Final Report 2009 - 2012

# Understanding dispersal and oviposition behaviour of *Diabrotica virgifera* in non-maize crops to improve advice and guidelines for crop rotation

## (Untersuchungen zum Flug- und Eiablageverhalten des Maiswurzelbohrers in verschiedenen Ackerkulturen, um die Fruchtfolge-Empfehlungen für die bayerische Landwirtschaft zu verbessern)

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**Thema:**
Untersuchungen zum Flug- und Eiablageverhalten des Maiswurzelbohrers in verschiedenen Nicht-Mais-Ackerkulturen, um die Fruchtfolge-Empfehlungen für die bayerische Landwirtschaft zu verbessern

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**Für:**
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**In Zusammenarbeit mit:**
Staatlicher Pflanzenschutzdienst des Bezirkes Csongrad in Hodmezovasarhely in Ungarn,
Staatlicher Sortenregistrierungsdienst in Szekkutas in Ungarn,
Landwirtschaftsbetrieb Hodmezogazda Rt in Hodmezovasarhely in Ungarn.

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**Endbericht**
1. April 2009 bis 30 Dezember 2012

# Untersuchungen zum Flug- und Eiablageverhalten des Maiswurzelbohrers in verschiedenen Ackerkulturen, um die Fruchtfolge-Empfehlungen für die bayerische Landwirtschaft zu verbessern

## Laut Arbeitsplan geplante Arbeitsschritte während des abgelaufenen Berichtszeitraums

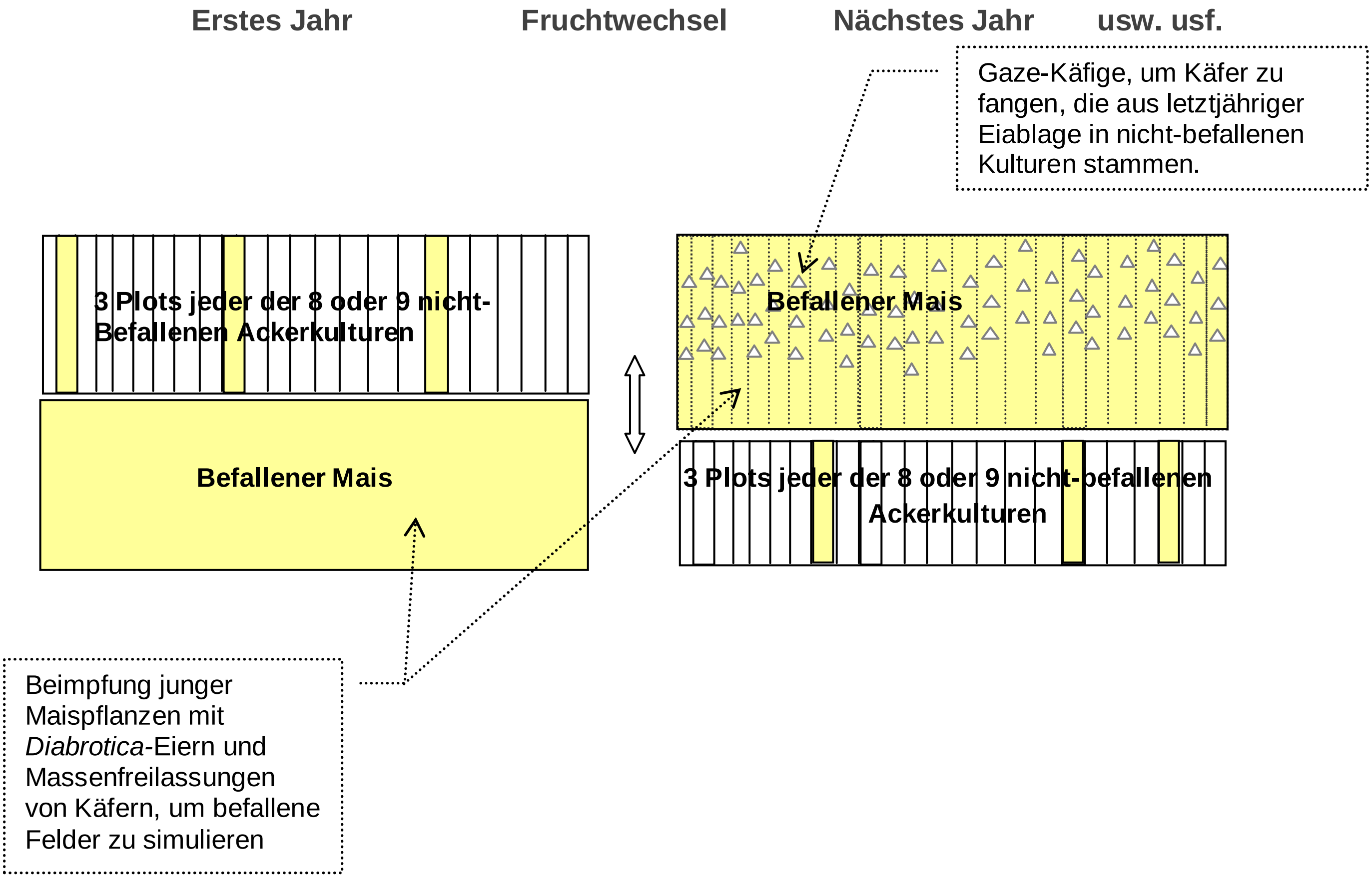
- Anlegen und Durchführen eines Fruchtfolge-Feldversuches in Ungarn, um das Flug- und Eiablageverhalten des Maiswurzelbohrers in verschiedenen Ackerkulturen zu untersuchen.
- Jedes Versuchsfeld wird in zwei Sektoren eingeteilt. Ein Sektor ist ein mit Maiswurzelbohrer befallenes Maisfeld, der zweite Sektor enthält kleinere Versuchsflächen mit mindestens 8 verschiedenen Ackerkulturen. Diese werden Winterweizen (Stoppelbehandlung mit Grubber), Winterweizen (keine Stoppelbehandlung), Raps (Stoppelbehandlung mit Grubber), Hirse, Sudangras, Zuckerrüben, Kartoffeln und Erbsen (Stoppelbehandlung mit Grubber) als auch Mais sein. Jede dieser Ackerkulturen wird in 3 Versuchsparzellen angelegt, das heisst auf insgesamt 27 Parzellen.
- Eine Maiswurzelbohrer Population wird im grossen Maisfeld-Sektor freigelassen. Der Maiswurzelbohrer kann dann zwischen den Versuchsflächen hin- und herfliegen und Eier ablegen.
- Der Flug der Käfer wird mit gelben Klebfallen von Juli bis September jedes Jahr gemessen.
- Die zwei Sektoren des Experimentierfeldes werden jährlich gewechselt, das heisst Mais wird auf die Versuchsflächen gepflanzt, wo im Vorjahr die anderen Ackerkulturen waren.
- Es werden Schlupf-Käfige aufgestellt, um die eventuell schlüpfenden Käfer zu fangen. Wird ein Käfer gefangen, so wurden im Vorjahr in den jeweiligen Ackerkulturen Eier abgelegt. Es kann dann analysiert werden, welche Kulturen am meisten zur Eiablage aufgesucht wurden, und damit in einer Fruchtfolge eventuell nicht empfohlen werden sollten.
- Da es methodisch schwierig ist, Eiablagen in Nicht-Mais-Ackerkulturen mit einem Freiland-Feldversuch zu quantifizieren, werden zusätzlich Käfigversuche mit hohen künstlichen Käferdichten im gleichen Versuchsfeld durchgeführt.
- Grosse Gazekäfige werden über die Schnittstellen zwischen Mais und den jeweiligen Nicht-Mais-Ackerkulturen gestellt. Käfer werden darin freigelassen, und diese können dann im Mais Teil oder in der anderen Ackerkultur innerhalb der Käfige Eier ablegen.
- Im folgenden Jahr wird dann der Schlupf von Käfern wieder mit Käfigen bestimmt. Mit dieser standardisierten Versuchsmethode wird sichergestellt, dass quantitativ wertvolle Daten erhalten werden, im Fall, dass die natürliche Eiablage im Nicht-Mais im Freiland-Feldversuch gering ist.
- Alle Experimente werden mindestens an einem, aber besser zwei Standort(en) in Südungarn durchgeführt und über vier Jahre wiederholt, d.h. von 2009 bis 2012.

