

## Seasonal Dispersal of the Western Corn Rootworm (*Diabrotica virgifera virgifera*) Adults in Bt and non-Bt Maize Fields

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### Abstract

CAGÁŇ L., ROSCA I. (2012): **Seasonal dispersal of the Western corn rootworm (*Diabrotica virgifera virgifera*) adults in Bt and non-Bt maize fields.** Plant Protect. Sci. (Special Issue), **48**: S36–S42.

32 experimental plots were sown at Borovce (district Trnava) in Slovakia during 2008 with 8 maize hybrids, including coleopteran-protected MON 88017, the lepidopteran-protected MON 89034, the stacked product, MON 89034 × MON 88017, that same hybrids isolate that did not have the Bt trait or traits, and 4 commercial hybrids. Part of the fourth repetition was unexpectedly infested and damaged with the Western corn rootworm (WCR). High numbers of the WCR adults were found in the damaged plots on July 8. Adult WCR abundance remained concentrated in this area until August 12–19. The beetles apparently began to disperse during August 19–September 2, and after September 2. The trap captures increased in the uninfested areas up to September 16, no significant difference having been found in the adult WCR captures between the infested and uninfested plots, including the plots with MON 88017. Bt-maize hybrids containing MON 88017 strongly influenced the level of the WCR damage as assessed by lodging of the maize plants, and they also influenced the numbers of the WCR adults before maize flowering. We found that intensive movement of the WCR adults from the place of their origin started later in the vegetation season.

**Key words:** Cry3Bb1; yellow sticky traps; Chrysomelidae; adult dispersal

Genetically modified maize was developed for the regulation of the Western corn rootworm (WCR) (*Diabrotica virgifera virgifera* LeConte). The first transgenic corn hybrids expressing the *Bacillus thuringiensis* (Bt) Cry3Bb1 protein to control corn rootworm (*Diabrotica* spp.) larvae were registered for the commercial use in 2003 (RICE 2003). Cry3Bb1 protein does not persist or accumulate in soil and is degraded rapidly (ICOZ & STOTZKY 2008). At present, many genetically modified *Diabrotica*-active Bt maize transforma-

tion events are grown commercially worldwide (DEVOS *et al.* 2012).

Despite the substantial amount of research already performed (e.g. AL-DEEB & WILDE 2005; NOWATZKI *et al.* 2006; MARQUARDT & KRUPKE 2009) there are still many open questions on how Bt-maize hybrids influence the bionomy of the WCR. It seems clear that Bt maize hybrids at least decrease the larval development (OYEDIRAN *et al.* 2007) or damage by larvae (AL-DEEB & WILDE 2005).

Supported by the Slovak Grant Agency of the Ministry of Education and Slovak Academy of Science – VEGA, Grant No. 1/0894/11.

In this case, an unexpected WCR infestation occurred in a part of trial including the plots with both conventional maize hybrids and Bt maize that suppresses the feeding by WCR larvae. This situation offered an opportunity to investigate the movement of WCR adults during the season. The aim of this study was to determine, if Bt maize containing the Bt WCR trait is a barrier for the WCR adult movement.

## MATERIAL AND METHODS

The field trial was initiated at Borovce, Slovakia (48°34.831'N, 17°43.302'E) in 2008. The trial included 8 maize hybrids (MON 89034 × MON 88017, MON 89034, MON 88017, Conventional Parental (that same hybrid isolate that did not have the Bt trait or traits)– DKC 5143, Conventional hybrid 1 – CISKO, Conventional hybrid 2 – FORTIUS, Conventional hybrid 3 – PR36D79, Conventional hybrid 4 – MERIDIEN) randomly arranged in 4 repetitions. The plot size was 33.6 m (48 rows at 70 cm) × 30 m (Table 1). Three repetitions were sown in plots with a preceding crop other than maize and a portion of one repetition was sown in plots with maize as the preceding crop.

