

INTEGRATED PEST MANAGEMENT

Food and oviposition preferences of *Diabrotica v. virgifera* in multiple-choice crop habitat situations

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Abstract

Multiple-choice field cage experiments were used to clarify to what extent adults of one of the most destructive maize pest, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera:

Chrysomelidae), use non-maize crop habitats as alternative food sources or oviposition sites in situations of non-maize/maize rotations. Between 2009 and 2012, *D. v. virgifera* adults were released into large walk-in gauze cages each containing different combinations of three out of ten crop habitats in southern Hungary. Maize was planted the following year, allowing the development of larvae and the emergence of adults that were then captured in small cages. Results indicate that the polyphagous nature of *D. v. virgifera* adults is, under field situations, not as important as often stated. The generational, *i.e.* annual growth rate of populations, an indicator of crop habitat quality for food and oviposition, appeared highest when the entire multiple-choice cage had been planted with maize (populations nearly doubled). When Sudan grass and *Sorghum* millet were combined with maize, a slight population growth was still possible. When maize was combined with any other habitat type, populations decreased from year to year, suggesting that non-maize crop habitats are suboptimal, and their role as alternative food sources under field conditions might be overestimated. As for oviposition, also maize was found to be the most attractive. Of medium proportional attractiveness for oviposition were *Sorghum* millet, Sudan grass, and ploughed bare soil. Harvested and grubbed winter rape with some regrowth, harvested and grubbed or not grubbed winter-wheat with regrowth, and potatoes were comparatively less attractive for oviposition, suggesting that regrowth or volunteer crops play no role for *D. v. virgifera*. Least suitable were harvested and grubbed peas and soybean. The presence of particular weed species had no detectable influence on oviposition, but vegetation coverage of crops and/or weeds positively influenced oviposition. In conclusion, there is no indication that the rotation-tolerant *D. v. virgifera* strain had been introduced from the USA. Crop rotation, no matter what crop combination in an agricultural area, remains an effective control measure for *D. v. virgifera* populations in Europe, and should be communicated as such by regional or country wide decision makers in agri-policy.

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Introduction

Multiple-choice experiments are powerful tools in insect ecology and behavioral sciences (Dent & Walton, 1998; Krebs, 1999). The multiple-choice technique is mainly applied in controlled environmental setups in the laboratory, for example to assess foraging behaviors (Ball, 1982) and feeding preferences of pest insects (Jalali *et al.*, 1988) or host preferences of biological control agents (Minot & Leonard, 1976; Alphen *et al.*, 1982), and even for studies about tri-trophic interactions (Jansson & Lashomb, 1988; Derridj *et al.*, 1989; Easwaramoorthy *et al.*, 1992; Micha *et al.*, 2000). Some researchers

also use multiple-choice experiments in the field, particularly to assess host specificity and thus safety of potential weed biological control agents (Hinz, 1998).

Here, we use this powerful technique under field conditions to determine the importance of host and supposed non-host habitats for one of the major North American and European pests of maize, *Zea mays* L. This is the western corn rootworm, *Diabrotica virgifera* ssp. *virgifera* LeConte (Coleoptera: Chrysomelidae). This pest is hypothesized to have originated in Mexico, where several pestiferous *Diabrotica* species occur (Branson & Krysan, 1981; Krysan & Smith, 1987). However, with the expansion of maize growing areas in the 20th century, *D. v. virgifera* became a major pest of maize in large areas of North America (Krysan & Miller, 1986; Levine & Oloumi-Sadeghi, 1991). *Diabrotica v. virgifera* was accidentally introduced from North America into Europe on several occasions between the 1980s and the early 2000s (Miller *et al.*, 2005; Szalai *et al.*, 2011). It is now a threat to maize production in many European countries (Kiss *et al.*, 2005). Should all maize production regions in the 27 European Union member states become infested, the potential losses are estimated to be as high as 580 million USD per year (Wessler & Fall, 2010).

Diabrotica v. virgifera is a univoltine species which overwinters as eggs in the soil (Krysan and Miller, 1986). After the maize has germinated, the eggs hatch and the three larval instars feed almost exclusively on maize roots (Moeser & Hibbard, 2005a). Feeding damage on maize roots often causes plant lodging and economically significant yield losses. Adults emerge between mid-June and early August in Central Europe (Hemerik *et al.*, 2004; Toepfer & Kuhlmann, 2006), and can occasionally further reduce yields through intensive silk feeding, which interferes with maize pollination (Chiang, 1973). In contrast to the larvae, adults use alternative food sources, particularly pollen from weeds or other crops (Moeser *et al.*, 2006, 2007). However, also for adults, maize seems to be the preferred source of food, although hybrids may differ in their attractiveness (Reissig & Wilde, 1971; Spencer *et al.*, 2009). From laboratory studies, it is obvious that the diet of adults influences their fecundity and fertility (Mabry *et al.*, 2004). Adults live several months and can lay eggs continuously (Branson & Johnson, 1973; Hill, 1975) depending on food availability. How relevant food uptake from non-maize sources is for *D. v. virgifera* oviposition and population growth has not been assessed under field conditions.