## Tatsächlich durchgeführte Arbeitsschritte und erreichte Ziele.

*Durchgeführte Arbeitsschritte:*

- Zwei Fruchtfolge-Feldversuche wurden in Ungarn angelegt, um das Flug- und Eiablageverhalten des Maiswurzelbohrers in verschiedenen Ackerkulturen zu untersuchen. Ein ca. 2 ha grosser Versuch wurde in Szekkutas angelegt, und ein ca. 0.5 ha grosser Versuch in Hodmezovasarhely.
- Jedes Feldexperiment wurde in zwei Sektoren eingeteilt. Ein Sektor ist ein Maisfeld, worin ein Maiswurzelbohrer-Befall durch Massenfreilassungen erreicht wurde. Im Versuch in Szekkutas enthält der zweite Sektor 8 bis 9 verschiedene Ackerkulturen: Weizen

(Stoppelbehandlung mit Grubber), Weizen (keine Stoppelbehandlung), Raps (Stoppelbehandlung mit Grubber), Hirse, Sudangrass, Zuckerrüben, Kartoffeln und Erbsen (Stoppelbehandlung mit Grubber) als auch Mais. Jede dieser Ackerkulturen wurde in 3 Parzellen angelegt. Im Versuch in Hodmezovasarhely enthielt der zweite Sektor jedes Jahr 4 verschiedene Ackerkulturen. Jede dieser Ackerkulturen wurde in 2 Parzellen angelegt.



Gaze-Käfige, um Käfer zu fangen, die aus letztjähriger Eiablage in nicht-befallenen Kulturen stammen.

Beimpfung junger Maispflanzen mit *Diabrotica*-Eiern und Massenfreilassungen von Käfern, um befallene Felder zu simulieren

*Fig. Versuchsaufbau des Experimentierfeldes von einen zum nächsten Jahr (In jeder einzelnen Versuchsfläche und in jedem Jahr stehen 3 bis 4 gelbe Klebfallen, um den Flug von Käfern aus dem befallenen Maisfeld in andere Flächen zu bestimmen).*

*Fig. Versuchsflächen verschiedener nichtbefallener Ackerkulturen und befallener Mais*

- Maiswurzelbohrer-Eier wurden im Maisfeld- Sektor an die Wurzeln im Frühsommer ausgebracht, als auch Käfer im Juli jedes Jahr freigelassen. Der Maiswurzelbohrer konnte dann zwischen den Versuchsflächen hin- und herfliegen, fressen, und gegebenenfalls Eier ablegen.

*Fig. Massensammlung von über 30000 Käfern und dann Freilassung*

- Der Flug der Käfers wurde mit gelben Klebfallen von Mitte Juli bis Anfang September gemessen (wöchentliche Kontrollen von 4 Fallen pro Versuchsparzelle in Szekkutas, 3 Fallen pro Versuchsparzelle in Hodmezovasarhely).

*Fig. Käferflug zu Hirse*

*Käferfang mit Klebfalle*

*Wöchentliche Fallenkontrolle*

- Die zwei Sektoren des Experimentierfeldes wurden im Herbst gewechselt. Das heisst, Mais wird im folgenden Jahr immer auf die Versuchsflächen gepflanzt, wo im Vorjahr die anderen Ackerkulturen standen.
- Im folgenden Jahr wurden kleine Schlupfkäfige (1.30 x 0.4, x 1.20 m) auf die Maisflächen aufgestellt, wo im Vorjahr die Parzellen der nicht-befallenen Ackerkulturen waren. Der Käferschlupf wurde im Juli und August gemessen.

*Fig. Käfige um den Schlupf des Käfers zu erfassen und auf Eiablagen im vorherigen Jahr zu schliessen*

- Da es recht schwierig ist, Eiablagen in Ackerkulturen mit einem Feldversuch gut zu quantifizieren, wurden zusätzlich Käfigversuche mit hohen künstlichen Käferdichten in beiden Experimentierfeldern durchgeführt.
- Grosse Gazekäfige (2 x 4.5 x 2 m) wurden über die Schnittstellen zwischen Mais und den jeweiligen anderen Ackerkulturen gestellt (20 bis 23 Käfige in Szekkutas, 7 bis 9 Käfige in Hodmezovasarhely, 2009, 2010 und 2011). 100 Käfer wurden darin 2009 und 2011, bzw. 150 Käfer 2010 freigelassen. Diese konnten dann im Mais oder in den anderen Ackerkulturen innerhalb des Käfigs Eier ablegen.
- Im folgenden Jahr wurde Mais über die Käfigflächen gepflanzt.
- Im folgenden Jahr wurden kleine Schlupfkäfige (1.30 x 0.4, x 1.20 m) auf die Maisflächen aufgestellt, wo im Vorjahr die anderen Ackerkulturen waren. Der Käferschlupf wurde im Juli und August gemessen.

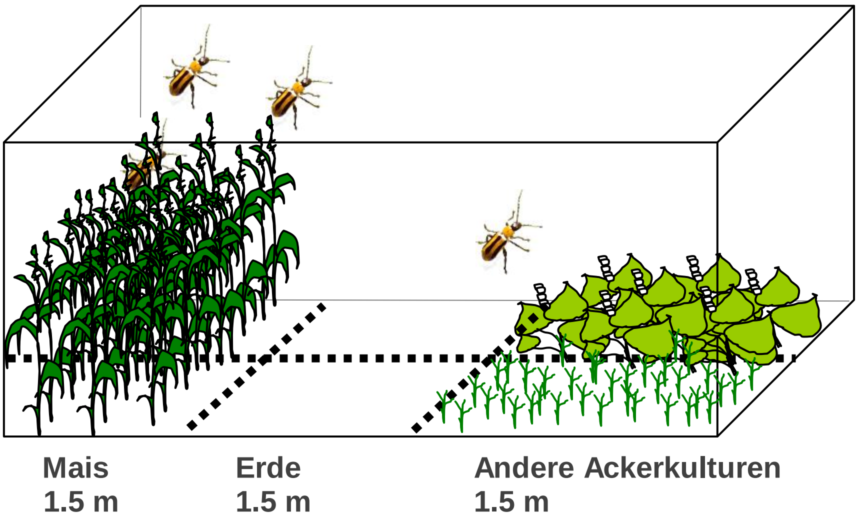


*Fig. Gazekäfige über Schnittstellen zwischen Mais und anderen Ackerkulturen*

*Fig. Gazekäfige über Schnittstellen zwischen Mais und anderen Ackerkulturen*

*Erreichte Ziele:*

- Die max. mögliche Anzahl an Feldexperimenten in Ungarn wurde organisiert und angelegt.
- Die maximale Anzahl an geplanten Versuchsflächen wurde realisiert.
- Maisfelder wurden erfolgreich mit dem Maiswurzelbohrer infiziert.
- Der Flug von Käfern wurde erfolgreich mit Klebtafeln gemessen.
- Grosse Gazekäfige wurden aufgestellt, und Käfer darin freigelassen.
- Kleinere Schlupf- Gazekäfige wurden aufgestellt, und der Käferschlupf als Folge von Eiablagen im vorherigen Jahr erfolgreich gemessen.
- Es wurde sichergestellt, dass alle Versuche mindestens über vier Jahre an den gleichen Standorten laufen konnten.

## Vergleich des Vorhabenstandes mit dem verbindlichen Arbeits-, Zeit- und Finanzierungsplan

*Angabe von Gründen, falls sich die Aussichten für die Erreichung der Ziele des Vorhabens innerhalb des angegebenen Berichtszeitraumes gegenüber dem verbindlichen Arbeitsplan geändert haben*:

- Änderungen wurden in den Projekt-Zielsetzungen nicht vorgenommen.