An unexpected infestation of the Western corn rootworm (WCR) occurred in a portion of replicate four where maize had been planted in the previous year. WCR abundance (based on the WCR adult captures) was monitored in each plot throughout the study area nine times over the season to evaluate the adult beetle movement patterns. WCR density was monitored nine times between June and September

2008 using Pherocon<sup>®</sup> AM yellow sticky traps (Great Lakes IPM, Inc., Vestaburg, USA). The WCR amount was collected using yellow sticky traps. Three traps were placed per plot in rows 18, 27, and 35 of each plot. The sticky traps were placed approximately halfway into the plot. They were collected after seven days and the number of trapped individuals was counted. The collection dates included June 10, June 24, July 8, July 22, July 29, August 5, August 19, September 2, and September 16.

The differences between the numbers of the WCR beetles collected in yellow sticky traps were calculated by one-way analysis of variance (Table 2). Simple linear regression was calculated to explain the correlation between the numbers of the WCR adults at different dates (Table 3).

## RESULTS

The results show that high numbers of the WCR adults were found in plots without the coleopteran-protected trait, MON 88017, where the preceding crop had been maize. Within the infested portion of repetition four, significantly fewer WCR adults were captured in the treatments containing MON 88017 during the period of adult emergence (Table 1). Plant lodging observations were used as an indication of the WCR larval feeding. The hybrids containing MON 88017 (plots 404 and 406) exhibited no lodging while the remaining treatments in the infested area revealed 25.3, 57.7, and 68.8% lodged plants for plots with non-Bt maize hybrids, respectively (Table 1).

Table 1. Experimental design of the plots

1.3 ± 1.2	6.0 ± 4.6	4.7 ± 2.1	6.3 ± 4.5
3.3 ± 4.2	5.7 ± 4.2	4.3 ± 5.1	2.3 ± 2.1
2.7 ± 2.5	5.3 ± 3.8	4.3 ± 4.5	5.0 ± 3.6
2.7 ± 0.6	1.3 ± 1.5	2.7 ± 2.5	4.7 ± 2.1
3.3 ± 3.2	8.3 ± 3.8	7.3 ± 2.1	84.7 ± 33.3
5.3 ± 1.5	5.0 ± 1.0	9.0 ± 6.6	12.3 ± 1.2
4.7 ± 3.1	5.7 ± 3.2	9.3 ± 2.9	161.7 ± 54.0
12.7 ± 3.8	12.3 ± 5.9	19.3 ± 9.5	124.3 ± 29.0

Number of the WCR adults in yellow sticky traps at locality Borovce (Slovakia) during July 8, 2008. The date inform about the time when traps were collected after 7 days of their installation in plot. At each plot there were installed three traps. Numbers show average number of the WCR adults in one yellow sticky trap and standard deviation. Grey plots show WCR-resistant maize genotypes (MON 88017). Thick lines indicate plots with maize as preceding crop heavily infested with WCR larvae

Table 2. Average number of the WCR adults per one plot calculated from number of adults collected in three yellow sticky traps after 7 days of their installation