Adults of *D. v. virgifera* are known to primarily lay their eggs into the soil of maize fields (Spencer *et al.*, 2009). The behavioral sequence in the choice of certain oviposition habitats is, however, not entirely understood. Hibbard *et al.* (1994) concluded from olfactorial bioassays that adults can recognize a maize field. Lance (1992) reported that maize root odor, CO₂ emission from the soil, soil bacterial odors and soil moisture might be cues for *D. v. virgifera* females to select oviposition sites. Kirk (1981) determined that cracks and holes in the maize field surface are crucial for oviposition. The maize plant itself, particularly the stem base, seemed to be less important.

In any case, the adult oviposition preference for maize, and the restriction of larval feeding to maize roots make crop rotation the most powerful management option against this pest (Glass, 2011). Crop rotation is largely successful in Europe, not only for the management of established populations, but also for eradication and containment purposes (Lipa, 2004; Reynaud *et al.*, 2005; Furlan, 2008; Glass, 2011). In the USA, some populations, however, have evolved to more frequently lay their eggs in non-maize crops where maize is grown the following year which allows larval development and adult emergence (Gray *et al.*, 1998; Levine *et al.*, 2002). The evolution of this behavioral resistance is hypothesized to be a result in loss of fidelity to maize rather than any particular attractivity of another crop (Knolhoff *et al.*, 2006). Whether such populations have been introduced into Europe during the multiple introductions of this species is unknown. Other studies from Europe as well as from North America suggest that it belongs to the natural survival strategy of *D. v.*

virgifera, to oviposit to some extent into non-maize crops (Barcic *et al.*, 2007; Spencer *et al.*, 2008). In terms of pest management, this might be of importance for small holder maize production (Barcic *et al.*, 2012).

Therefore, the feeding and oviposition behaviour of *D. v. virgifera* adults was studied in large multiple-choice field cages at two field sites under European cropping conditions. Between 9 and 23 large gauze cages with female and male adults were placed into each study field, with each cage covering three different crop habitats. This allowed *D. v. virgifera* adults to feed on maize and other crops, as well as to oviposit into different habitats. The importance of this feeding was assessed by recording the intensity of annual population growth depending on the previous year's habitat combination. Oviposition was assessed by measuring the adult emergence from maize that was grown over the areas where different crop habitats had been grown the previous year. Results will confirm crop rotation as a major pest management tool for this species, or suggest adaptations in the advice for certain crop rotation schemes. This is particularly helpful for regional or country wide decision makers in agri-policy as well as for developers of integrated pest management guidelines.

Materials and Methods

Experimental setup

The oviposition preference of *D. v. virgifera* adults was studied in field cages at two field sites in southern Hungary in 2009, 2010 and 2011, referred to as site A and B (Table 1). Both sites were situated within intensively used agricultural land, primarily for maize, sunflower and winter-wheat production. Site A was of 2 hectare size and site B of 0.3 hectares. Both sites were divided into two halves, with one half planted with maize (1 and 0.15 hectare respectively). The other half was subdivided into two to three plots of 10 crop habitats differing in replicate numbers and spatial sequence from year to year (each plot 6 m wide and 25 to 50 m long). The experiments were repeated according to minimum experimentation standards suggested by Begley (2013), *i.e.* over three *D. v. virgifera* generations from 2009 to 2010, 2010 to 2011 and 2011 to 2012.

At both sites, large walk-in gauze field cages of 2×4.5×1.9 m in size (covering 9 m²) were used to create enclosures for *D. v. virgifera* to oviposit (Figure 1). The gauze was of white UV-resistant cloth with 2 mm meshes that allowed aeration, but prevented adult beetle from escape (Gardenia Ltd, Győr, Hungary). The cage frames consisted of grey PVC tubes. At site A, 23 field gauze cages were placed from 22 July until 28 August 2009, 20 cages from 22 July until 23 September 2010, and 19 cages from 13 July until 21 September 2011. At site B, 7 field gauze cages were placed from 22 July until 19 August 2009, 9 cages from 16 July until 24 September 2010 and from 9 July until 22 September 2011.

The field cages were set up across the intersections of field plots so that they covered plants from the maize crop section (about 4 m²) and from two other adjacent crops (each about 2 m²) (Figure 1). Crop combinations were assessed in 1 to 6 replicates per site and/or year (Tables 2 and 3). The crop habitats were as follows: ploughed bare soil (present next to maize and one other crop in 13 cages), potatoes (18 cages), sugar beet (4 cages), *Sorghum bicolor* (L.) Moench (24 cages), *Sorghum sudanese* (Steud.) deWet (8 cages); as well as peas (21 cages), winter rape (23 cages), winter wheat that had been harvested and grubbed, *i.e.* the vegetation cover opened with a deep-tine cultivator (19 cages); and finally winter wheat that had been only harvested, but not grubbed (24 cages). A total of 87 cages were established at the two sites over the course of the study, all of which had at least half of the area planted with maize. Six of the cages contained only maize and no other habitat type.

