate 2) but with visible damages were registered on 6.25% control (C) plants. Nine plants or 18.75% were with at least one root chewed to within 3.8 cm, rated as level of damage 3, while only one plant (2.1%) was rated as damage level 4, with one entire node destroyed. 16.7% were with severe root damages (rate 5), and only 4.2% control (C) plants had root injuries rated as level 6, representing one or more nodes destroyed.

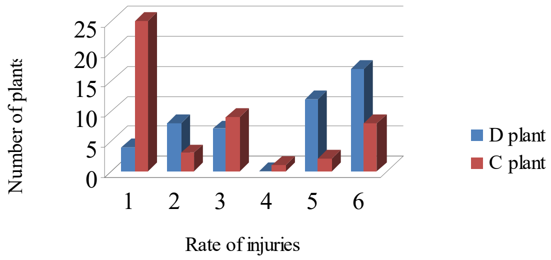

Fig. 1 The level of root damages on D and C plants according to traditional scale [18] in 2015

Roots are extremely important plant organs and development and mass vary between stages of plant growth and development. The most rapid development of maize roots occurs during the first 8 weeks after sowing [19]. As corn plants age, the growth of roots generally increases at slower rates than shoots [20]. After silking stage, corn root length declines [21]. This decline in root length after silking is most probably due to the high demand of grains for carbon. This increased demand of grains in C and N results in their enhanced translocation to grains, and lack of translocation to the roots [22]. The decline of root length is also a consequence of root feeders like WCR.

The majority of the infested plants (35.5% of D plants), suffered severe root injuries and damage level was evaluated as rate 6. From the total number of D plants, 25% were with two entire nodes destroyed (rate 5), and 14.6% were rated as level of injury 3. Only seven or 16.7% of D plants were with visible damages caused by larvae (rate 2), while only 8.3% infested plants were with healthy root systems (rate 1). D plants with one node destroyed (rate 4) were not registered in the experiment during 2015.

Statistical analysis indicates at highly significant differences between the level of root damages on infested (D) and the control (C) plants (Z=4.85**, p<0.01).

TABLE I
Differences between Root Biomass and Root Damages of *D. v. virgifera* Infested Plants and the Control Plants in Two Consecutive Years and the Correlation between Mentioned Parameters

Year	Parameter	Mean Values D	Mean Values C	Z	Sig.
2015	root mass	69,62 ±16.33 a	30,16 ±10.81 b	7,66**	0.00
	root damage	4.25	2.37	4.85**	0.01
2016	root mass	267.92 ±125.86 a	234.18 ±132.64 a	1.28ns	0.20
	root damage	3.81	4.01	4.77ns	0.61

Year	Regression Analysis	Plants	R	R Square	t	Sig.
2015	root mass/ root damage	D	0.609	0.371	-5.210	0.000
		C	-0.725	0.725	-7.140	0.000
2016	root mass/ root damage	D	0.512	0.262	-1.032	0.060
		C	-0.473	0.224	-1.340	0.135

Mean values ±SD; Values with the same small letter are on the same level of significance;
NS – non significant difference for the confidence level 95%, D – infested plants; C – control plants

Inspection of the rest of the field indicates at the presence of 6% of natural WCR infestation, i.e. between 100 randomly chosen plants in the row, six plants were recorded with the goose neck symptoms. In experimental field, 24 GN plants were recorded between infested and neighboring plants.

B. Root Biomass in 2015

The level of root damages was very high in 2015, and the differences in root biomass between D and C plants was statistically significant (Z=7.66**, p<0.01) (Table I). The smallest measured root biomasses of C and D plants were 26.88 g and 22.17 g, respectively (Fig. 2). The highest root biomass of C and D plants were 142.2 g and 144.4 g respectively. The average values of root biomasses of C and D plants were 30.16 g and 69.62 g, respectively.

The performed regression analysis registers highly significant negative correlation (t=-5.210**; -7.140**, p<0.01) between root damage and root mass for D and C plants. It reflects that root damage significantly affected root biomass of D and C plants in 2015.

C. Root Damages in 2016

The results from 2016 are presented in Fig. 3. In 2016, the lowest number of C plants, only three i.e. 6.25%, were with healthy root systems (rate 1). Visible damages caused by larvae (rate 2) were registered on three C plants (6.25%). Root injuries of rate 3 (with at least one root chewed to within 3.8 cm (1½ inches)) were on 13 plants (27.08%), while only six plants (12.5%) were with one entire node destroyed (rate 4). 13 plants (27.08%) were rated as the level of damage 5, and 10 C plants (20.83%) were with three or more nodes destroyed (rate 6).