Date of trap installation–collection	Growth stage		No of plots					P-value
			3	3	4	5	17	
			x1	x2bt	x2	x3bt	x3	
June 17–24	V4	$\bar{x}$	38.67 <sup>b</sup>	13.33 <sup>a</sup>	8.50 <sup>a</sup>	7.20 <sup>a</sup>	7.12 <sup>a</sup>	0.0001
		SD	17.84	4.93	3.70	4.87	2.89	
July 1–8	V9–V10	$\bar{x}$	370.67 <sup>b</sup>	26.33 <sup>a</sup>	28.75 <sup>a</sup>	9.20 <sup>a</sup>	17.18 <sup>a</sup>	0.0001
		SD	229.34	11.59	11.59	5.72	8.78	
July 15–22	V18–VT	$\bar{x}$	411.00 <sup>b</sup>	26.00 <sup>a</sup>	26.75 <sup>a</sup>	9.80 <sup>a</sup>	16.65 <sup>a</sup>	0.0001
		SD	252.93	18.08	20.32	5.36	10.05	
July 22–29	VT–R1	$\bar{x}$	349.67 <sup>b</sup>	117.33 <sup>a</sup>	80.00 <sup>a</sup>	34.80 <sup>a</sup>	44.82 <sup>a</sup>	0.0001
		SD	174.06	69.76	38.13	14.27	29.35	
July 29–August 5	R1–R2	$\bar{x}$	264.67 <sup>b</sup>	60.33 <sup>a</sup>	43.75 <sup>a</sup>	39.20 <sup>a</sup>	37.88 <sup>a</sup>	0.0001
		SD	153.30	42.19	19.82	11.39	9.47	
August 12–19	R3–R4	$\bar{x}$	97.00 <sup>b</sup>	58.00 <sup>ab</sup>	41.25 <sup>a</sup>	24.60 <sup>a</sup>	29.41 <sup>a</sup>	0.0007
		SD	53.81	40.71	13.33	5.90	12.69	
August 26–September 2	R5	$\bar{x}$	42.33 <sup>a</sup>	33.33 <sup>a</sup>	46.25 <sup>a</sup>	19.00 <sup>a</sup>	37.12 <sup>a</sup>	0.1289
		SD	19.83	21.94	13.05	3.16	14.81	
September 9–16	R6	$\bar{x}$	8.67 <sup>a</sup>	20.67 <sup>a</sup>	25.00 <sup>a</sup>	18.00 <sup>a</sup>	16.24 <sup>a</sup>	0.0744
		SD	8.38	1.15	15.34	6.89	5.12	

Means in succeeding rows followed by the same letter are not significantly different (ANOVA Tukey test  $P > 0.05$ ); growth stage – growth stage on the date of trap installation;  $\bar{x}$  – average; SD – standard deviation; x1 – non-Bt maize plots attacked by WCR; x2bt – Bt maize plots directly connected to attacked plots; x2 – non-Bt maize plots directly connected to attacked plots; x3bt – Bt maize plots not directly connected to attacked plots; x3 – non-Bt maize plots not directly connected to attacked plots

After the emergence, the adult beetles generally remained in the area of emergence and there were significant differences between plots with MON 88017 hybrids and the other plots (Table 1). Later in the season, in August and September, the WCR adults began to disperse from the area of infestation. The trap captures increased in the uninfested areas until September 16 when there was MON 88017 single and no significant difference in the adult WCR captures between the infested and uninfested plots, including the plots with the stack (Tables 2 and 3).

Table 2 shows that the first WCR adults were collected in the yellow sticky traps before June 24. The numbers of the adults collected in the yellow sticky traps indicated that there was a movement of the WCR adults immediately after their emergence, the WCR adults having been found on plots which were more than 100 m away from their assumed origin. Table 3 shows the plots which were the source of the WCR adults. The same table also shows that the

emergence of further WCR adults continued during June 24–July 1, but the movement of the adults was significantly retarded during June 24–July 8. It is also clear that the emergence of the WCR adults finished in the period of July 1–8, because there was no difference between the captures during this period and the next captures (Table 2).

Table 3 shows the correlation coefficients between the numbers of the WCR adults on different dates. The correlation coefficients indicate the changes in the population of the WCR adults in each plot. It is obvious from the table that the captures of the WCR adults in the yellow sticky traps almost had not changed from July 1–8 until July 22–29. These data confirmed that during July 8–29, the WCR adults did not move very much from the place of their emergence.

A very small change of adult captures was found during July 22–29 and July 29–August 5 (a strong significant correlation between the dates was main-

Table 3. Correlation coefficients among numbers of *D. virgifera virgifera* adults in 32 plots (as a sum collected from 3 yellow sticky traps) in different dates at the locality Borovce (Slovakia) 2008