The approximate start of natural emergence of *D. v. virgifera* adults in the region was observed around 20 June 2009, 29 June 2010, and 20 June 2011 during visual *D. v. virgifera* surveys in highly infested fields in the region twice per week throughout the second half of June each year. The emergence period in 2009 and 2011 was in line with long year averages, the emergence in 2010 was delayed (Toepfer *et al.*, 2006). In each of these years, the peak of emergence was two to three weeks after the first detection of emergence.

In 2009 and 2011, 100 *D. v. virgifera* adults (*i.e.* 50 females and 50 males) were released onto the maize plants in each gauze cage to simulate a high adult density (equals approx. 11 adults/m² of cage or 10 adults per maize plant). All released adults were of a defined, young age to allow sufficient development time and full oviposition. This is, they were collected newly emerged from field cages of other experiments, and transferred. Or they were field-collected from natural populations

of heavy infested fields in the area latest about 14 days after emergence (timing based on measurements of adult emergence of natural populations in the region). This also means that females were still in process of developing their ovaries and eggs when released (Branson & Johnson, 1973). Adults were released into the cages on 22 July 2009 and 8 July 2011 at site A, and on 17 July 2009 and 13 July 2011 at site B. In 2010, 75 females and 75 males were released in each gauze cage on 22 July 2010 at site A, and on 16 July 2010 at site B (equals approximately 17 adults/m² cage or 16 adults per maize plant). Data from these releases were standardized to 50:50 released adults to allow for comparison between sites and years.

After release in the cages, live female and male adults were visually searched for and counted inside the cages weekly until no adults were found any more, *i.e.* until mid to end of September. To assure most adults were counted, all plants in a cage were heavily shaken so that

Table 1. Characteristics of the two study sites in Csongrad county in southern Hungary.

Location	Szekskutas			Hodmezovasarhely		
Coordinates	N 46°31.00.0 - E 20°30.25.8			N 46°25.998 - E 20°20.348		
Elevation (m)	87			83		
Soil types	heavy gleyic solonetz			heavy gleyic chernozem		
Sand content (%)	61			29		
Silt content (%)	21			35		
Clay content (%)	16			32		
Humus content (%)	2			4		
CaCO ₃ (%)	0.1			6.7		
C organic (%)	1.3			2.3		
PH (H ₂ O)	7.3			8.1		
Year	2009	2010	2011	2009	2010	2011
Mean weed cover per plot in August (%)	17±5.1	13±13.5	18±3.5	38±1.9	13±8.3	35±8.8
Mean daily air temperature May (C)	17	17	17	17	17	17
Mean daily air temperature June (C)	20 _{max 27}	21 _{max 29}	21 _{max 28}	20 _{max 27}	21 _{max 29}	21 _{max 28}
Mean daily air temperature July (C)	23 _{max 35}	24 _{max 32}	22 _{max 35}	24 _{max 36}	24 _{max 33}	22 _{max 36}
Mean daily air temperature August (C)	23 _{max 33}	23 _{max 33}	22 _{max 32}	23 _{max 33}	23 _{max 33}	22 _{max 33}
Sum rainfall May (mm)	6	16	60	6	20	67
Sum rainfall June (mm)	34	3	21	34	3	21
Sum rainfall July (mm)	19	20	30	19	22	34
Sum rainfall August (mm)	20	15	2	22	15	4

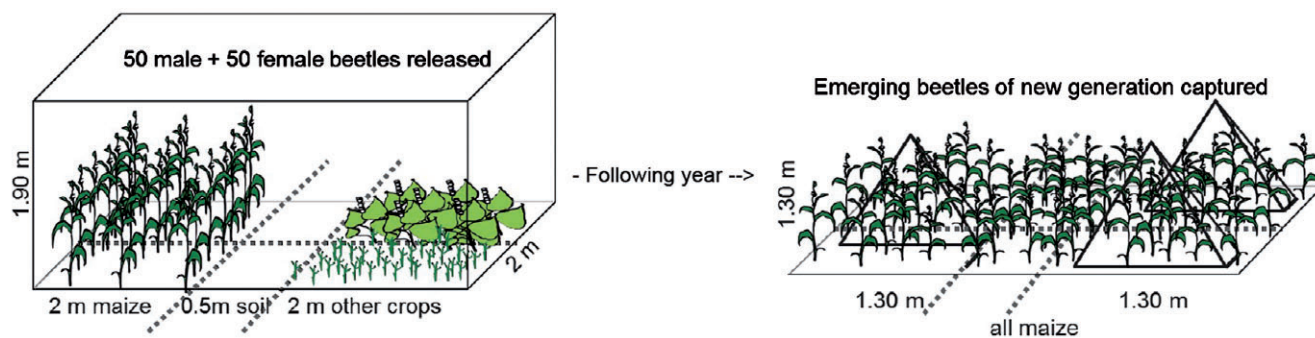


Figure 1. Multiple-choice walk-in gauze cages with released and ovipositing adult *Diabrotica v. virgifera*. Oviposition assessed by recording adult emergence from three small emergence cages placed over maize grown the subsequent year.

adults fell to the ground. They subsequently crawled up plants and the gauze cloths of the cage and were easily counted.

