

## Evaluation of corn hybrids for tolerance to corn rootworm (*Diabrotica virgifera virgifera* LeConte) larval feeding

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### Summary

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte (WCR)) is a major pest of corn in USA. The pest arrived in Croatia in 1995, and today over 250.000 ha are infested. Insecticides are regularly used to control WCR, but the cost is high, can pose environmental risks, and may become ineffective due to resistance. Growing corn that is resistant to corn rootworms would be a valuable alternative to insecticides. Nine Croatian (Institute of Agriculture, Osijek) and two Pioneer Hi-Bred Int. Inc. (Johnston, Iowa, USA) commercial corn hybrids were evaluated for WCR resistance at two locations using complete randomized block design with four replications. One location was in east Croatia (Osijek) and one was in the USA (Iowa). The hybrids' tolerance to larval feeding was rated using root injury, root regrowth and root size. Root injury was rated using the Node-Injury Scale 0-3 and evaluation of root size and root regrowth were done by scale 1-6. In an analysis of variance combined across locations, root injury didn't show any significant differences among hybrids, but root regrowth and root size were significantly different.

**Key words:** western corn rootworm, root node injury, root size, root regrowth, corn hybrids

### Introduction

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte (WCR)) is a major corn pest (Tollefson, 1998). Corn rootworms cost US growers an estimated \$ 1 billion annually in crop losses and control costs (Metcalf, 1986). More than 50% of fields under continuous corn production are treated with insecticides for control of corn rootworm. However, many fields are unnecessarily treated each year (Gray et al. 1993). Investigations on resistance to corn rootworm started 60 years ago as an alternative to insecticides, and continue today. Even in 1920' the difference in reaction to larval feeding was noticed (Bigger et al., 1938). Bigger et al., 1941 noticed that certain lines had superior tolerance to rootworms. The resistance by Owens et al. 1974, has been found due to larger root system and greater secondary root development.

Tolerance, conferred by larger root size and greater root regrowth, has been identified as the primary mechanism. A tolerant plant sustains as much feeding damage as a susceptible plant, but is able to develop and produce high grain yield regardless of the injury (Riedell and Evenson 1993). Studies in Missouri in 1997 and 1998 identified several crosses that had

significantly less damage caused by corn rootworm larvae (Hibbard *et al.* 1999). Identifying commercial hybrids with tolerance to corn rootworm would reduce the amount of insecticides that needed to be applied.

In Europe the first record of WCR was from Yugoslavia in 1992 (Baca 1994). The pest was identified in Croatia in 1995, and today is infested approximately 23.500 km<sup>2</sup> ha (Igrc-Barcic and Bažok, 2004). During 2000 near the border to Yugoslavia, some cornfields were infested with 0.37 to 0.51 beetles per plant (Ivezic *et al.* 2001). There was no visible lodging. Similar situation at the east of Croatia was in 2001 and in 2002, while in 2003, lodging was noticed at many cornfield near the border with Yugoslavia and also in Baranya County, near the border with Hungary. In Osijek there was no visible lodging yet. This pest has been discovered in several other European countries and is spreading very fast. European scientists in the infested countries are trying to discover the best control measures for their production systems.

The objectives of this research were to evaluate commercial Croatian hybrids and two standard hybrids, under natural infestations to obtain information about their tolerance to corn rootworms larval feeding in different environmental conditions.

## Material and methods

Field experiments were conducted in 2001, 2002 and 2003 at two locations. One of them was in USA (Sutherland, Iowa) and another was in Osijek, Croatia. Locations used natural rootworm infestations. The experimental design at each location was a randomized complete block with four replications. In Croatia 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. In the USA, the plots were machine planted with a 0.76 m row spacing with plants spaced approximately 23 cm apart. In Iowa the plots were two rows wide and 25 m long. Because they are the most popular hybrids grown by Croatian farmers, the following nine Croatian hybrids were selected (Institute of Agriculture, Dept. for Maize Breeding and Genetics, Osijek, Croatia), and two commercial hybrids from Pioneer, one (P 3563) with known tolerance to WCR and other (P 3573) known to be susceptible (Pioneer Hi-Bred Int. Inc. Johnston, Iowa, USA), were chosen as standards. The hybrids are as follow: 1.Os 499, 2.OsSK 444, 3.OsSK 552, 4.OsSK 596 R, 5.OsSK 602, 6.OsSK 617, 7.OsSK 644, 8.OsSK 654, 9.OsSK 659, 10.P 3563, 11.P 3573.