	July 1–8	July 15–22	July 22–29	July 29– August 5	August 12–19	August 26– September 2	September 9–16
June 17–24	0.901	0.912	0.920	0.896	0.672	0.156	–0.245
<i>P</i> -value	0.0000	0.0000	0.0000	0.0000	0.0000	0.3929	0.1758
July 1–8	–	0.999	0.919	0.848	0.556	0.126	–0.424
<i>P</i> -value	–	0.0000	0.0000	0.000	0.0010	0.4911	0.0156
July 15–22	–	–	0.928	0.870	0.584	0.141	–0.415
<i>P</i> -value	–	–	0.0000	0.0000	0.0005	0.4405	0.0183
July 22–29	–	–	–	0.911	0.763	0.348	–0.334
<i>P</i> -value	–	–	–	0.0000	0.0000	0.0513	0.062
July 29–August 5	–	–	–	–	0.845	0.369	–0.266
<i>P</i> -value	–	–	–	–	0.0000	0.0376	0.1415
August 12–19	–	–	–	–	–	0.589	–0.076
<i>P</i> -value	–	–	–	–	–	0.0004	0.6791
August 26– September 2	–	–	–	–	–	–	–0.078
<i>P</i> -value	–	–	–	–	–	–	0.6705

For field plan see Table 1

tained) (Table 3). It means that there was hardly any movement of the WCR adults in the maize field during July 22 and August 5.

There was significant correlation between the numbers of the WCR adults during July 29–August 5 and August 12–19 (Table 3). It means that during August 5–August 19 the WCR adults almost did not disperse in the maize field.

During August 26–September 2 the distribution of the WCR adults in the maize field did not correlate (no significant relation) with that in former periods (Table 3). Thus, the migration of the WCR adults was very strong during August 19–September 2.

The distribution of the WCR beetles in the yellow sticky traps changed significantly when comparing the dates August 26–September 2 and September 9–16. It means that the disperse of adults was very strong after September 2. The negative correlations between the numbers of the collected adults just show that beetles moved from the destroyed plots to those well developed.

During the time of the trap installation there were determined the maize growth stages according to RITCHIE *et al.* (1993). The flowering started during the week July 22–July 29 and it was finished during July 29–August 5.

## DISCUSSION

Our results confirmed the efficacy of the Bt maize against the WCR larvae which had been already proven by AL-DEEB and WILDE (2005) or HIBBARD *et al.* (2009), who demonstrated that Bt maize (MON 863) expressing the Cry3Bb1 toxin was extremely effective in reducing the damage caused by the WCR larvae, and it also influenced the WCR larval development (CLARK *et al.* 2006).

The plant damage and number of adults found were the significantly different on susceptible maize hybrids and on the hybrids containing MON 88017. We suppose that the differences between the susceptible hybrids were caused by the level of the soil infection by the pest eggs and subsequently by the number of hatched larvae rather than by the differences between the hybrid susceptibility. Similarly, HIBBARD *et al.* (2009) mentioned that the plant damage, the number of western corn rootworm larvae recovered, and the adult emergence from MON 863 did not significantly differ between egg hatching times from widely varying phenologies.

The suppression of WCR by MON 88017 was succeeded by a low adult emergence and the lack of plant lodging in MON 88017 and MON 89034 ×

MON 88017 plots. Additionally, a similar occurrence of the WCR adult beetles at the end of the season in all plots, including the treatments with MON 88017, supports the previous findings that the maize plants producing the Cry3Bb1 protein do not act as a deterrent against WCR adults (AL-DEEB & WILDE 2005; NOWATZKI *et al.* 2006). Even later experiments in laboratory conditions (MEISSLE *et al.* 2011) discovered that male, but not female, survival was reduced in the Bt-leaf treatment as compared with the control, or that the female weight was lower when fed Bt maize. The authors concluded that direct effects of Cry3Bb1 on adult beetles could explain the observed effects, however, varietal differences between Bt and control maize are also possible and the impact of Bt maize on the adult populations is likely to be limited.

There was a movement of the WCR adults in the maize field immediately after their emergence in June, as there was hardly any movement of the WCR adults in the maize field from July 8 to August 19 while the disperse of the WCR adults was very strong in the period of August 19–September 2 and later on.

The information on the mating behaviour of the WCR partially explains our results. The results of MARQUARDT and KRUPKE (2009) indicate that females do not move far from the site of emergence before mating and do not mate within the first four hours of their emergence, but their later behaviour is not defined. Significant numbers of males are sexually mature and available to mate with females as they emerge (QUIRING & TIMMINS 1990; SPENCER *et al.* 2009).