*Begründung für notwendige Änderungen in der Zielsetzung:*

- N/A

*Hinweise auf Ergebnisse, die inzwischen von dritter Stelle bekannt wurden und die für die Durchführung des Vorhabens von Bedeutung sind:*

- Keine

## Wichtige Ergebnisse und andere wesentliche Ereignisse des Berichtszeitraumes

- Massenfreilassungen des Käfers und deren Wiederfang mit gelben Klebtafeln zeigten, dass ca. 5.7 ± 2.9% der Käferpopulation vom befallenen Maisfeld in nichtbefallene Ackerkulturen einfliegt (nur 2.9 ± 1.2% wenn nicht befallene Nicht-Mais-Kulturen einbezogen werden) (Daten von zwei Feldern, 2009, 2010, 2011, 2012).
- Nicht befallener Mais war die attraktivste Ackerkultur für einfliegende Käfer aus dem befallenen Maisfeld. Einige Käfer flogen auch über anderen Ackerkulturen.
- Fänge schlüpfender Käfer in kleinen Gazekäfigen im jeweiligen Folgejahr zeigten, dass 6 ± 3.1 % der gesamten Käferpopulation auf Mais-Flächen schlüpft, die im Vorjahr mit nicht-befallenen Kulturen bepflanzt waren (ca. 3.1 ± 3.9 % wenn nicht-befallener Mais als Vorjahreskultur nicht mit einberechnet wird).

## Zusammenfassung

Es ist bekannt, dass Käfer des westlichen Maiswurzelbohrers (*Diabrotica virgifera virgifera*, Coleoptera: Chrysomelidae) auch in andere Ackerkulturen ausser Mais einfliegen, wahrscheinlich primär um Nahrung zu suchen. In den USA gibt es auch Maiswurzelbohrer-Populationen, die vermehrt Eier in Ackerkulturen ablegen, wo im nächsten Jahr auf Grund von Fruchtfolgen Mais angebaut wird, und sich somit die Larven entwickeln können. Zwei Fruchtfolge-Feldversuche wurden in Südungarn über 4 Jahre angelegt, um dieses Flugverhalten als auch mögliche Eiablagen des Maiswurzelbohrers in 10 nichtbefallene Ackerkulturen unter europäischen Bedingungen zu untersuchen. Dabei wurden vor allem für Bayern typische Kulturen berücksichtigt.

Massenfreilassungen des Käfers und deren Wiederfang mit gelben Klebtafeln zeigten, dass ca. 5.7 ± 2.9% der Käferpopulation vom befallenen Maisfeld in nichtbefallene Ackerkulturen einfliegt (nur 2.9 ± 1.2% wenn nicht befallene Nicht-Mais-Kulturen einbezogen werden). Nicht befallener Mais war die attraktivste Ackerkultur für einfliegende Käfer aus dem befallenen Maisfeld. Einige Käfer flogen auch über anderen Ackerkulturen.

Fänge schlüpfender Käfer in kleinen Gazekäfigen im jeweiligen Folgejahr zeigten, dass 6 ± 3.1 % der gesamten Käferpopulation auf Mais-Flächen schlüpft, die im Vorjahr mit nicht-befallenen Kulturen bepflanzt waren (ca. 3.1 ± 3.9 % wenn nicht-befallener Mais als Vorjahreskultur nicht mit einberechnet wird).

Es kann abschliessend festgestellt werden, dass die beobachtete Eiablage des Wurzelbohrers in nicht-Maiskulturen zu gering ist, um im nächsten Jahr im Fall von einer Fruchtfolge zu Mais, in diesem dann Schäden zu verursachen. Deshalb kann man davon ausgehen, dass alle Nicht-Maiskulturen für die Kontrolle von Maiswurzelbohrer-Populationen geeignet sind.

**Detaillierte Informationen in Englisch**

# Understanding dispersal and oviposition behaviour of *Diabrotica v. virgifera* in non-maize crops to improve advice and guidelines for crop rotation in Bavaria in southern Germany

## Final report 2009-2012

Submitted 31 October 2012

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## Dispersal and oviposition behaviour of *Diabrotica v. virgifera* in maize and non-maize crop habitats

### Abstract

Adults of the maize pest *Diabrotica virgifera virgifera* (Western corn rootworm, Coleoptera: Chrysomelidae) are known to perform inter-field movements to crops other than maize, supposedly to access protein-rich food sources. In the USA, rotation tolerant populations also lay significant numbers of eggs in non-maize crops where maize is grown the following year, which then allows larval development. Crop rotation experiments were carried out in Hungary to investigate to what extent adults disperse out of infested maize fields and lay eggs in uninfested 10 different habitat types typical for agricultural areas in Western Europe, and consequently may reduce the efficacy of rotation as a control measure. Mass-releases and recaptures of adults in crop rotation experiments at two study sites in southern Hungary between 2009 and 2012, revealed that only 5.7 ± 2.9% of the entire population disperses from infested maize fields towards adjacent habitats, including maize. When adjacent maize was not included in the analyses, then only 2.9 ± 1.2 % of the population from the infested maize fields dispersed into adjacent non-maize habitats. Based on the trap catches, the adults of both sexes dispersed into maize with the greatest frequency. Adult males dispersed into millet with the second greatest frequency. After maize, females were trapped with the next greatest frequency in millet, ploughed bare soil, Sudangrass, harvested and grubbed winter wheat, harvested and grubbed winter rape, or potatoes. Dispersing adults also laid eggs, i.e. 6 ± 3.1% of the population oviposited in previously uninfested habitats. The adults oviposited most frequently in maize, and the results indicate that the majority of the *D. v. virgifera* population from infested maize had dispersed and oviposited over the entire maize area, regardless of whether the maize was their natal field (the infested field simulated by mass releases) or not (the adjacent uninfested field). When previously uninfested maize was not included in the analyses, only 3.1± 3.9 % of the entire *D. v. virgifera* population oviposited into uninfested crop habitats. In general, fields that were not harvested or at least not grubbed after harvest during *D. v. virgifera* `s oviposition period such as millet, Sudan grass, sugar beet and harvested but not grubbed winterwheat appeared more attractive for oviposition (together 5.8 ± 1.3% oviposition) than crop habitats that had experienced soil preparations, such as the ploughed bare soil, harvested and grubbed peas, harvested and grubbed winterwheat or harvested and grubbed winter rape (together 1 ± 0.3%). Despite the known average grow rate of *D. v. virgifera* generations by factor 4, our findings indicate that it is unlikely

that economic damage will occur in first-year maize following rotation with non-maize crops. Damage may occasionally appear in second year maize, in cases of small scale farms where the first year of maize was grown in fields adjacent to sites with large populations of *D. v. virgifera*, combined with an optimal year of *D. v. virgifera* generational growth. In general, it can be concluded that *D. v. virgifera* population do not have a strong preference for ovipositing in non-maize crops and therefore, this alien invasive pest can be successfully managed by rotating maize with any other crop.

## Introduction

*Diabrotica virgifera* ssp. *virgifera* LeConte (Western corn rootworm*,* Coleoptera: Chrysomelidae) is a maize pest in North America and Europe. It is hypothesized to have originated in Mexico, where several pestiferous *Diabrotica* species occur (Branson and Krysan 1981; Krysan and Smith 1987). With the expansion of maize growing areas in the 20th century, *D. v. virgifera* became a major pest of maize, *Zea mays* (L.), in North America (Krysan and Miller 1986, Levine and Oloumi 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). It is now a threat to maize production in many European countries, particularly Austria, Hungary, Serbia, Romania, Slovakia, and Italy (Kiss *et al*. 2005b). It recently arrived in Germany, France, Poland and Belorussia (Ciosi *et al*. 2008) and is expected to become a threat to maize production areas in these countries as well.

*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 and Hibbard 2005). Feeding damage 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 and Kuhlmann 2006), and can occasionally further reduce crop yields through intensive silk feeding, which interferes with maize pollination (Chiang 1973). Crop rotation is a successful control measure in Europe, and largely used in Hungary, Serbia and Romania (Kiss et al. 2005).  To keep populations below the economic threshold, it is not even necessary to rotate 100% of the maize fields (Kruegener et al. 2011, Szalai et al. 2013).

