

## ROOT COMPENSATION OF SEVEN MAIZE HYBRIDS DUE TO WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA* *VIRGIFERA* LECONTE) LARVAL INJURY

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### Abstract

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The western corn rootworm is the major pest of maize in the USA, and from 1992 became major threat in Europe as well. During 2007, 2008 and 2009 field investigation was conducted in continuous maize to evaluate root injury and tolerance associated traits (root regrowth and root size) of seven commercial hybrids after rootworm larval feeding. Each year adult populations were monitored by pheromone traps that attract both males and females. The pheromone traps were monitored weekly. The greatest adult activity was recorded in 2008 with the capture of 9.60 beetles per trap per day. In 2007 and 2009, captures were 2.60 and 6.00 beetles per trap per day, respectively. Significant differences were observed every year among hybrids for all traits. All hybrids proved to be moderately tolerant to rootworm larval injury in the first year. Hybrid OsSK 602 was significantly different with the least damaged roots (an average root injury rating 1.24), good development of compensatory roots following larval root injury (an average root regrowth rating of 2.66), and this was the highest yielding hybrid. Hybrid OsSK 617 significantly differed as it was the most able to compensate rootworm larval injury with well developed new roots. Hybrids OsSK 602 and OsSK 617 had the best development of compensatory roots in the two years of continuous maize and can be considered as the most tolerant among the commercial maize hybrids evaluated.

*Key words:* *Diabrotica*, monitoring, root regrowth, root injury, tolerance

*Abbreviations:* WCR – western corn rootworm

### Introduction

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (WCR) is the major pest

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of maize in the USA, and since 1992 became a major threat in Europe as well. It belongs to the list of the 100 worst invading species as described by the European invasive species database DAISIE (EU

6th FP SSPI-CT-2003-511202). Adults emerge in July and August. The males emerge before the females. Soon after their emergence, WCR adults copulate, and the females start ovipositing after 2 weeks. The maximum life span of females is from 96 to 154 days, depending on temperatures (optimum 23.5°C) (Elliott et al., 1990). Adults feed on maize leaves, silks and pollen. *D. virgifera virgifera* is a univoltine species. The main damage to maize is caused by rootworm larval feeding on the roots. The loss of root tissue reduces the uptake of water and nutrients, thus affecting plant growth (Chiang, 1973). Heavily damaged maize plants are lodged, causing symptom called *goose necking*, which impairs harvest. Root injury and grain yield losses from rootworm injury vary depending on agronomic and environmental conditions (Spike and Tollefson, 1989).

This pest has shown an ability to evolve resistance to several control measures, including insecticides (Meinke et al., 1998 and Wright et al., 2000) and cultural practices (Levine et al., 2002). Therefore, efforts for identifying sources of resistance or tolerance to corn rootworm in maize cultivars are crucial. Often in breeding programs the selection for increased yield leads to reduced levels of resistance and tolerance in crops compared to their wild relatives (Rosenthal and Dirzo, 1997). Tolerance has been traditionally considered as an alternative to plant resistance in breeding programs, and most of the studies have focused on this mechanism of resistance. Tolerance indicates the plant's ability to withstand or compensate for insect damage (Smith, 1989). However, environmental factors may affect tolerance more than other types of resistance (Pedigo, 1989). Different phenotypic traits evaluating resistance traits against WCR larval damage are used in the US and Europe (root injury; root pull resistance; root regrowth; root size; root volume and plant lodging). Investigation of resistance to corn rootworm started 60 years ago in the USA Corn Belt as an alternative to insecticides or other management tactics, and continues today. Even in 1920 dif-