There are many pieces of information on the influence of the maize growth stage on the seasonal movement of the WCR adults. The populations of beetles in the fields peaked while corn was flowering, but the capture in the malaise traps remained low until silks had dried. The capture of the WCR then increased and remained high through the season. The malaise traps were constructed to permit separate collections of beetles that entered on either of the two opposite sides (LANCE *et al.* 1989). The authors wanted to say that the movement of the WCR adults was low during silking. The directional movements of beetles towards pollinating corn and the associated semiochemicals could be inferred from both 1994 and 1995 data analyses (DARNELL *et al.* 2000). It was found (PIERCE & GRAY 2006), that the adult captures increased in soybean plots after the maize

developed past the maize R2 stage of development (RITCHIE *et al.* 1993). Thus, it was later in the maize vegetation season, which partially agrees with our information.

There was also substantial emigration from the early-planted field while most of the beetles in the late-planted field were immigrants and the net seasonal emigration out of the early planted maize towards the late-planted maize was about 62% (NARANJO 1991). This was not the case with our field, because all plots were sown at the same time and the hybrids had the same phenology during the season.

It is not easy to determine how the weather conditions influence the movement of the WCR adults. It was found that at temperatures above 30°C, the adults were hidden at the base of plants, among the adventive roots, under lump soil from the superficial layer, and under husk leavies. Also lower temperatures (below 20°C) negatively influence the adults' flight and their feeding (IOANA & MARGHITAS 2003). The flight activity of the WCR adults occurred in the 2–3 h periods after sunrise and before sunset. Flight activity peaks were further characterised by a temperature range of 22.2°C to 27.0°C (WITKOWSKI *et al.* 1975). Such temperatures were usual at the observed locality, which suggests that the local climatic conditions should not significantly influence the capture of the WCR adults in the traps.

We came to the question which is better for the WCR future population development – to mate and to lay the eggs in the place of emergence, or to mate and afterwards to try to find new areas for the development of the new population? We found that the intensive movement of the WCR adults from the place of their origin started later in the vegetation season. The field studies in Virginia (KUHAR & YOUNGMAN 1998) showed that the adult population estimates obtained in the calendar week 33 (mid-August) revealed the highest correlations with subsequent root damage. These results indicate that the WCR females laid the highest numbers of eggs later in the season, similarly as found during our study. Pherocon AM trapping and visual counting are two commonly used methods for identifying the maize fields in the USA that contain the number of adult WCR sufficient to cause economic root damage by larvae in the following year. The most appropriate period for sampling was during the first half of August (BAŽOK *et al.* 2011). According to our results,

during the first half of August almost the whole WCR population did not move from its place of emergence, and the real migration to new plots (fields) started after August 19. Now, the question arises, when do the females lay their eggs? And why do they wait so long for migration? If the bulk of the WCR adults move after August 19, then it is necessary to consider that they will find new areas for the egg laying. And probably it is important to calculate the relation between the late population of the WCR and the damage caused by the larvae in the following year.

The distribution of the WCR adults in our plots (based on the captures from the yellow sticky traps) at the end of the maize vegetation season was equable. This confirms the formerly achieved data that approximately the same density of WCR adults was recorded along the edges of the maize fields as that recorded in the centres of those fields. Pherocon AM non-baited yellow sticky traps in (IGRC BARČIĆ *et al.* 2007). WCR adult habitat management cannot be suggested as a means of preventing the beetles initial dispersal because their movement was usually non-directional, and alternative food plants were used prior to reaching maize. Even the beetle movements were slightly correlated with the wind direction (TOEPFER *et al.* 2006).

MATIN and YULE (1984) published an observation which seems similar to our findings: The spatial distribution of adult *Diabrotica longicornis barberi* Smith and Lawrence in a Quebec cornfield varied with the host phenology. The initial distribution in the second half of July was “contagious”; the distribution was random during the first 2 weeks of August when the crop was in full bloom. The distribution was again “contagious” when most of the corn silks dried up after mid-August.

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Received for publication November 1, 2012

Accepted after corrections December 7, 2012

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