However, the adults are active fliers (Spencer *et al*. 2005) and readily perform inter-field movements to crops other than maize, mostly to access protein-rich food sources, such as pollen from lucerne flowers or sunflowers (Toepfer *et al*. 2005). It was demonstrated that a small proportion of these dispersing adults also lay eggs in the non-maize crops, even when these did not contain any Poacean weeds (grasses) (Kiss *et al*. 2005a, Barcic *et al.* 2007) that are usually considered to be suitable alternative hosts of *D. v. virgifera* larvae (Moeser and Hibbard 2005).

Taking this information into consideration, crop rotation trials were conducted in Hungary and Croatia between 2000 and 2004 (Kiss *et al*. 2005a), testing maize, soybean, sunflower, winter wheat, and oat. The main result of the study was that only a few adults emerged from maize fields that had been planted with soybean, sunflower, winter wheat or oat in the previous year. For these crops, at least, it can be concluded that crop rotation remains a powerful tool in interrupting the life cycle of *D. v. virgifera* and in controlling *D. v. virgifera*.

It is still not known to what extent *D. v. virgifera* may lay eggs into non-maize crops typical for Western Europe, i.e. in regions of southern Germany or eastern France that have recently been invaded by *D. v. virgifera*.  Such crops include, for example, winter wheat, winter rape, sugar beet, millet or Sudan grass (the latter for biogas), potatoes and to some extent peas. Furthermore, clarification was needed as to whether field management of winter wheat (stubble grubbing and then ploughing in autumn, or no stubble management and thus only ploughing in autumn) may affect the oviposition behaviour of dispersing adults.

This four-year study therefore aimed to investigate the dispersal and oviposition behaviour of adult *D. v. virgifera* in 10 different habitat types (other crops and bare fields) typical for agricultural areas in Western Europe at two study sites in southern Hungary. The findings of this study have implications for the development of guidelines and standards for crop rotation as a means to control *D. v. virgifera* in European regions where the populations have been spreading.

## Material and methods

### *Study sites and experimental design*

This study is carried out at two study sites in southern Hungary between 2009 and 2012 (Table C1) (referred to as site A: Szekkutas, N46° 31 00.0, E20° 30 25.8, 87 m above sea level; site B: Hodmezovasarhely, N 46° 25.998, E 20° 20.348, 83 m). None of the fields had a geographic relief drift, and the soil texture was assumed homogenous across the study sites. Field site A was on gleyic solonetz soil, site B on gleyic chernozem. Site A was surrounded by agricultural land, site B was surrounded half by agricultural land half by buildings. In previous years before the study was initiated, winterwheat was grown at sites A and maize at site B.

Each site was divided into two sections: half of the site was planted in maize and artificially infested with *D. v. virgifera* (described below) and the other half of the site was divided into plots with other habitats typical of agricultural areas in Western Europe(the habitat types in the plots are listed in Table C1). At site A, in 2009 the large maize section (144 x 55 m) was planted with 53000 maize plants; in 2010, the large maize section (156 x 60 m) was planted 63000 plants, in 2011, the large maize section (156 x 50 m) was planted with 65000 maize plants; in 2012, the large maize section (156 x 60 m) covering 75000 plants. In site B, the large maize section was 50 x 25 m covering 8000 plants in 2009, 50 x 30 m covering 10000 plants in 2010 and 2012, and 50 x 25 m covering 10000 plants in 2011.

At site A, three plots of 8 to 9 habitat types were established: i.e. maize (*Zea mays*), ploughed bare soil (only 2009 and 2010), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris var. altissima*; not in 2009), millet (*Sorghum bicolor*) and Sudan grass (*Sorghum Sudanese*; not in 2009 and 2010); peas (*Pisum sativum (partim)*), winter rape (*Brassica napus (partim*), not in 2012) and winter wheat (*Triticum aestivum var. lutescens*) that had been harvested and grubbed prior the dispersal period of *D. v. virgifera,* and finally winter wheat that had been harvested but not grubbed prior to the dispersal of *D. v. virgifera* (Tab. C1). At site B, two plots of four habitat types were planted: i.e. in 2009 maize, Sudan grass, and winter rape that had been harvested and grubbed prior to the dispersal period of *D. v. virgifera*; in 2010 maize, millet, Sudan grass, and winter wheat that had been harvested but not grubbed prior to the dispersal period of *D. v. virgifera*; in 2011 maize, peas that had been harvested, and ploughed bare soil, and 2012 maize, peas that had been harvested, ploughed bare soil and sugar beet.

The two sections of the study sites were rotated annually, in order to assess how the previous year's habitat type influences *D. v. virgifera* emergence in maize.

### *Infestation with D. v. virgifera*

In order to achieve a homogenous population of *D. v. virgifera* in the maize section of the study sites, (i) young maize plants were infested with *D. v. virgifera* eggs, and (ii) *D. v. virgifera* adults were mass released.

(i) The soil around young maize plants at 2nd – 3rd leaf stage was infested with a total of 15,000 ready to hatch *D. v. virgifera* eggs (50 per 300 plants) in site A on 12 May 2009; 178000 eggs (200 per 890 plants) between 5 and 26 May 2010; and 64,000 eggs (400 eggs per 160 plants) 16 May 2011, but none in 2012. In site B, soil was infested with 10,000 eggs (50 per 200 plants) on 12 May 2009, 76000 eggs (200 per 380 plants) between 5 and 26 May 2010, and 16,000 eggs (400 per 40 plants) 16 May 2011, but none in 2012. Eggs were obtained from a laboratory culture of adults collected in the field in southern Hungary the preceding year (for procedures see Singh and Moore 1999). *Diabrotica v. virgifera* eggs were overwintered at 6-8°C in moist sand and diapause was broken in the second half of April by transferring them to 25°C. To collect the eggs, the sand was sieved through a 250 micrometre mesh. Recovered eggs were mixed into a solution of water and 0.15% agar. About 2 to 3 ml aqueous agar (containing approximately 100 to 200 of ready-to-hatch eggs) was applied using a standard pipette (Eppendorf, Hamburg, Germany) into 100-140 mm-deep holes at a distance of 50-80 mm from each maize plant. Only 5 to 10 % of eggs were expected to survive until adulthood due to natural mortality (Toepfer *et al*. 2006).

(ii) *D. v. virgifera* adults were mass collected in naturally infested maize fields near Karduskut and Hodmezovasarhely in southern Hungary and transported in a cool box to the study sites. Batches of 500 adults were released at about 20-metre intervals along the middle row of the maize section at each study site. In site A, a total of 30,000 adults were released (~ 0.6 to 0.5 adults per plant) between 14 and 28 July 2009 and between 19 and 27 July 2010; 40,000 (~ 0.5 adults per plant) between 8 and 20 July 2011; 39,000 (~ 0.45 adults per plant) between 6 and 11 July 2012. In site B, 3,000 adults were released (~ 0.4 adults per plant) 17 July 2009; 10,000 adults were released (~ 1 adult per plant) between 14 and 17 July 2010; 8,000 (~ 0.8 adults per plant) 5 and 6 July 2011; 10,700 (~ 1 adults per plant) 5 and 6 July 2012.

The eggs and the adult releases resulted in populations of about 0.7 to 0.9 adults per plant in site A in 2009, ca. 0.6 to 0.8 per plant in 2010, ca. 0.5 to 0.6 per plant in 2011 and ca. 0.4 to 0.5 per plant in 2012. In site B, the adult releases resulted in approximately 0.6 to 0.7 adults per plant in site B in 2009, 1.2 to 1.3 adults per plant in 2010, and 0.9 to 1 adults per plant in 2011 and 2012.

Based on field observations in highly infested fields, the approximate start of natural emergence of *D. v. virgifera* in the region was around 20.6.2009, 29.6.2010, 20.6.2011, and 22.6.2012, with 2009, 2011 and 2012, been normal years and 2010 been a delayed year (Toepfer et al. 2006).