In mid-August, crop related factors were visually estimated and recorded as follows: maize plant density (plants per m²), crop phenology, approximate percent vegetation cover of crops and weeds, weed species numbers, and weed flowering status. The most common weeds were

Abutilon theophrasti Medik., *Amaranthus blitoides* S.Wats., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., *Datura stramonium* L., *Hibiscus trionum* L., *Malva spp.*, *Lamium spp.*, *Setaria glauca* (L.) P.Beauv., *Sonchus arvensis* L., *Sorghum halepense* (L.) Pers., and *Xanthium orientale* L. Moreover, maximum, minimum and mean daily

Table 2. Average adult *Diabrotica v. virgifera* emergence per m² of maize due to oviposition into different pre-crops the previous year. Each large multiple-choice gauze cage with female and male adults the previous year covered three of 10 different crop habitats in different combinations in two field sites in southern Hungary in July and August 2009, 2010, and 2011. Oviposition assessed by recording adult emergence from three small emergence cages placed over maize grown the subsequent years, i.e. in 2010, 2011, and 2012. Data standardized to 11 released adults per m² the corresponding previous years.

Site	Y1	Y2	Emergence of adult <i>D. v. virgifera</i> per m ² maize due to oviposition into different pre-crops the previous year																													
			Maize			Sorghum Millet			Sorghum Sudan grass			Ploughed bare soil			Peas harvested, grubbed*			Rape harvested, grubbed*			Wheat harvested, non-grubbed*			Wheat harvested, grubbed*			Potatoes			Soybean		
			M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N	M	SD	N
A	2009	2010	8.6	15.1	13	1.9	1.6	4				1.6	1.9	6	0.6	1.6	6	0.3	0.8	6	0.3	0.8	6	0.6	1.0	6	1.2	1.7	5			
B	2009	2010	1.7	2.3	6				2.6	2.2	3							3.2	2.2	3										0.96	1.1	4
A	2010	2011	12.2	14.8	13	8.1	8.8	6							1.0	1.9	4	1.1	1.5	6	4.9	5.8	5	3.2	2.7	6	4.2	3.0	4			
B	2010	2011	2.5	2.2	7	2.1	0.7	3	1.9	0.7	4	2.6	1.8	2										0.6	1.3	4						
A	2011	2012	22.0	18.4	14	7.2	7.3	4	15.4	0	2				1.4	1.0	4	1.3	1.0	6	1.9	1.7	6	3.5	3.2	5	1.9	1.9	3			
B	2011	2012	0.5	1.1	7							1.9	2.7	4	0.0	0.0	4										0.5	1.0	4			
		Mean	7.9	8.3	60	4.8	3.3	17	6.6	7.6	9	2.0	0.5	12	0.8	0.6	18	1.5	1.2	21	2.4	2.3	17	2.0	1.6	21	1.9	1.6	16	0.96	1.1	4
		Weighted mean	10.2	7.8	60	5.4	2.9	17	4.5	5.1	9	1.9	0.4	12	0.7	0.5	18	1.5	1.1	21	2.2	1.9	17	2.0	1.4	21	1.9	1.4	16	0.96	0	4

Y1, Year of release and oviposition; Y2, Year of adult emergence; M, mean; SD, standard deviation; N, number of small cages per pre-crop weighted by n. *Some regrowth observed few weeks after harvest.

Table 3. The generational (=annual) growth rate of *Diabrotica v. virgifera* populations in dependence of oviposition into different crop habitat combinations in large multiple-choice cages each covering three of ten habitats in two field sites in Hungary from 2009 to 2010, 2010 to 2011, 2011 to 2012. Growth rate assessed as the ratio of number of emerged adults versus the number of adults released the previous year. Letters in last column indicate significant differences according to Tukey *post hoc* multiple comparison at P<0.05. 14 other crop combinations had only 1 replicate over sites and years and are not presented.

Crop combination	Generational growth rate				Diff.
	Mean	SEM	n		
Maize only	↑↑	2.1	0.9	5	f
Maize and Sudan grass	↑	1.7	0.9	4	e
Maize and <i>Sorghum</i> millet	↑	1.4	0.6	8	e
Maize, potatoes, harvested and grubbed peas	↓	0.8	0.8	2	d
Maize, harvested grubbed and harvested non-grubbed winterwheat	↓	0.7	0.1	9	cd
Maize, harvested grubbed winter rape, harvested grubbed winterwheat	↓	0.6	0.2	4	cd
Maize, potatoes, harvested non-grubbed peas	↓	0.6	0.2	5	cd
Maize, <i>Sorghum</i> millet, harvested non-grubbed winterwheat	↓↓	0.5	0.0	2	bc
Maize, harvested grubbed peas, harvested grubbed winterwheat	↓↓	0.5	0.3	3	bc
Maize, potatoes, harvested grubbed winter rape	↓↓	0.4	0.0	2	bc
Maize, harvested grubbed peas, harvested grubbed winter rape	↓↓	0.4	0.2	8	bc
Maize and potatoes	↓↓	0.3	0.1	2	bc
Maize, bare ploughed soil, harvested grubbed winter rape	↓↓↓	0.2	0.0	3	b
Maize and bare soil	↓↓↓	0.2	0.2	4	b
Maize and harvested non-grubbed winterwheat	↓↓↓	0.2	0.1	3	b
Maize, potatoes, <i>Sorghum</i> millet	↓↓↓	0.2	0.0	2	b
Maize, potatoes, bare ploughed soil	↓↓↓	0.1	0.1	2	ab
Maize and soya	↓↓↓	0.1	0.1	2	ab
Maize and harvested grubbed peas	↓↓↓	<0.01	<0.01	2	a