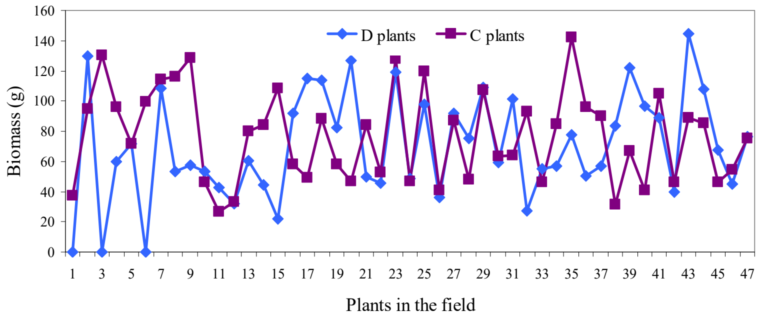

Fig. 2 The root biomass of maize plants in 2015

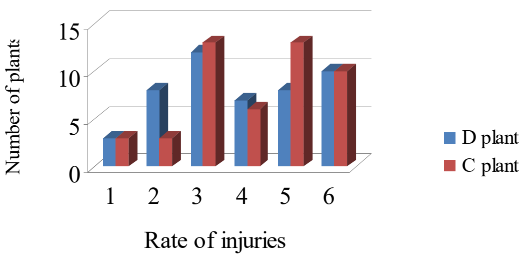

Fig. 3 The level of root damages on D and C plants according to traditional scale [18] in 2016

Of all infested D plants, 10 (20.83%) were with the highest root damages (rate 6), and 8 plants (16.66%) with two nodes destroyed (rate 5). From the total number of infested plants, 7 plants (15.58%) were with one entire node destroyed (rate 4), 12 plants (25%) with at least one root chewed to within 3.8 cm (1½ inches) of the plant (rate 3), while visible damages caused by larvae (rate 2) were on 8 plants (16.7%). Only 3 D plants (6.25%) from 48 plants were with healthy root system (rate 1).

The difference between the level of root damages, during the last observation, on D and C plants (Table I) was not statistically significant, in 2016, (Z=4.77ns, $p<0.01$).

Inspection of the rest of the field indicates at the presence of 14% of natural WCR infestation, i.e. between 100 randomly chosen plants in the row, 14 plants were recorded with the GN symptoms. In experimental field, 45 GN plants were recorded between infested and neighboring plants.

D.Root Biomass in 2016

Although the level of root damages in 2016 was very high, the differences in root mass between D and C plants were not statistical significant (Z=1.28ns, $p>0.05$). The smallest measured root biomasses of C and D plants were 67.7 g and 93.12 g, respectively (Fig. 4). The highest root biomass of C and D plants were 708.8 g and 532.43 g respectively. The average values of root biomasses of C and D plants were 234.18 g and 267.62 g, respectively (Table I).

The results of regression analysis shows significant negative correlation (Table I) (t=-1.032ns; -1.340ns, $p<0.05$) between root damage and root mass for D and C plants. Obtained results indicate that root damage did not significantly affect root biomass of D and C plants in 2016.

The level of damages caused by the presence of WCR larvae in maize monoculture can increase the percent of lodged plants from 3% to 15% [23] and yield losses caused by the lodging of plants, up to 75% [24]. Reference [25] also points out that larval presence in maize field leads to a decrease in yield. The same authors [25] indicate that larvae cause more severe root injuries than adults on maize silk. Monoculture in maize field represents one of the main reasons for the increase in WCR population and contributes to bigger plant damages and root injuries [26], [27]. The maize is a plant with a high ability to recover, and maize root tolerance is associated with its capability to grow new roots after injury from WCR larvae [28]. The results of this work are in compliance with [29] indicating that larval presence in soil can cause different root damages as a consequence of different climatic condition or soil structure.