Moreover, maximum, minimum and mean daily air temperature at 1.5 m height, and rainfall was recorded hourly from July to September of each study year (Davis Instruments Corp., Hayward, CA, USA). In 2009, mean temperature in July was 24 C (max 36, min 13) and the sum of rainfall 6 mm. In 2010, mean temperature in July was 24 C (max 33, min 17) and the sum of rainfall 25 mm. In 2011, mean temperature in July was 19 C (max 36, min 13) and the sum of rainfall 17 mm. In 2012, mean temperature in July was 25 C (max 38, min 13) and the sum of rainfall 36 mm. In 2009, mean temperature in August was 24 C (max 33, min 15) and sum of rainfall 8 mm. In 2010, mean temperature in August was 23 C (max 33, min 14) and rainfall 5 mm. In 2011, mean temperature in August was 22 C (max 33, min 14) and rainfall 4 mm. In 2012, mean temperature in August was 24 C (max 39, min 10) and rainfall 6 mm.

### ***Assessment of the dispersal from infested maize into uninfested habitats***

Adult dispersal from the infested maize fields into the other habitat types (other crops and bare fields) was measured by placing three to four unbaited yellow sticky traps (type PheroconAM of Trece Company, USA) systematically on the middle row of each plot (Schroeder *et al*. 2005). In site A, 4 traps were placed per plot at 7, 14, 20 and 30 m distances to the adjacent infested maize field in 2009, and at 7, 14, 30 and 40 m in 2010 and 2011, and 10, 20, 30, and 40 m in 2012. In site B, 3 traps were placed at 7, 14 and 20 m distances in 2009 and 2010; at 9, 18 and 23 m in 2011, and at 2, 9, and 18 m in 2012. Traps were fixed onto wooden sticks just above the ground (15 cm) with the yellow sides facing rectangular to the infested maize field. This allowed monitoring of the adults since they are assumed to move on or near the ground for oviposition (Kiss *et al*. 2005a). Six traps were also placed in the infested large maize field section to estimate the source population of *D. v. virgifera*. All traps were checked weekly after the mass releases had been finalised (see above) and over the time period when mature *D. v. virgifera* were likely to perform dispersal for oviposition. This is usually from mid-July to September in southern Hungary (Toepfer and Kuhlmann 2006) following the 10 to 21 day pre-oviposition period (Branson and Johnson 1973; Hill 1975; Short and Hill 1972; Sherwood and Levine 1993). In site A, traps were checked from 23 July to 3 September 2009, from 29 July to 31 August in 2010, from 19 July to 21 September 2011 and from 17 July to 4 September 2012. In site B, traps were checked from 16 July to 2 September 2009, from 22 July to 30 August 2010, from 12 July to 21 September 2011, and from 16 July to 5 September 2012. Traps were exchanged once per month in 2009, and every 3 weeks in 2010, 2011 and 2012. The number of captured adults was recorded per trap, plot, crop habitat and week. Captured adults were sexed according to colour and antenna length (Staetz *et al*. 1976; Kuhar and Youngman 1995).

Capture data were standardised to captures per trap per week. The captures of females had been weighted by a factor of 3, due to the 3 fold higher capture probability of males in traps than females when not placed onto a maize stem (Godfrey and Turpin 1983, Kuhar and Youngman 1998). The distribution of capture data was visually investigated using histograms and Q Q plots, as well as through Kolmogorov-Smirnov statistics (Kinnear and Gray 2000). Most capture data remained non-normally distributed even after sqrt(x+1) or lg (x+1) transformation (Krebs 1999) because of the relatively high frequency of zero-captures of traps and the low dispersal into certain crops compared to others. Therefore captures were compared between crops using multiple pairwise contrast comparisons with a sequential Sidak procedure following GLZM (Kinnear and Gray 2000). Due to the large number of compared crops, a false discovery rate analysis was applied to reduce the number of false p-values < 0.05. P-values obtained from the Sidak tests were adjusted to q-values using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) via the software Q-VALUE (Storey, 2002; 2004) in R (R Development Core Team, 2009).

The population density in the infested maize field was defined by the number of released *D. v. virgifera* (see above). However, in terms of trap capture analyses, the population density in the infested maize field was defined by week with the highest average capture of the 6 traps, which was usually shortly after finalisation of mass releases.

To calculate the average proportion of a *D. v. virgifera* population dispersing from the infested maize into uninfested crops, capture data from the uninfested crops of the two study sites needed to be pooled. Therefore, capture data from both sites were standardised towards the overall population density in a study site, i.e. the density in the infested maize field plus the density in all uninfested crop habitats. This proportion of dispersing *D. v. virgifera* was calculated for each of the uninfested crops for a 4 week period (between the 4th and 8th week after first detection of *D. v. virgifera* emergence in the region) as well as for an 8 week period (4th to 12th week). The latter reflects the entire time period of dispersal activities of mature *D. v. virgifera*. Moreover, all proportions of dispersing *D. v. virgifera* were calculated for sexes separately and sexes pooled (Table C1). Proportional dispersal was compared between crop habitats using multiple pairwise Games Howell comparisons following GLM (Kinnear and Gray 2000). Due to the large number of compared crops, a false discovery rate analysis was applied to reduce the number of false p-values < 0.05. P-values obtained from the Games Howell tests were adjusted to q-values using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) via the software Q-VALUE (Storey, 2002; 2004) in R (R Development Core Team, 2009).

### *Assessment of factors influencing dispersal from infested maize into uninfested habitats*

At weekly intervals in July, August and September, the following habitat related factors were recorded for the infested maize field as well as for each of the other habitat types: (1) plant phenology of all crops, e.g. the % male and female flowering of maize, (2) % of crop plants with green foliage per crop, (3) % dried non-green plants per crop, (4) harvest, (5) soil preparations, (6) % crop cover. The flower ratio as well as the green plant ratio between the infested maize field and the uninfested crop habitats was calculated weekly. Moreover, weed species numbers, weed coverage and the most abundant weeds species were recorded from each plot in mid-July and mid-August. The most common weeds were *Abutilon theophrasti, Amaranthus blitoides, Amaranthus retroflexus, Ambrosia artemisifolia, Chenopodium album, Cirsium arvense, Convolvulus arvensis, Datura stramonium, Hibiscus trionum, Malva sp., Lamium sp., Setaria glauca, Sonchus arvensis, Sorghum halepense,* and *Xanthium orientale.*

Several independent GLM Univariate Analyses of Variances were used to detect which independent factors, such as study site, crop habitat, time periods, study year, and crop habitat related factors, influenced the captures per trap per week and the proportions of dispersing adults (both dependent factors). Associations between crop habitats related factors and dispersal was assessed through Pearson Correlation analyses.

### *Assessment of oviposition in uninfested habitats*

It was assumed that eggs oviposited in uninfested habitats hatch the following spring and its larvae develop on the roots of maize, i.e. in the maize field that replaced the different habitats due to rotation. Adult *D. v. virgifera* emergence was measured in two to three gauzed emergence cages (130 x 40 inner size x 120 cm height) each covering 6 to 7 plants, per plot of each of the habitats of previous year (Fig. C2). Emerging adult *D. v. virgifera* were collected and recorded weekly in site A from 28 June to 19 August 2010, 29 June to 8 August and from 21 June to 9 August 2012; in site B from 29 June to 14 August 2010, from 27 June to 10 August 2011, and from 22 June to 6 August 2012. The accumulated number of totally emerged adults were standardised as numbers per row metre per plot per crop habitat and site and year. Therefore, the proportional emergence in each crop habitat (reflecting the oviposition in the previous year) was calculated through standardising emergence data towards the overall absolute population density of emerged adults in each study site.

Proportional emergence (= oviposition in previous year) was compared between crop habitats using multiple pairwise Games Howell comparisons following GLM (Kinnear and Gray 2000). Due to the large number of compared crops, a false discovery rate analysis was applied to reduce the number of false p-values < 0.05. P-values obtained from the Games Howell tests were adjusted to q-values using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) via the software Q-VALUE (Storey, 2002; 2004) in R (R Development Core Team, 2009).

### *Assessment of factors influencing oviposition into uninfested habitats*

Several independent GLM Univariate Analyses of Variances were used to detect which independent factors (study site, crop habitat, time periods, study year, and habitat related factors) influenced the proportional emergence in the following year (dependent factors).