↑ population growth, ↓ population decline. SEM, standard error of mean; n, replicates of large multiple-choice cages over sites and years.

air temperature at 1.5 m height and rainfall were recorded hourly from July to September of each study year (Davis Instruments Corp., Hayward, CA, USA) (Table 1).

Assessing feeding and oviposition preferences

The year following the adult releases in large multiple-choice field cages, maize was planted at each location. Then, three small pyramidal gauze cages were positioned in the maize over the areas where each of the different habitat types had been established the previous year (130×40 cm inner size with wooden frame base × 130 cm height of gauze around wire frame, each cage covering 6 to 7 plants or 0.5 m², same gauze materials as mentioned above). These small cages were used to collect and record the emerging adult *D. v. virgifera* to estimate oviposition in each habitat type the previous year. These small cages were placed before the start of adult emergence in the experimental plots, and checked weekly for emerging adults (at site A: from 28 June to 19 August 2010, from 2 July to 8 August 2011 and from 6 July to 9 August 2012; at site B: 29 June to 14 August 2010, from 2 July to 10 August 2011, and from 3 July to 9 August 2012). Adult records were performed blinded, *i.e.* by students that did not know the multiple-choice history of a certain field area the previous year, to improve likelihood that data are unbiased (Begley, 2013). Emerged adult numbers were calculated as captures per crop habitat per m² and standardized to 11 adults released/m² in the cages the previous year. Overall averages of adult emergence across years and sites were weighted by the number of cages placed per crop habitat combination in a certain site and year (Table 2).

Relative oviposition preferences were estimated by calculating the proportional emergence of adults from each of the previous year's habi-

tats compared to all adults emerged from the total area of each of the previous year's large multiple-choice cages.

The generational (=annual) growth rate of *D. v. virgifera* populations (ratio of number of emerged adults *versus* released adults the previous year) was estimated as an indicator of crop habitat quality for food, shelter and oviposition. If a certain combination had led to a significantly higher annual growth than others, then the considered crops habitat were estimated more optimal as feeding source than others.

Analyzing factors influencing feeding and oviposition preferences

Distributions of emergence and generational growth rate data were investigated using histograms as well as normal and detrended normal probability Q - Q plots (Kinnear & Gray, 2000). One-way ANOVA was conducted to detect whether the independent factors site location, year, habitat or any of their interactions affected the emergence of *D. v. virgifera*, and thus the proportional oviposition by adults the previous year (dependent factor). The independent-samples Kruskal-Wallis H test (nonparametric analogue of one-way ANOVA) was used to detect whether the independent factors of crop or weed vegetation cover, maize plant density, weed species numbers, amount of flowering weeds, weed species present and whether flowering or not, as well as maximum, mean, minimum temperature and rain during the main oviposition period, *i.e.* August, influenced the dependent factor of adult emergence. Pearson's correlation was used to test how oviposition might be associated with weather conditions.

One-way ANOVA was conducted to detect whether independent factor crop habitat of year x affected the generational growth rate of *D. v.*