ferences in plant response to larval feeding were noticed (Bigger et al., 1938). Bigger et al. (1941) reported that certain lines had superior tolerance to rootworms. The resistance reported by Owens et al. (1974) was due to larger root systems and greater compensatory root development. Riedell and Evenson (1993) stated that a tolerant plant sustains as much feeding damage as a susceptible plant, but is able to develop and produce high grain yields regardless of the injury. Studies in Missouri, USA, in 1997 and 1998 identified several crosses that had significantly less corn rootworm larval damage (Hibbard et al. 1999). Bohn (2006) reported that there were no germplasm sources with superior WCR resistance under moderate to high insect pressure, even though some genotypes were characterized by large root systems and superior compensatory root development after rootworm injury. Besides the lack of highly resistant maize cultivars, only limited information is available about the organized defence responses of maize against root feeding and wounding. Dashiell et al. (2006), reviewed rootworm resistant varieties in 14 maize genotypes by assessing the resistance and tolerance to WCR larvae. They found that genotypes with the least damage had an average root injury rating of 2.10, which exceeds economic injury level of one node of roots destroyed. Abel et al. (2000), in their investigations of fifteen experimental lines of maize, were not able to identify root feeding resistance to western corn rootworms. Ivezic et al. (2006) conducted investigations in the US and Croatia comparing Croatian commercial maize hybrids and two hybrids declared as WCR tolerant and susceptible standards. Several Croatian hybrids revealed tolerance that was comparable, or even greater, than the WCR tolerant standard hybrid. Yet, no genetically-engineered maize for corn rootworm control is registered for use in Europe. Experiments and practical experiences proved that one-year crop rotation (or two in case of high beetle numbers) is efficient enough for keeping WCR populations at low levels. Two years without maize growing is costly for farmers,

yet only marginally increases WCR population reduction.

This paper reports results of three years of field investigation in Osijek, Croatia. The objectives were to monitor WCR beetle populations and evaluate the tolerance (root injury) and associated traits (root size and root regrowth) of seven commercial maize hybrids in continuous growing of maize.

## Materials and Methods

Studies were conducted at the Agricultural Institute Osijek, Croatia (45.3°N, 18.4°E). Investigation included seven commercial maize hybrids: OsSK 444, Os 499, OsSK 552, OsSK 596, OsSK 602, OsSK 617 and OsSK 713. These hybrids are preferred by most maize producers in Croatia. The experiments were planted in 2007, 2008, and 2009 under continuous maize growing conditions in a major maize production area in Croatia with natural WCR infestations. The experimental design was a randomized complete block with four replications. Hybrids were planted in plots 6 m long with 2 seeds per hill, 25 cm between hills, and 0.70 m row spacing. Plots were thinned to one plant per hill. Conventional maize tillage systems, weed control and fertility programs were applied.

Osijek monthly precipitation totals for three consecutive years (2007 to 2009) were obtained from the Croatian meteorological and hydrological service.

Western corn rootworm populations were monitored weekly with pheromone traps (Csalomon). The traps were placed in the maize fields during early June of each year, depending on weather conditions. Traps were positioned at ear height of the maize plant, or approximately one m above the soil surface. Three traps were placed in 2007, six traps in 2008, and two traps in 2009 in the whole field where experiment was settled. WCR beetles were captured on the adhesive surface of the trap. Beetles were counted on the traps weekly. Traps

were replaced with new ones every 25 days. Each year WCR populations were monitored until the middle of October (126 days of monitoring in 2007, 129 in 2008 and 118 in 2009).

In mid July of each year, five plants from each plot were tagged for identification, and dug from the soil. The roots were cleaned with pressurized water and evaluated for three maize root traits associated with WCR native resistance (tolerance): rootworm larvae feeding injury, root size, and root regrowth. Root injury was evaluated according to the Iowa State University 0-3 Node-Injury Scale as follows: 0.00 – no feeding damage; 1.00 – one node, or the equivalent of an entire node, eaten back to within approximately 5 cm of the stalk; 2.00 – two nodes eaten; 3.00 – three or more nodes eaten. If one and ½ nodes are destroyed, the rating would be 1.50 (Oleson et al., 2005). The Eiben 1-6 Scale was reversed (Rogers et al., 1975), and used to rate root size and regrowth. On the reversed scales a rating of 1 indicated a large root system or well developed compensatory roots, and a rating of 6 indicated a small root system or poorly developed compensatory roots. Plots were harvested each year in October. The statistical analysis was performed using SAS/STAT (SAS Institute Inc., 2000). The general linear models procedure was used to perform analysis of variance (ANOVA) of root injury, root regrowth, and root size for each year. Means were tested by least significant difference test (LSD) ( $P < 0.05$ ).

## Results and Discussion

The average number of beetles captured per trap per day over the entire monitoring period provides information of adult rootworm beetle activity. Many authors compared number of captured beetles over the season to root injury. WCR captures as high as five beetles per visual (yellow sticky) trap per day have been proposed as a threshold predicting economic rootworm injury (Hein and Tollefson, 1985; Kuhar and Youngman, 1998; O'Neal et al., 2001 and Rondon and Gray, 2003).