## Results

### *Dispersal from infested maize fields into uninfested habitats*

During the major *D. v. virgifera* dispersal activity (4 to 8 weeks after the start of emergence), on average **5.7 ± 2.9%** SEM of the released *D. v. virgifera* population dispersed into other habitat types (n = 8 sites and/or years, Fig. C1). The greatest percentage (30.7 ± 5%) of these dispersing adults moved into the uninfested maize plots.

When uninfested maize is not included in the analyses, then only **2.9 ± 1.2 %** of the entire population in the study sites dispersed into uninfested non-maize habitats (Fig. C1).

The population density of infested and released *D. v. virgifera* reached 52 adults per trap per week in the source (=infested) maize field of study site A in 2009, 7 adults in 2010, 12 in 2011 and 22 in 2012. In site B, the population density in the source field reached 8 adults per trap per week in 2009, 28 in 2010, 1 in 2011 and 2 in 2012. Far fewer adults were captured in the adjacent uninfested habitats (for details see Table C1, Fig. C1).

*Table C1 Captures of* ***dispersing D. v. virgifera*** *from infested maize into uninfested crop habitats in study site A and B (captures of adults on un-baited yellow sticky traps per week), and* ***emergence of D. v. virgifera*** *adults from maize in the subsequent year (adults totally emerged per row metre maize).*

*SD = standard deviation shown; N = 12 (=3 plots with four traps) for each uninfested crop habitat in study site A, 6 (=2 plots with three traps) for each uninfested crop habitat in study site B, 6 traps for infested maize field in study site A and B each year. Adult captures and corresponding time period only presented for the main oviposition period, i.e. between 4and 8 weeks after detection of first emergence in the regions. The captures of females were weighted by factor 3 due to the 3 fold higher capture probability of males in this trap type than females(Kuhar and Youngman 1998). Small letters in columns indicate significant differences of mean values of captured adults of both sexes between crops according to q values < 0.05 of the false discovery analyses of p values < 0.05 from multiple pairwise contrast comparisons with a sequential Sidak procedure following GLZM.*

| **Study site A 2009 to 2010** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 16 July – 12 August 2009) | | | | (Adults emerged / row metre 29 June – 19 August 2010) | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 21.12 | 18.40 | 2.72 | e | 4.07 | 2.1 | 2.0 | c |
| | SD | 7.28 | 7.02 | 2.97 | | 2.3 | 1.6 | 1.1 | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 6.46 | 5.21 | 1.25 | d | 6.91 | 3.0 | 4.0 | c |
| | SD | 3.19 | 2.44 | 1.14 | | 3.0 | 1.7 | 1.7 | |
| Peas harvested and grubbed | Mean | 0.17 | 0.08 | 0.08 | a | 0.12 | 0.1 | 0.0 | b |
| | SD | 0.34 | 0.21 | 0.29 | | 0.3 | 0.3 | 0.0 | |
| Ploughed bare soil | Mean | 0.37 | 0.28 | 0.10 | ab | 0.00 | 0.0 | 0.0 | a |
| | SD | 0.52 | 0.29 | 0.35 | | 0.0 | 0.0 | 0.0 | |
| Potatoes | Mean | 0.93 | 0.68 | 0.25 | b | 0.00 | 0.0 | 0.0 | a |
| | SD | 0.95 | 0.67 | 0.45 | | 0.0 | 0.0 | 0.0 | |
| Rape harvested and grubbed | Mean | 0.38 | 0.05 | 0.33 | ab | 0.25 | 0.0 | 0.2 | b |
| | SD | 0.47 | 0.12 | 0.49 | | 0.6 | 0.0 | 0.6 | |
| Sorghum (Millet) | Mean | 3.26 | 2.41 | 0.85 | c | 0.99 | 0.5 | 0.5 | b |
| | SD | 1.21 | 0.95 | 1.21 | | 0.9 | 0.6 | 0.4 | |
| Wheat harvested and grubbed | Mean | 0.63 | 0.18 | 0.45 | ab | 0.37 | 0.1 | 0.2 | b |
| | SD | 0.78 | 0.15 | 0.73 | | 0.6 | 0.3 | 0.4 | |
| Wheat harvested not grubbed | Mean | 0.53 | 0.44 | 0.08 | ab | 0.25 | 0.1 | 0.1 | b |
| | SD | 0.60 | 0.60 | 0.29 | | 0.4 | 0.3 | 0.3 | |

| **Study site A 2010 to 2011** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 23 July - 29 August 2010) | | | | (Adults emerged / row metre 29 June – 8 August 2011) | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 5.63 | 2.33 | 3.30 | d | 3.7 | 5.9 | 9.63 | d |
| | SD | 2.08 | .32 | 1.87 | | 2.7 | 3.4 | 6.1 | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 2.75 | 2.18 | 0.59 | c | 1.6 | 1.7 | 3.29 | c |
| | SD | 3.20 | 2.83 | 1.21 | | 2.2 | 2.0 | 4.1 | |
| Peas harvested and grubbed | Mean | 0.07 | 0.07 | 0.00 | a | 0.1 | 0.0 | 0.08 | a |
| | SD | 0.13 | 0.13 | 0.00 | | 0.2 | 0.0 | 0.2 | |
| Ploughed bare soil | Mean | 0.38 | 0.15 | 0.23 | b | 0.0 | 0.0 | 0.00 | a |
| | SD | 0.47 | 0.23 | 0.42 | | 0.0 | 0.0 | 0.0 | |
| Potatoes | Mean | 0.08 | 0.08 | 0.00 | a | 0.0 | 0.2 | 0.25 | ab |
| | SD | 0.14 | 0.14 | 0.00 | | 0.0 | 0.5 | 0.5 | |
| Rape harvested and grubbed | Mean | 0.14 | 0.00 | 0.14 | ab | 0.2 | 0.2 | 0.41 | ab |
| | SD | 0.33 | 0.00 | 0.33 | | 0.4 | 0.3 | 0.5 | |
| Sorghum (Millet) | Mean | 0.12 | 0.05 | 0.08 | ab | 0.4 | 0.3 | 0.74 | b |
| | SD | 0.32 | 0.12 | 0.26 | | 0.7 | 0.4 | 0.6 | |
| Sugar beet | Mean | 0.02 | 0.02 | 0.00 | a | 0.4 | 0.9 | 1.3 | b |
| | SD | 0.07 | 0.07 | 0.00 | | 1.2 | 1.4 | 2.3 | |
| Wheat harvested and grubbed | Mean | 0.26 | 0.08 | 0.18 | ab | 0.0 | 0.2 | 0.25 | ab |
| | SD | .44 | 0.16 | 0.33 | | 0.0 | 0.4 | 0.4 | |
| Wheat harvested not grubbed | Mean | 0.09 | 0.03 | 0.07 | a | 0.1 | 0.0 | 0.08 | a |
| | SD | 0.24 | 0.09 | 0.23 | | 0.2 | 0.0 | 0.2 | |