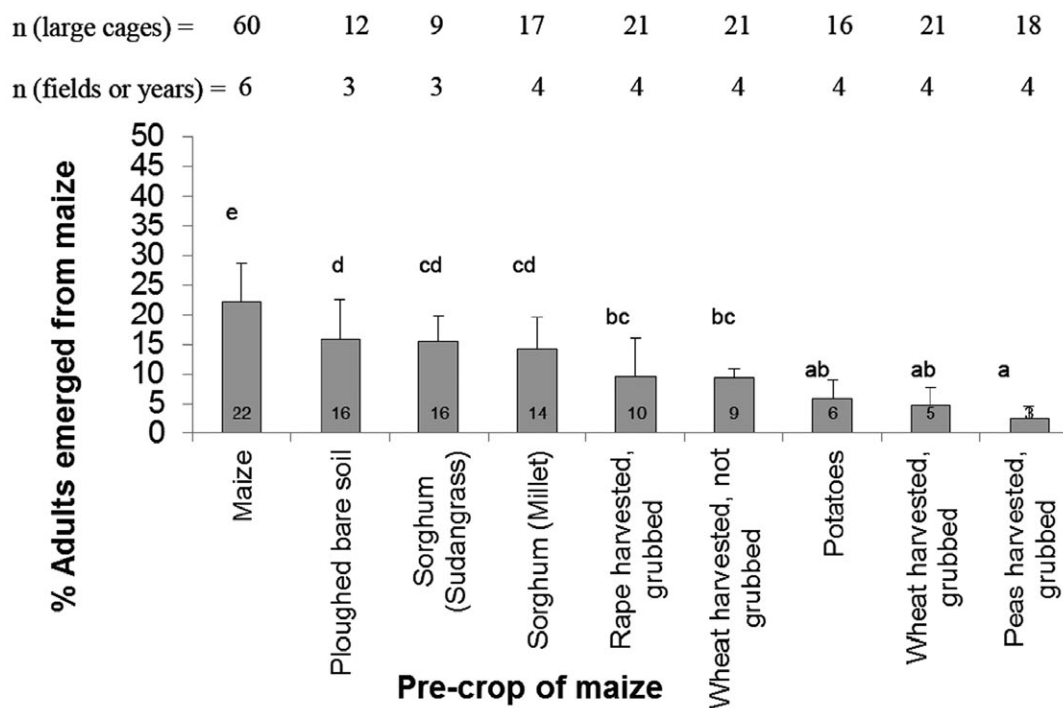


Figure 2. Proportional oviposition of *Diabrotica v. virgifera* into different crops in large multiple-choice field gauze cages each covering three different crop habitats in two field sites in southern Hungary in 2009, 2010, and 2011. Oviposition assessed by recording adult emergence from maize in three small cages the following year estimating % emergence in each crop habitat per m² compared with all adults emerged per m² from the area of each large multiple-choice cage placed the previous year. Data for soybean not presented, as this crop was only tested in one site and one year. Letters on bars indicate significant differences according to Tukey *post hoc* multiple comparison at $P < 0.05$; error bars=SEM.

virgifera populations from year x to $x+1$, as an indicator of crop habitat quality for food and oviposition sites (dependent factor). Pearson's correlation was used to test how growth rate might be associated with weather conditions.

Results

Diabrotica v. virgifera adults emerged from maize as a result of oviposition into different habitats in multiple-choice situations the previous year. The numbers of adults emerging from the cages ranged from 0 to 22 adults per m^2 , compared with 11 adults released per m^2 the previous year (Table 2).

The habitat type significantly affected the proportional oviposition of *D. v. virgifera* (ANOVA: $F_{7,184}=50.5$, $P<0.0001$, Figure 2). Maize was found most attractive, which is reflected (i) by 10.2 emerged adults per m^2 that is more than double the number of adults emerging from the other habitat types (weighted mean with all different adjacent crop combinations, SD 7.8; Table 3), and (ii) by the proportionally higher percentage of adult emergence from maize after exclusively maize than from maize after other crops habitat combinations (Figure 2).

Of medium proportional attractiveness were *Sorghum* millet, Sudan grass, and ploughed bare soil (Figure 2). Harvested and grubbed winter rape with regrowth, harvested and grubbed or not grubbed winter wheat with regrowth, as well as potatoes were less attractive for oviposition. Least suitable were harvested and grubbed peas, although they usually regrew during the oviposition period of *D. v. virgifera*. Absolute emergence from soybean was less than from most other crops (Tables 2 and 3), but proportional attractiveness of this crop across site and years could not be calculated due to limited replicate numbers.

Maize plant density positively affected oviposition (Kruskal Wallis: Chi-square 8.45, $P=0.038$). Also, increased crop coverage, regardless of the crop habitat type, positively affected oviposition (Kruskal Wallis: Chi-square 9.2, $P=0.027$), as did weed coverage (Kruskal Wallis: Chi-square 10.2, $P=0.017$). Total weed species numbers, as well as species of flowering or non-flowering weeds did not affect oviposition (Kruskal Wallis: Chi-square 4.2 to 7.7, all $P>0.05$). None of the 15 most abundant weed species had, in terms of their specimen numbers, a detectable effect on oviposition (Kruskal Wallis: Chi-squares 0.1 to 5.3, all $P>0.05$).

Neither the study location (ANOVA, $F_{1,189}=0.4$, $P=0.54$) or the study year (ANOVA, $F_{2,188}=2.7$, $P=0.07$) affected the proportional oviposition of *D. v. virgifera* into the different crop habitats. Increasing daily minimum and daily average of the usually high weekly temperatures in Hungary in August was negatively correlated with oviposition (Pearson $r=-0.54$, two tailed $P<0.0001$ and $r=-0.36$ and $P=0.001$ respectively; $n=87$). No effects were found for the daily maximum temperature in August ($r=0.19$, $P=0.77$) or for the sum of weekly rainfalls in August ($r=-0.17$, $P=0.125$).

The generational (=annual) growth rate of *D. v. virgifera* populations, as an indicator of crop habitat quality for food and oviposition sites, primarily depended on the crop types maize and Sudan grass (only maize in multiple-choice cages: ANOVA, $F_{1,78}=17.7$, $P<0.0001$; maize and Sudan grass in cages, $F_{1,78}=19.8$, $P<0.0001$). There were no significant differences among the other crop habitat combinations.