Five competitive plants in each plot were chosen, tagged for identification, and dug from the soil. The root systems were cleaned with pressurized water and rated for rootworm larvae feeding injury, root size and root regrowth. The root ratings were conducted on the middle of July at both locations in all years of investigation. At the Iowa location, addition experiment was done in all years of investigations. Roots were cut off and dried, so regrowth could be measured quantitatively (in grams). The measurements have been made during and after larval feeding and calculating the difference. It was compared with visual ratings by correlation.

Root injury was rated on the Iowa State University 0-3 Node-Injury Scale. The Iowa State University Node-Injury Scale is 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. Damage between complete nodes eaten is noted as the percentage of the node missing, e.g., 1.50= one and half nodes eaten and 0.25 = one quarter of node eaten (Oleson and Tollefson 2000; Nowatzki *et al.* 2000).

The Eiben 1-6 Scale, reversed, (Rogers et al. 1975) was used to rate root size. On the reversed scales a rating of 1 indicated a large root system and a rating of 6 indicated a small root system.

The root regrowth was also rating according to the Eiben 1-6 Scale, reversed, (Rogers et al. 1975). On the reversed scales a rating of 1 indicated a well developed secondary roots and a rating of 6 indicated poorly developed secondary roots (Fig.1.).

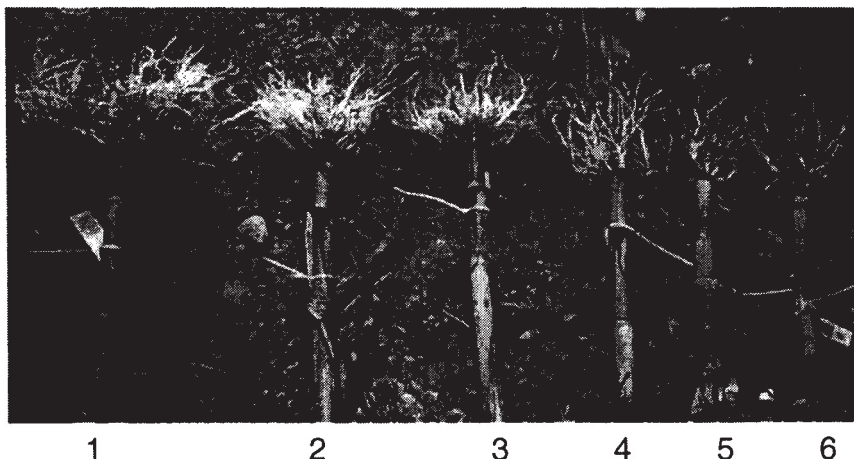


Figure 1. Root regrowth rating scale (1-6)

#### Statistical analysis

Data for root injury, root size, and secondary root regrowth rating were analyzed by location and hybrids using analysis of variance. Response variables were analyzed as least squares means, and means were separated using least significant differences (LSD) of the least squares means. At the Iowa location, linear regression was used and coefficients of determination ( $R^2$ ) calculated for relationship between secondary root regrowth rating (1-6), and roots weight (g).

#### Results and discussion

In an analysis of variance combined across locations and years, root injury wasn't significantly different, but root regrowth and root size differed significantly among hybrids (Table 1.).