Furthermore, it is proposed to consider one beetle per maize plant as an economic rootworm larval injury threshold. No economic injury threshold was determined for captures on pheromone traps. Few studies compared captures on yellow sticky and pheromone traps (Hesler and Sutter, 1993 and Toth et al., 2003). Pheromone traps are more sensible and catch more beetles than yellow sticky traps. Toth (2005) report on some locations over 200% more captured beetles on pheromone traps compared to yellow sticky traps. The lowest capture of WCR beetles was recorded in 2007 with an average of 2.60 beetles per trap per day (Table 1). In 2008, the greatest adult activity and captures of 9.60 beetles per trap per day were detected. Pest population's level was smaller in 2009 when compared to year 2008, whereas averages of 6.00 beetles were captured per trap per day. Ivezich et al. (2009a) reported varying monitoring results over a four year period (2002 – 2005) in Osijek, when more beetles were captured compared to the years in this investigation.

**Table 1**  
**Captures of western corn rootworm beetles on pheromone traps in the period of 2007 – 2009**

	2007	2008	2009
Total WCR number	976	7421	1422
WCR/trap/day*	2.60	9.60	6.00

\*Calculated on the base of total WCR capture per number of traps placed in experimental plots and number of days monitored

Monthly precipitation was the greatest throughout the growing season in 2008. An unusually wet period was recorded at the beginning of the maize growing season for that year (Table 2). In July 2008, when the root traits were evaluated, nearly 80 mm of rain fell. The amount of precipitation in 2008 can be considered as favorable for all maize hybrids to compensate for root injury by regrowing new roots. In 2007 and 2009 in the period of

**Table 2**  
**Monthly precipitation (mm) for Osijek (Croatia) in the period of 2007 – 2009**

	2007	2008	2009
March	75.70	85.20	26.50
April	0.70	49.90	18.70
May	48.50	66.90	39.40
June	60.60	76.30	62.80
July	31.70	79.30	13.80
August	89.00	46.20	60.60
September	71.20	86.30	10.00
October	97.70	29.80	55.30
Total	475.10	519.90	287.10

May and June, when the most larval injury occurs, the amount of precipitation was similar (102 and 109 mm, respectively), and about 40 mm less than it was measured in the same period 2008. Year 2009 was considered as dry since the amount of precipitation was below 30 years average and differed among the years of investigation by having the lowest rainfall. Several factors are important in determining maize response to corn rootworm injury. Some authors (Branson et al., 1980 and Chiang et al., 1980) suggest more severe grain yield losses under drought conditions.

**Table 3**  
**Grain yields ( $t\ ha^{-1}$ ) of seven hybrids in the period of 2007 - 2009**

	2007	2008	2009	Mean
OsSK 444	9.00	7.37	8.94	8.44
Os 499	8.85	12.99	11.38	11.07
OsSK 552	11.81	12.64	10.71	11.72
OsSK 596	9.49	12.7	11.91	11.37
OsSK 602	12.03	15.18	13.48	13.56
OsSK 617	9.62	11.89	7.58	9.7
OsSK 713	10.37	12.71	12.73	11.94

All hybrids, in all years, produced acceptable grain yields. The greatest yields were achieved in 2008 for all hybrids except OsSK 444 (Table 3), which suggests favorable growing season. The influence of rootworm larval feeding on grain yields can vary considerably. Chiang et al. (1980) reported 40 to 50% yield losses caused by WCR attack. Hybrid OsSK 602 proved to be able to produce the highest yield in all years, indicating this hybrid as a tolerant to different agronomic and environmental conditions.