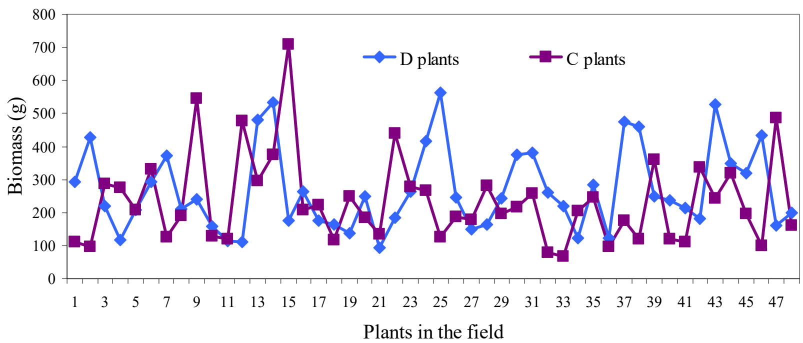

Fig. 4 The root biomass of maize plants in 2016

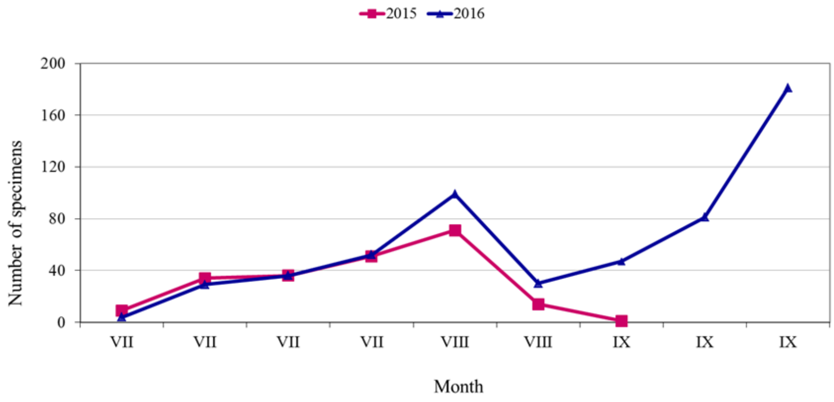

Fig. 5 Flight dynamics of WCR in 2015 and 2016

E. WCR Flight Dynamics 2015 and 2016

Inspection of sticky bases in 2015 indicates that WCR flight fluctuated during vegetation period (Fig. 3). The highest number of caught specimens was in middle of vegetation (6th August 2015) when the presence of 71 WCR adults was recorded. At the end of vegetation (10th September) the smallest number of WCR adults (1 specimen) was recorded.

In 2016 (Fig. 6), continuous flight was registered from 2nd July until 30th September. At the beginning of August (4th August) the first peak (99 specimens) was registered. The highest number of caught specimens in weekly inspections (181) was registered on 30th September. During 2016 vegetation, the highest total number of caught WCR males recorded on sticky bases was 559, while during 2015, the number of caught males in pheromone traps was 216 specimens (Fig. 5).

The results of [30] indicate that the highest number of caught adults in pheromone traps was in the period of 25th July - 15th August in Serbia. The highest number of caught WCR adults in 2015 was on 6th August (71 adults) and in 2016 on 30th September (181 specimens). The results are presented on Fig. 1. In 2015 the first catch in Bečej was on 9th July and the last was on 10th September. Reference [31] shows that the highest efficiency of pheromone traps was in the mid vegetation, with daily catch of six WCR adults. On the other hand, during the vegetation period from 27th August to 10th September, not a single imago was registered in traps in Zemunpolje [32]. Our experiments and many other studies indicate the progressive and fluctuating catch of adults of WCR in different vegetation periods.

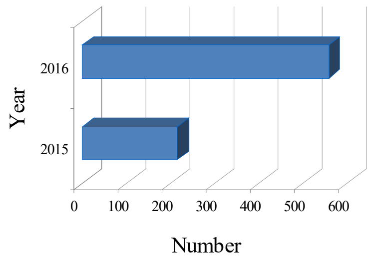

Fig. 6 Total number of caught males per year

F. Climatic Conditions in 2015 and 2016

The results of this work are in compliance with [25] indicating that larval presence in soil can cause different root damages as a consequence of different climatic conditions or soil structure.

Climatic conditions differed between these two years (Figs. 5 and 6).