| **Study site A 2011 to 2012** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 19 July - 15 August 2011) | | | | (Adults emerged / row metre 21 June – 9 August 2012) | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 2.7 | 2.6 | 5.3 | c | 2.0 | 1.2 | 0.9 | c |
| | SD | 1.6 | 1.0 | 2.0 | | 2.1 | 1.3 | 0.8 | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 6.4 | 11.9 | 18.3 | d | 1.5 | 0.9 | 0.6 | bc |
| | SD | 5.1 | 10.3 | 14.5 | | 1.2 | 0.8 | 0.5 | |
| Peas harvested and grubbed | Mean | 0.1 | 0.0 | 0.1 | a | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.1 | 0.0 | 0.1 | | 0.1 | 0.0 | 0.1 | |
| Potatoes | Mean | 0.0 | 0.1 | 0.1 | a | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.1 | 0.3 | 0.3 | | 0.1 | 0.1 | 0.0 | |
| Rape harvested and grubbed | Mean | 0.0 | 0.1 | 0.1 | a | 0.1 | 0.1 | 0.0 | ab |
| | SD | 0.1 | 0.3 | 0.3 | | 0.1 | 0.1 | 0.1 | |
| Sorghum (Millet) | Mean | 0.3 | 0.4 | 0.7 | ab | 0.1 | 0.1 | 0.0 | ab |
| | SD | 0.3 | 0.5 | 0.6 | | 0.2 | 0.2 | 0.1 | |
| Sorghum (Sudangrass) | Mean | 0.2 | 0.7 | 0.9 | b | 0.8 | 0.6 | 0.2 | b |
| | SD | 0.4 | 1.5 | 1.6 | | 0.4 | 0.5 | 0.2 | |
| Sugar beet | Mean | 0.0 | 0.1 | 0.1 | a | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.0 | 0.3 | 0.3 | | 0.1 | 0.1 | 0.1 | |
| Wheat harvested and grubbed | Mean | 0.1 | 0.1 | 0.2 | ab | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.1 | 0.3 | 0.3 | | 0.1 | 0.1 | 0.0 | |
| Wheat harvested not grubbed | Mean | 0.2 | 0.2 | 0.4 | ab | 0.1 | 0.0 | 0.0 | ab |
| | SD | 0.2 | 0.4 | 0.4 | | 0.1 | 0.1 | 0.1 | |

| **Study site A 2012** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 17 July - 14 August 2012) | | | | No data for 2013 | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 23.1 | 7.6 | 15.5 | e | | | | |
| | SD | 12.5 | 2.26 | 10.32 | | | | | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 7.9 | 3.3 | 4.59 | d | | | | |
| | SD | 12.8 | 5.04 | 7.82 | | | | | |
| Peas harvested and grubbed | Mean | 0.26 | 0.15 | 0.08 | a | | | | |
| | SD | 0.29 | 0.22 | 0.26 | | | | | |
| Potatoes | Mean | 0.75 | 0.46 | 0.3 | bc | | | | |
| | SD | 1.15 | 0.58 | 0.62 | | | | | |
| Sorghum (Millet) | Mean | 0.82 | 0.52 | 0.3 | c | | | | |
| | SD | 0.92 | 0.72 | 0.44 | | | | | |
| Sorghum (Sudangrass) | Mean | 0.45 | 0.23 | 0.23 | b | | | | |
| | SD | 0.6 | 0.27 | 0.64 | | | | | |
| Sugar beet | Mean | 0.51 | 0.31 | 0.2 | bc | | | | |
| | SD | 0.44 | 0.26 | 0.37 | | | | | |
| Wheat harvested and grubbed | Mean | 0.37 | 0.24 | 0.13 | ab | | | | |
| | SD | 0.36 | 0.24 | 0.29 | | | | | |
| Wheat harvested not grubbed | Mean | 0.23 | 0.23 | 0.00 | a | | | | |
| | SD | 0.34 | 0.34 | 0.00 | | | | | |

| **Study site B 2009 to 2010** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 16 July - 12 August 2009) | | | | (Adults emerged / row metre 29 June – 14 August 2010) | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 2.25 | 1.85 | 0.40 | b | 2.0 | 1.3 | 0.7 | c |
| | SD | 1.19 | 1.26 | 0.70 | | 2.0 | 1.3 | 0.9 | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 1.97 | 1.02 | 0.95 | b | 0.7 | 0.2 | 0.6 | ab |
| | SD | 1.96 | 0.71 | 1.27 | | 1.5 | 0.4 | 1.1 | |
| Rape harvested and grubbed | Mean | 0.30 | 0.07 | 0.23 | a | 0.2 | 0.2 | 0.0 | a |
| | SD | 0.48 | 0.16 | 0.36 | | 0.4 | 0.4 | 0.0 | |
| Sorghum (Sudan grass) | Mean | 0.23 | 0.23 | 0.00 | a | 1.5 | 0.7 | 0.7 | bc |
| | SD | 0.27 | 0.27 | 0.00 | | 2.1 | 1.0 | 1.0 | |

| **Study site B 2010 to 2011** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 22 July - 14 August 2010) | | | | (Adults emerged / row metre 26 June – 10 August 2011) | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 4.5 | 3.0 | 1.5 | d | 0.0 | 0.4 | 0.37 | b |
| | SD | 2.25 | 1.06 | 1.20 | | 0.0 | 0.5 | 0.52 | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 1.50 | 1.20 | 0.30 | c | 0.9 | 0.9 | 1.85 | c |
| | SD | 0.94 | 0.60 | 0.33 | | 0.7 | 1.1 | 1.28 | |
| Ploughed bare soil | Mean | 0.07 | 0.07 | 0.00 | a | 0.0 | 0.0 | 0.00 | a |
| | SD | 0.14 | 0.14 | 0.00 | | 0.0 | 0.0 | 0.00 | |
| Sorghum (Millet) | Mean | 0.13 | 0.13 | 0.00 | ab | 0.0 | 0.0 | 0.00 | a |
| | SD | 0.22 | 0.22 | 0.00 | | 0.0 | 0.0 | 0.00 | |
| Sorghum (Sudan grass) | Mean | 0.22 | 0.11 | 0.11 | b | 0.2 | 0.0 | 0.19 | ab |
| | SD | 0.44 | 0.17 | 0.29 | | 0.4 | 0.0 | 0.37 | |
| Wheat harvested not grubbed | Mean | 0.07 | 0.07 | 0.00 | a | 0.4 | 0.0 | 0.37 | b |
| | SD | 0.10 | 0.10 | 0.00 | | 0.4 | 0.0 | 0.43 | |

| **Study site B 2011 to 2012** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 12 July - 17 August 2011) | | | | (Adults emerged / row metre 22 June - 6 August 2012) | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 0.1 | 0.1 | 0.2 | b | 0.1 | 0.0 | 0.0 | ab |
| | SD | 0.2 | 0.3 | 0.3 | | 0.1 | 0.1 | 0.1 | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 0.0 | 0.0 | 0.0 | a | 0.2 | 0.2 | 0.0 | b |
| | SD | 0.1 | 0.0 | 0.1 | | 0.2 | 0.2 | 0.0 | |
| Ploughed bare soil | Mean | 0.1 | 0.0 | 0.1 | ab | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.3 | 0.0 | 0.3 | | 0.0 | 0.0 | 0.0 | |
| Potatoes | Mean | 0.0 | 0.0 | 0.0 | a | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 | 0.0 | |
| Peas harvested and grubbed | Mean | 0.1 | 0.0 | 0.1 | ab | 0.0 | 0.0 | 0.0 | a |
| | SD | 0.2 | 0.0 | 0.2 | | 0.0 | 0.0 | 0.0 | |

| **Study site B 2012** | | **Dispersing adult *D. v. virgifera*** | | | | **Oviposition of dispersing *D. v. virgifera*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | (Adults / trap / week 16 July - 14 August 2012) | | | | No data for 2013 | | | |
| **Crop habitat** | | **Both Sexes** | **Males** | **Females *** | **Diff.** | **Both Sexes** | **Males** | **Females** | **Diff.** |
| **Infested maize field** | | | | | | | | | |
| | Mean | 0.18 | 0.18 | 0.0 | b | | | | |
| | SD | 0.21 | 0.21 | 0.0 | | | | | |
| **Uninfested crop habitats** | | | | | | | | | |
| Maize | Mean | 0.1 | 0.1 | 0.0 | ab | | | | |
| | SD | 0.17 | 0.17 | 0.0 | | | | | |
| Ploughed bare soil | Mean | 0.0 | 0.0 | 0.0 | a | | | | |
| | SD | 0.0 | 0.0 | 0.0 | | | | | |
| Potatoes | Mean | 0.0 | 0.0 | 0.0 | a | | | | |
| | SD | 0.0 | 0.0 | 0.0 | | | | | |
| Peas harvested and grubbed | Mean | 0.0 | 0.0 | 0.0 | a | | | | |
| | SD | 0.0 | 0.0 | 0.0 | | | | | |
| Sugar beet | Mean | 0.1 | 0.1 | 0.0 | ab | | | | |
| | SD | 0.15 | 0.15 | 0.0 | | | | | |