Significant differences between the hybrids were observed for all tolerance traits in all years of investigation (Table 4). Root injury ratings increased over the years of growing maize continuously. A significant difference was observed in 2007 for root injury (df=6; F=6.03; P<0.0001), but the injury was low with mean root injury ratings of 0.58 for all hybrids. In the following years mean root injury ratings for all hybrids were significantly different (in 2008: df=6; F=2.97; P=0.009; in 2009: df=6; F=1.32; P=0.254) and more severe (1.70 and 2.04, respectively), indicating all hybrids to become more susceptible in every subsequent year of continuous maize. Compared to the first year experiment, more damaged roots in second year

(2008) for all hybrids can be related to a higher pest population density. However, in 2009 root injury increased but WCR population density was smaller compared to the 2008. Environmental conditions were of greater significance in this investigation. Year 2008 was wet while 2009 was considered as dry. Drought conditions induce stress in plants and decrease plants ability to defend against herbivory, revealing increased susceptibility to rootworm larval injury in 2009. In 2007, a significant hybrid effect was recorded for the least damaged hybrid OsSK 617 (0.37) and the most damaged hybrid OsSK 713 (0.70). Different root injury scales have been proposed to assess rootworm damage to maize that indicates economic yield losses (Grey and Steffey, 1998). The level of root injury of one and more eaten root node is considered as economically significant (Mayo, 1986; Sutter et al., 1990 and Oleson et al., 2005). In 2007, the level of root injury was below the economic index of one destroyed root node. An economic injury index was exceeded in 2008 and 2009 for all hybrids. Significant differences were determined between the hybrids in 2008 and 2009, whereas hybrid OsSK 602 retained the smallest root-injury rating in 2008 and 2009, with rating of 1.30 and 1.83,

**Table 4**

**Root injury, regrowth and size ratings of seven maize hybrids in the period of 2007-2009**

	Root injury				Root regrowth				Root size			
	2007	2008	2009	Mean	2007	2008	2009	Mean	2007	2008	2009	Mean
OsSK 444	0.53b	1.71abc	1.88b	1.38ab	3.20b	4.70a	2.55c	3.48bc	2.30d	1.85d	2.40c	2.18a
Os 499	0.69a	1.84ab	2.16ab	1.56b	3.15b	4.45a	3.40ab	3.67bc	2.70cd	3.35bc	2.60bc	2.88b
OsSK 552	0.65b	1.42bc	1.96ab	1.33ab	4.15a	3.65b	3.84a	3.89c	3.05bc	2.95bc	3.32ab	3.10bc
OsSK 596	0.55b	2.13a	2.11ab	1.59b	2.90b	4.45a	3.30ab	3.55bc	2.65cd	4.75a	3.05abc	3.48c
OsSK 602	0.55b	1.30c	1.83b	1.24a	3.25b	1.65c	3.05bc	2.66a	3.80a	1.79d	3.05abc	2.90b
OsSK 617	0.37c	1.66bc	2.29a	1.44ab	1.80c	1.45c	3.35ab	2.20a	3.65ab	2.65c	3.75a	3.35bc
OsSK 713	0.70a	1.84ab	2.03ab	1.52ab	2.80b	3.65b	3.50ab	3.32b	3.10bc	3.50b	3.40ab	3.33bc
Mean	0.58	1.7	2.04	1.44	3.04	3.43	3.28	3.25	3.04	2.99	3.08	3.04

Values within columns followed by the same letter are not significantly different with least significant differences test (P<0.05)

respectively. This result suggests this hybrid to be tolerant or the least susceptible in continuous maize. In 2008, hybrid OsSK 596 was the most damaged in terms of root injury ratings, with an average of more than two root nodes destroyed. In 2009, root injury ratings of all hybrids were close to or above two root nodes destroyed. Yield losses at damage levels of over one eaten root node may vary, since grain yield depends on various interacting factors such as genotype yielding potential and environmental conditions (Gray and Steffey, 1998). Several authors reported yield losses of 6 to 9% with one to two eaten root nodes (Kahler et al., 1985; Sutter et al., 1990; Spike and Tollefson, 1991 and Riedell et al., 1996). But yield losses are expected to be more severe with root injury rating of one and more, and several studies confirmed losses of 20 to 30% (Godfrey et al., 1993; Davis, 1994 and Roth et al., 1995). Chiang et al. (1980) reported 40 to 50% yield losses caused by WCR attack. Godfrey et al. (1993) reported similar results of root injury in continuous maize, no yield loss in first year with less than one root-node eaten, and a 30% yield loss with 1.5 root nodes damaged in the following year. Urias-Lopez and Meinke (2001) also reported no yield losses with less than one root node eaten in the first year, and 13% yield loss with more than one root node destroyed in the second year of continuous maize.