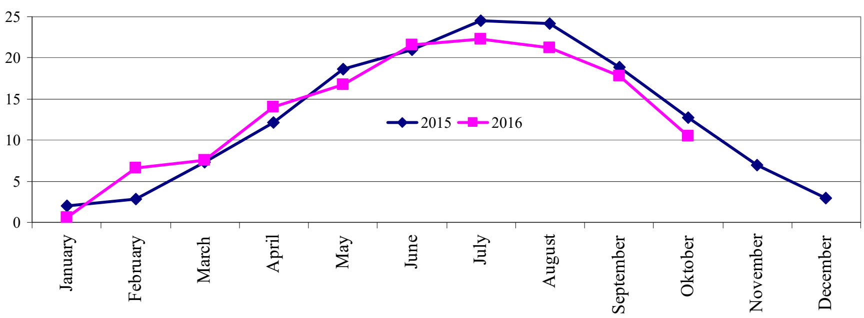

Fig. 7 Average temperatures during 2015 and 2016

Average daily temperatures in 2015 were higher compared to temperatures in 2016 (Fig. 7). Moderate temperature during spring and summer in 2016, and the mild winter temperatures in 2015 affected the natural population density in 2016, which was higher compared to previous year. Also, the precipitation levels between these years significantly differed. The total amount of precipitation in 2015 was 511.2 mm and in 2016 582.2 mm. During 2015, there were periods with total absence of rain, in March and April, (Fig. 8) which affected the WCR density and behavior.

Insects are poikilothermic organisms, whose body temperature vary and depend on the surrounding temperature. Also, their development is temperature-dependent and occurs within specific temperature ranges, between lower and upper developmental thresholds.

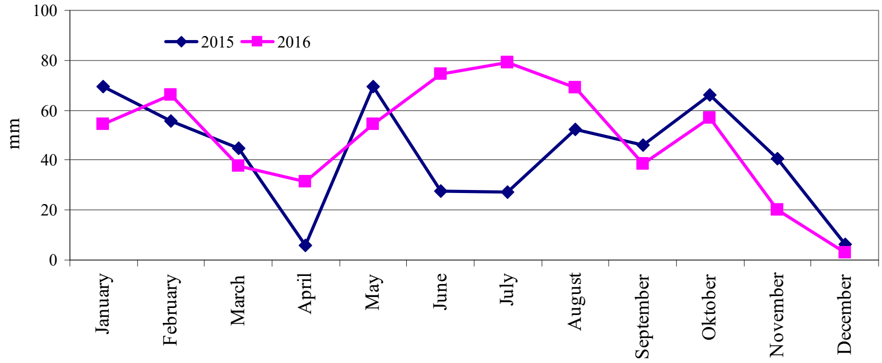

Fig. 8 Average precipitation in 2015 and 2016

The life cycle of many insects is synchronized with the phenology of the host plant, which also depends on temperature. This is the reason why the temperature determines the lower and upper limits of the insect distributional range, by affecting development and availability of both insect pest and host plant [33].

The impact of climatic parameters, primarily the temperature, on insects can be direct and these parameters can affect their development, reproduction, survival, behavior, distribution, migration and adaptation. Or, it can be indirect influence which occurs through the effects on the host plant, interacting species, natural enemies and competitors [34].

The rise of average temperatures during spring and summer months, which is a result of climate changes, may induce the faster insect development, increase the number of generations per year, cause changes in population abundance, provide longer period suitable for the development, increase overwintering survival rate, enable spreading of distribution range to higher latitudes and altitudes and cause introduction of alternative and/or temporary hosts [34].

According to [35], dry and warm conditions generally lead to increase in insect number. These authors presented results showing positive correlations between daily average air temperature and daily average adult number caught on pheromone traps, which is in consistence with the results presented in our work. However, there are limits from which these correlations become negative. The rainfall is an important factor that influences adults' dynamics in maize fields. Excessive rainfall leads to a decrease of the number of adult *D.v.v.* Daily observations showed a decreasing number of beetles while rainfall increases, as presented by mentioned authors. The same trend was registered in our work, where the lowest number of caught adults was caught in phero-traps during rainy periods, in both years.

WCR represents one of the most important factors affecting maize production worldwide. Also, the presence of soil dwelling pests, including *D.v.v.* larvae threatens root systems, and decreased root development caused by different environmental and biological agents causes loss and damage of yield. According to [36], in the USA, WCR infestation promoted root re-growth and brace root development. Based on our results it could be concluded that maize hybrid, soil moisture, presence of aboveground insects are contributing factors in WCR larval survival, level of harmfulness and recovery of the maize plant root system. This is in accordance with the results reported by [37], indicating that low levels of rootworm injury did not significantly affect grain yield even though reductions in photosynthetic rate and growth response patterns could be measured during vegetative growth stages. This supports the idea [38], [39] that maize can tolerate and compensate for some level of early season larval injury without sustaining significant yield loss.

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