Table 1. Results of combined ANOVA and LSD test

| Source of variation | df | Root injury | Root size | Root regrowth |
|---------------------|----|-------------|-----------|---------------|
| Hybrids             | 10 | n.s.        | *         | **            |
| Location            | 1  | *           | *         | n.s.          |
| Years               | 2  | *           | **        | **            |

\* $P < 0.05$ ; \*\*  $P < 0.01$ ; n.s. no significant differences.

**Root injury**

Root injury had average of 0,664 in Osijek, Croatia, and 1,242 in Iowa, USA. In Osijek locality significant differences ( $P < 0,01$ ) in root injury occurred in third year in compare to first and second year. The corn rootworm larval feeding increased in 2003 and become very similar to USA infestations. There were not any significant differences between hybrids comparing root injury.

Average values and LSD test for root injury were given in Table 2.

Table 2. Average values of root injury for hybrids, location and years with LSD test.

| Hybrids    |       | Location |       | Years   |       |
|------------|-------|----------|-------|---------|-------|
| Os 499     | 0,675 | Croatia  | 0,664 | 2001    | 0,791 |
| Os SK 444  | 1,059 | USA      | 1,242 | 2002    | 0,777 |
| Os SK 552  | 0,910 |          |       | 2003    | 1,290 |
| Os SK 596R | 0,892 |          |       |         |       |
| Os SK 602  | 0,946 |          |       |         |       |
| Os SK 617  | 0,846 |          |       |         |       |
| Os SK 644  | 1,002 |          |       |         |       |
| Os SK 654  | 1,132 |          |       |         |       |
| Os SK 659  | 0,913 |          |       |         |       |
| P 3563     | 1,050 |          |       |         |       |
| P 3573     | 1,054 |          |       |         |       |
| LSD 0,05   | n.s.  | 0,24278  |       | 0,32670 |       |
| LSD 0,01   | n.s.  | n.s.     |       | n.s.    |       |

**Root size**

Root size show significant differences between hybrids, location and years (Table 3).

Table 3. Average values of root size for hybrids, location and years with LSD test.

| Hybrids    |         | Location |       | Years   |       |
|------------|---------|----------|-------|---------|-------|
| Os 499     | 2,517   | Croatia  | 2,226 | 2001    | 2,348 |
| Os SK 444  | 2,633   | USA      | 3,219 | 2002    | 2,778 |
| Os SK 552  | 2,658   |          |       | 2003    | 3,041 |
| Os SK 596R | 2,460   |          |       |         |       |
| Os SK 602  | 2,842   |          |       |         |       |
| Os SK 617  | 2,475   |          |       |         |       |
| Os SK 644  | 3,008   |          |       |         |       |
| Os SK 654  | 2,792   |          |       |         |       |
| Os SK 659  | 2,725   |          |       |         |       |
| P 3563     | 2,683   |          |       |         |       |
| P 3573     | 3,150   |          |       |         |       |
| LSD 0,05   | 0,44948 | 0,3949   |       | 0,33917 |       |
| LSD 0,01   | n.s.    | n.s.     |       | 0,44646 |       |

Root size of all hybrids didn't show significant differences in comparing to P 3563 with known tolerance to WCR.

Os SK 596R, Os SK 617 and Os 499 showed significant differences comparing to OsSK 644 and P 3573.

### **Root regrowth**

Root regrowth is one of the main parameters for evaluation the hybrid tolerance. Plants with better-developed secondary roots are able to give high yields even after WCR larvae feeding. In this investigation, root regrowth were significantly different between hybrids and between years (Table 4.).

Some hybrids developed secondary roots very well, in both location and in all years of investigation. Those hybrids, evaluated by visual scale below 3, and better than P 3563 are: OsSK 617, OsSK 602, OsSK 596R, OsSK 654 and OsSK 659.

Hybrid OsSK 617 was significantly better ( $p < 0,01$ ) than hybrid P 3563, while five other Os hybrids (OsSK 552, OsSK 596R, OsSK 602, OsSK 654 and OsSK 659) didn't show any statistically significant differences in compare to P 3563 hybrid, which means that they are tolerant.