Populations grew from year to year most successfully when the entire cage had been planted with maize, thus in a no-choice situation only with maize. In this case, the populations doubled on average across sites and years, however variability was high (mean 2.1 ± 1.99 SD) (Table 3). Sudan grass and *Sorghum* millet were the only crops that, combined with maize in multiple-choice cages, still allowed a slight population growth. When maize was combined with any other crop habitat, the oviposition of *D. v. virgifera* was so much decreased,

even into the maize-part of cages that the populations decreased from year to year.

An increased daily minimum of the usually high weekly temperatures in Hungary in August negatively affected population growth (Pearson $r=-0.31$, two tailed $P<0.0001$). No effects were found for the daily maximum temperature in August ($r=0.21$, $P=0.05$), the daily mean temperature ($r=-0.17$, $P=0.12$), or the sum of weekly rainfalls in August ($r=-0.01$, $P=0.79$).

Discussion and Conclusions

The larvae of *D. v. virgifera* are known to feed almost exclusively on maize roots (Mooser & Hibbard, 2005). This species is believed to have evolved millennia ago on maize together with its cultivation in Mexico (Krysan & Smith, 1987). It is unclear which groups of plants served as ancestral hosts of *D. v. virgifera*, as most of the other more than 300 Diabroticina species in the Americas feed on a wide range of plant species, a high proportion of which are dicotyledonous plants (Mooser & Hibbard, 2005b). Thus, there are many studies on the extent the *D. v. virgifera* larvae may feed on the roots of plants other than maize (Spencer *et al.*, 2009). Several laboratory and few field studies showed that *D. v. virgifera* larvae can indeed feed on other grasses and grassy crops, but not on dicotyledons. Most of these potential alternative larval hosts seem, however, suboptimal as food or in the timing of their growing season (Gloyna *et al.*, 2010). Consequently *D. v. virgifera* larvae were, so far, never reported to reach measurable populations in habitats other than maize (Spencer *et al.*, 2005; Spencer *et al.*, 2009; Gloyna *et al.*, 2011). Larvae remain restricted to maize under field conditions, and therefore crop rotation is used to control this pest.

In contrast to the larvae, adults of this species are polyphagous. They are reported to frequently search for and use alternative food sources, particularly pollen from weeds or other crops (see gut analyses by Mooser *et al.*, 2006; 2007). It was suggested that the adults need alternative pollens sources for their egg development, once the two to three week flowering period of maize is over (Trusca & Grozea, 2011). Adults live several months and can continuously lay eggs (Branson & Johnson, 1973; Hill, 1975) if sufficient food is available. Our results, however, indicate that the polyphagous nature of *D. v. virgifera* adults might be, under field situations, less important as often stated. It appeared that the feeding on alternative food plants (crops or weeds) frequently occurs, but that this does usually not improve fitness. This is reflected in the analyzed generational growth rates, which are indicators of quality of crop habitats for food. The growth rates were found highest when the entire agricultural habitat (here large cages) had been planted with maize (more than double yearly grow, mean 2.1 across fields and years). The standard error of 0.9 indicated that the generational growth rate in maize is highly variable, and can in some fields and years lead to a quick yearly population increase and in some years to dramatic decreases, a phenomenon also reported by Szalai *et al.* (2011) from fields in Hungary and Croatia. This indicates that, next to food, other factors might heavily influence the population dynamics of this species (Toepfer & Kuhlmann, 2006).

As our study was conducted in enclosed environments of field cages, they have prevented adults potentially flying larger distances to find a large variety of potential food sources in and outside the field, a phenomenon that could not be tested here. The here-used cages were however rather big for field studies (9 m^2 area); they covered different crops habitats in replicated combinations, and contained a high diversity and density of weeds. We therefore believe that results can reflect the comparative proportional use of non-maize plants and maize by *D. v. virgifera* for food, shelter and/or oviposition under field conditions. And this showed, astonishingly, that weeds or other crops than maize

had little advantage for *D. v. virgifera*. This is astonishing as we may assume from gut analyses that feeding on alternative food plants frequently occurs (Mooser *et al.*, 2006). In our study, only the *Sorghum* crops, *i.e.* Sudan grass and millet, combined with maize in a habitat, still allowed a slight population growth from year to year. This, however, should not be taken to contradict efficacy studies on *Sorghum* – maize rotations for controlling this pest, which clearly show that *Sorghum* is not a good food source for the larvae of this pest and can therefore be safely rotated (Saladini *et al.*, 2009). *Sorghum* crops seem, however, to serve to some extent as useful food sources for the adults, particularly because *Sorghum* is often flowering during the period of adult food uptake, and has a longer flowering period than maize (see also Mooser & Vidal, 2005). In contrast, when maize was combined with any other crop, the performance of *D. v. virgifera* in the multiple-choice cages decreased, even in the maize part of the cages; and this to an extent that populations declined from year to year. This reflects that non-maize crop habitats are generally suboptimal. Reissig & Wilde (1971) and Spencer *et al.* (2009) also reported from North American trials that maize seems to be the preferred source of adult food under field conditions, although hybrids may differ in their attractiveness. Considering the fact that only cages entirely grown with maize and no other crop attained considerable population growth in our study, it can be assumed that all other crop habitats pose low risk in pest population to reach threshold levels when maize is grown the following year. In conclusion, the importance of weeds and non-maize crops as alternative food sources seem to be, in terms of pest management strategies, often somewhat overestimated. In terms of invasion success, however, the polyphagous nature of the adults had likely favored the species to survive changing environments, and to employ large distance spread and colonizations of new regions (Toepfer *et al.*, 2006; Ciosi *et al.*, 2008).