Root regrowth ratings differed significantly among the hybrids each year (Table 4). Roots with regrowth rating of one are considered to have the best developed new compensatory roots, and six with the worst developed new roots. Simich et al. (2007) suggested root regrowth was the most reliable WCR tolerant trait due to small environmental variance. Root regrowth prevents yield reduction after larval injury (Spike and Tollefson, 1989). In 2007, root regrowth ratings were significantly different ( $df=6$ ;  $F=6.62$ ;  $P<0.0001$ ) and ranged from 1.80 to 4.15. In the same year, hybrid OsSK 617 compensated for the root injury the best with an average regrowth rating 1.80. A significant difference was observed for the same hybrid as it was the

only hybrid with an average root regrowth rating below two. In 2008, a significant difference was observed ( $df=6$ ;  $F=36.35$ ;  $P<0.0001$ ) but several hybrids had ratings above four (OsSK 444, OsSK 499 and OsSK 596), and failed to develop good compensatory roots although the year had favorable precipitation conditions. Hybrids OsSK 617 and OsSK 602 developed the best compensatory roots with an average rating 1.45 and 1.65, respectively. Good results of root regrowth of hybrid OsSK 617 suggest this hybrid was able to compensate for root injury caused by rootworm larval feeding in the first and second year of continuous maize. In 2009, significant differences among the hybrids occurred ( $df=6$ ;  $F=3.63$ ;  $P=0.002$ ), but root regrowth ratings of all hybrids except OsSK 444 were above three. This result indicates all hybrids are less able to compensate for root injury in the third year of continuous maize. Grey and Steffey (1998) reported positive relationship between the large root system and grain yield, and suggest soil moisture as important factor, as well as compensation of new roots after larval injury.

Root size ratings significantly differed among the hybrids, and the difference was inconsistent over the years (Table 4). The significantly largest root system ( $df=6$ ;  $F=6.46$ ;  $P<0.0001$ ) regardless of a years was recorded for hybrid OsSK 444. Root size of this hybrid was significantly different with ratings of 2.30, 1.85 and 2.40 in 2007, 2008 and 2009, respectively. Plants with large root system are considered to be more tolerant to WCR larval injury (Riedel and Evenson, 1993).

In our study, hybrid OsSK 602 proved to show tolerant characteristics to WCR larval feeding. Regardless of the year, hybrid OsSK 602 was significantly different with the least damaged roots (an average root injury rating of 1.24) and well developed compensatory roots following larval root injury (an average root regrowth rating of 2.66). Furthermore, this was the highest yielding hybrid for all three years with an average grain yield of 13.56 t ha<sup>-1</sup>. Hybrid OsSK 617 can be considered as more tolerant among the other hybrids, since

it had low injury ratings and good compensation of new roots. However, grain yield of this hybrid was in average  $9.70 \text{ t ha}^{-1}$ , but yielding potential depends greatly on environmental and other agronomical conditions as well (Stoyanova, 2009). Further studies are needed to conclude the larval injury as main cause for the second smallest yield among the hybrids. Simich et al. (2007) reported that hybrid OsSK 617 was the most tolerant to WCR larval injury in different environments. El Khishen et al. (2009) evaluated recently developed WCR resistant maize genotypes, and confirmed lesser damage and larvae survival with resistant genotypes.

## Conclusion

Maize plants are able to develop a native resistance against rootworm larval injury. Evaluating maize hybrids for resistance and tolerance to rootworm larval injury has been conducted for many decades in US and recently in Europe (Hibbard et al., 1999; Tollefson, 2007 and Ivezich et al., 2009b). Moeser and Hibbard (2005) report conventional breeding strategies for rootworm resistance in the past had limited success. Our study demonstrates variability among maize hybrids for different WCR tolerance traits. All hybrids proved to be moderately tolerant to rootworm larval injury in the first year. Hybrids OsSK 602 and OsSK 617 developed good compensatory roots in the two years of continuous maize, therefore they can be considered as the most tolerant among investigated maize hybrids. Both hybrids belong to the maturity group FAO 600. This reveals the need for further testing in different environments, of maize cultivars belonging to this group and to prove OsSK 602 and OsSK 617 hybrids to be more tolerant to rootworm larval feeding in different conditions. Our results, and findings of other similar studies, proved that recognized tolerant or resistant hybrids may be used as sources in WCR resistance breeding programs, which should be maintained since it might provide success in developing sustainable

and agronomical acceptable WCR resistant maize cultivars.

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