*Table 4.* Average values of root regrowth for hybrids, location and years with LSD test.

| Hybrids    |         | Location |       | Years   |       |
|------------|---------|----------|-------|---------|-------|
| Os 499     | 3,842   | Croatia  | 2,598 | 2001    | 3,111 |
| Os SK 444  | 2,950   | USA      | 2,950 | 2002    | 2,904 |
| Os SK 552  | 2,500   |          |       | 2003    | 2,307 |
| Os SK 596R | 2,181   |          |       |         |       |
| Os SK 602  | 2,083   |          |       |         |       |
| Os SK 617  | 1,800   |          |       |         |       |
| Os SK 644  | 4,050   |          |       |         |       |
| Os SK 654  | 2,300   |          |       |         |       |
| Os SK 659  | 2,358   |          |       |         |       |
| P 3563     | 2,475   |          |       |         |       |
| P 3573     | 3,975   |          |       |         |       |
| LSD 0,05   | 0,43559 | n.s.     |       | 0,38278 |       |
| LSD 0,01   | 0,59028 | n.s.     |       | 0,50387 |       |

In order to compare root regrow ratings by visual scale and weighting, in Iowa location the additional experiment were done. Root regrowth were measured quantitatively (in grams). Strong positive correlations ( $r=0,847^{**}$ ) were determined between weighting the roots in grams and visual scale, during all three years of investigations (Fig. 2.). Average grow of the secondary roots between the two weightings (two weeks), were 12,06g.

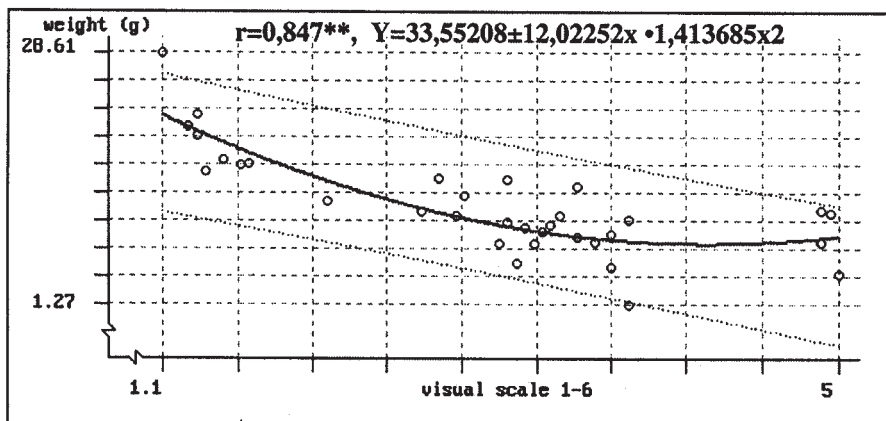


Figure 2. Correlation between root regrowth by visual scale and root weighting (g) in Iowa location

This results showed that visual scale is good enough to serve for root regrowth evaluation and it can replace the evaluation by weighting roots which means less of human work.

## Conclusions

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte (WCR)) is a major corn pest in USA, and becoming more and more threatened to Croatian and other European corn producers. Evaluation of corn hybrids for tolerance to corn rootworm (*Diabrotica virgifera virgifera* LeConte) larval feeding, in three years of investigations and two localities, showed that root root regrowth measuring is very effective tools for estimating the more tolerant hybrids. Root size measuring were also effective parameter for measuring tolerance, but root regrowth showed much more sensitivity, especially visible in statistical analyses. The results showed that some of Croatian commercial hybrids showed tolerance that was comparable, or better, than the Pioneer tolerant hybrid. Those hybrids are: OsSK 617, OsSK 602, OsSK 596R, OsSK 654, OsSK 659 and OsSK 552. Those hybrids can be recommended to farmers, in order to decrease the injury level caused by WCR. This can be valuable alternative to insecticides, and in European conditions can be combining with wide spectrum of crop rotation.

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