If only the oviposition of *D. v. virgifera* was considered in our study, a comparable picture can be drawn. Maize was clearly the most attractive crop habitat for the ovipositing *D. v. virgifera* adults, and maize density was positively correlated with adult emergence. This phenomenon is known since long, and from many other studies (Shaw *et al.*, 1978; Spencer *et al.*, 2009). Hibbard *et al.* (1994) concluded from olfactorial bioassays that adults can recognize a maize field. Kirk (1981) addressed that cracks and holes in the maize field surface are crucial for oviposition; but that the maize plant itself, particularly the stem base, seemed less important. It remains however crucial for *D. v. virgifera*'s survival to lay eggs into maize, as the larvae are restricted to maize in their feeding. This also made crop rotation the most powerful management option against this pest. Crop rotation is largely successful in Europe, and this not only for the management of established populations, but also for eradication and containment purposes (Lipa, 2004; Reynaud *et al.*, 2005; Furlan, 2008; Glass, 2011). In the USA, some populations, however, have evolved to more frequently lay their eggs in non-maize crops where maize is grown the following year which allows larval development and adult emergence (Gray *et al.*, 1998; Levine *et al.*, 2002). The evolution of this behavioral resistance was hypothesized to be a result in loss of fidelity to maize rather than any particular attractivity of another crop (Knolhoff *et al.*, 2006). That such populations had been introduced into Europe during the multiple introductions of this species (Miller *et al.*, 2005) cannot be supported by our study, at least not for the Central/South Eastern European genetic population (as defined by Ciosi *et al.*, 2008) that was studied here. This is because only a relatively small proportion of tested adults used non-maize habitats as oviposition sites despite that adults were enclosed and alternative crops were adjacent to maize. Thus, one would have expected an intensive use of these habitats by the insect. At least some adults emerged from maize after the non-maize habitats of *Sorghum* millet, Sudan grass, and ploughed bare soil. As this happened more frequently after these habitats than after any other non-maize habitat, it might be argued that this did not only occur because of the close proximity to maize, but that there were either vegetational characteristics

or soil conditions in these habitats which were suitable for adult oviposition. Harvested and grubbed winter rape with regrowth, harvested and grubbed or not grubbed winterwheat with regrowth and potatoes were comparatively less attractive for oviposition. This contradicts the hypothesis, that regrowth or volunteer wheat or other green fresh crop vegetation might favor *D. v. virgifera* adults. The least suitable crop habitats were harvested and grubbed peas and soybean, the latter being the most dominant crop in rotation with maize in the USA Corn Belt. All together it was surprising to see that regrowth of crops after harvest, and thus, moist, shaded and green microhabitats do not significantly increase *D. v. virgifera* oviposition, as proposed for example for soybean by Shaw *et al.* (1978). Meanwhile, an overall analysis of data across crops in our study indicated that vegetation coverage of crops and/or weeds positively influenced oviposition. Interestingly, single weed species, their flowering status, and their combination had no influence, as it was suggested for some weeds, such as for *Cirsium arvense* by Toepfer *et al.* (2007).

Finally, it must be stated, that from multiple-choice cage conditions, only relative comparisons can be made. No prediction of absolute levels of oviposition by *D. v. virgifera* in open field situations can be drawn, because of the enclosing nature of cages. We, however, assume that oviposition in non-maize habitats will be even less in open field situations, than in the enclosed cages with high *D. v. virgifera* densities. Still, it seems to belong to the natural survival strategy of *D. v. virgifera*, to oviposit to some extent into non-maize crops (Shaw *et al.*, 1978; Barcic *et al.*, 2007; Spencer *et al.*, 2009). This fact might occasionally become, in terms of pest management, a factor to be considered as border effects in small scale agriculture, such as Croatia (Barcic *et al.*, 2007), western and southern Germany, or Poland (Lipa, 2004; Stachow *et al.*, 2013).

In conclusion, despite the observed small oviposition activity in non-maize crops and subsequent pest development when maize is grown the following year, the limited extent of this behavior even under maize-adjacent situations, as well as the reduced generational population growth in multiple crop situations, indicate that crop rotation remains, no matter what crop combination in an agricultural area, an effective control measure of *D. v. virgifera* populations. Crop rotation is suggested to be possible with all tested crops to control *D. v. virgifera* populations. This underpins crop rotation as the major pest management tool for this species; and regional or country wide decision makers in agri-policy as well as developers of integrated pest management guidelines should clearly continue to take this fact into consideration, and reduce the hype around the potential destructive danger of this pest for European maize production.

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