### *Factors influencing dispersal from infested maize into uninfested habitats*

#### *Habitat type*

The habitat type influenced the proportion of dispersing *D. v. virgifera* adults regardless of whether both sexes were analysed together or separately, and regardless of whether a four week period of dispersal was considered or an eight week period, i.e. the entire period of dispersal (GLM: 4 weeks both sexes: $F_{(10, 567)} = 12.7$, Adjusted $R^2 = 0.22$, $P < 0.001$, males: $F_{(10, 567)} = 13.8$, Adjusted $R^2 = 0.24$, $P < 0.001$ females: $F_{(10, 567)} = 9.1$, Adjusted $R^2 = 0.17$, $P < 0.001$; 8 weeks both sexes: $F_{(10, 566)} = 14.3$, Adjusted $R^2 = 0.25$, $P < 0.001$, males: $F_{(10, 566)} = 13.8$, Adjusted $R^2 = 0.24$, $P < 0.001$ females: $F_{(10, 566)} = 10.1$, Adjusted $R^2 = 0.18$, $P < 0.001$;

For the dispersing *D. v. virgifera,* maize was the most attractive habitat among the 10 tested habitat types, regardless of whether males and female adults were considered separately or together (Fig. C1). The second most attractive for both sexes pooled was millet. On average across fields and years, significantly fewer dispersing *D. v. virgifera* adults were caught in all other habitat types., . Field management practices (stubble grubbing of winter wheat after harvest versus no stubble grubbing) did not affect the dispersal behaviour of the adults.

For males, the second most attractive crop was millet, followed by ploughed bare soil, Sudangrass, harvested and grubbed peas, harvested and grubbed winter wheat, sugar beet, and potatoes (Fig. C1). Harvested and grubbed winter rape, harvested but not grubbed winter wheat were, on average across fields and years, were the least visited by males.

For females, after maize, the next most attractive crops were was millet, ploughed bare soil, Sudangrass, harvested and grubbed winter wheat, harvested and grubbed winter rape, and potatoes (Fig. C1). On average across fields and years, few females dispersed into all other habitat types.

Harvesting an uninfested crop habitat slightly negatively influenced the proportion of immigrating adults (Pearson $r = -0.1$, $P= 0.002$, $n = 2624$). The larger the proportion of green maize plants in the infested maize field, the less adults dispersed into the adjacent habitats ($r = -0.1$, $P= 0.001$, $n = 2624$). The larger the proportion of dried maize plants in the infested maize field, the more adults emigrated ($r = 0.12$, $P< 0.001$, $n = 2624$). All other crop habitat related factors as well as weed characteristics had no detectable effect on the adult dispersal.

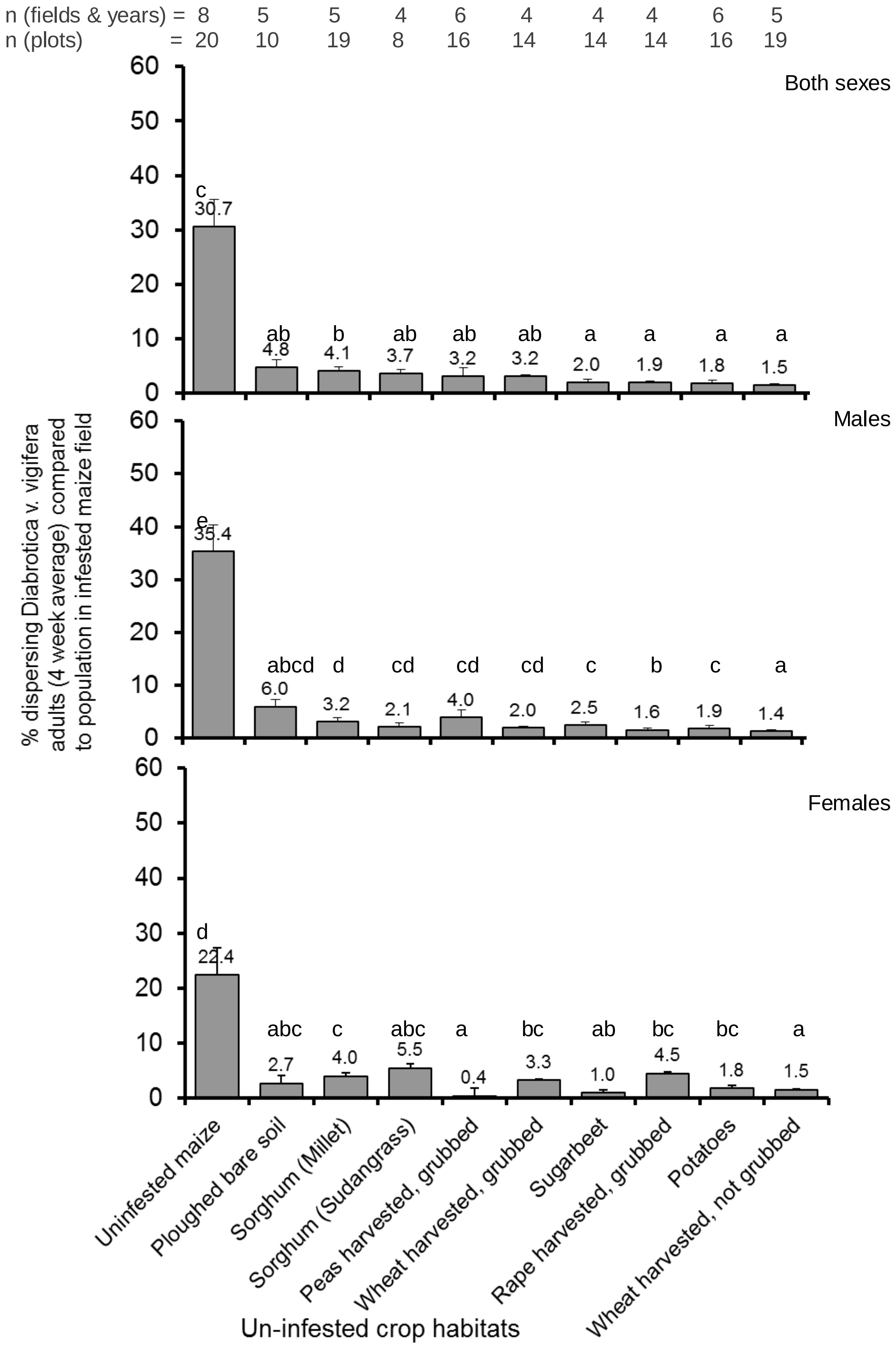

n (fields & years) = 8 5 5 4 6 4 4 4 6 5
n (plots) = 20 10 19 8 16 14 14 14 16 19
Both sexes
c 30.7
ab 4.8
b 4.1
ab 3.7
ab 3.2
ab 3.2
a 2.0
a 1.9
a 1.8
a 1.5
Males
e 35.4
abcd 6.0
d 3.2
cd 2.1
cd 4.0
cd 2.0
c 2.5
b 1.6
c 1.9
a 1.4
Females
d 22.4
abc 2.7
c 4.0
abc 5.5
a 0.4
bc 3.3
ab 1.0
bc 4.5
bc 1.8
a 1.5
0
10
20
30
40
50
60
% dispersing Diabrotica v. vigifera adults (4 week average) compared to population in infested maize field
Uninfested maize
Ploughed bare soil
Sorghum (Millet)
Sorghum (Sudangrass)
Peas harvested, grubbed
Wheat harvested, grubbed
Sugarbeet
Rape harvested, grubbed
Potatoes
Wheat harvested, not grubbed
Un-infested crop habitats

*Fig. C1* ***Average proportions of D. v. virgifera adults dispersing*** *from infested maize fields into small fields of different uninfested crop habitats. Experiments conducted in two sites in southern Hungary in 2009, 2010, 2011 and 2012. Dispersal assessed through captures of adults on un-baited yellow sticky traps placed 15 cm above ground. Small letters on bars indicate significant differences according to fdr-corrected Games Howell multiple pairwise comparisons at P<0.05 following GLM. Error bars = SEM.*

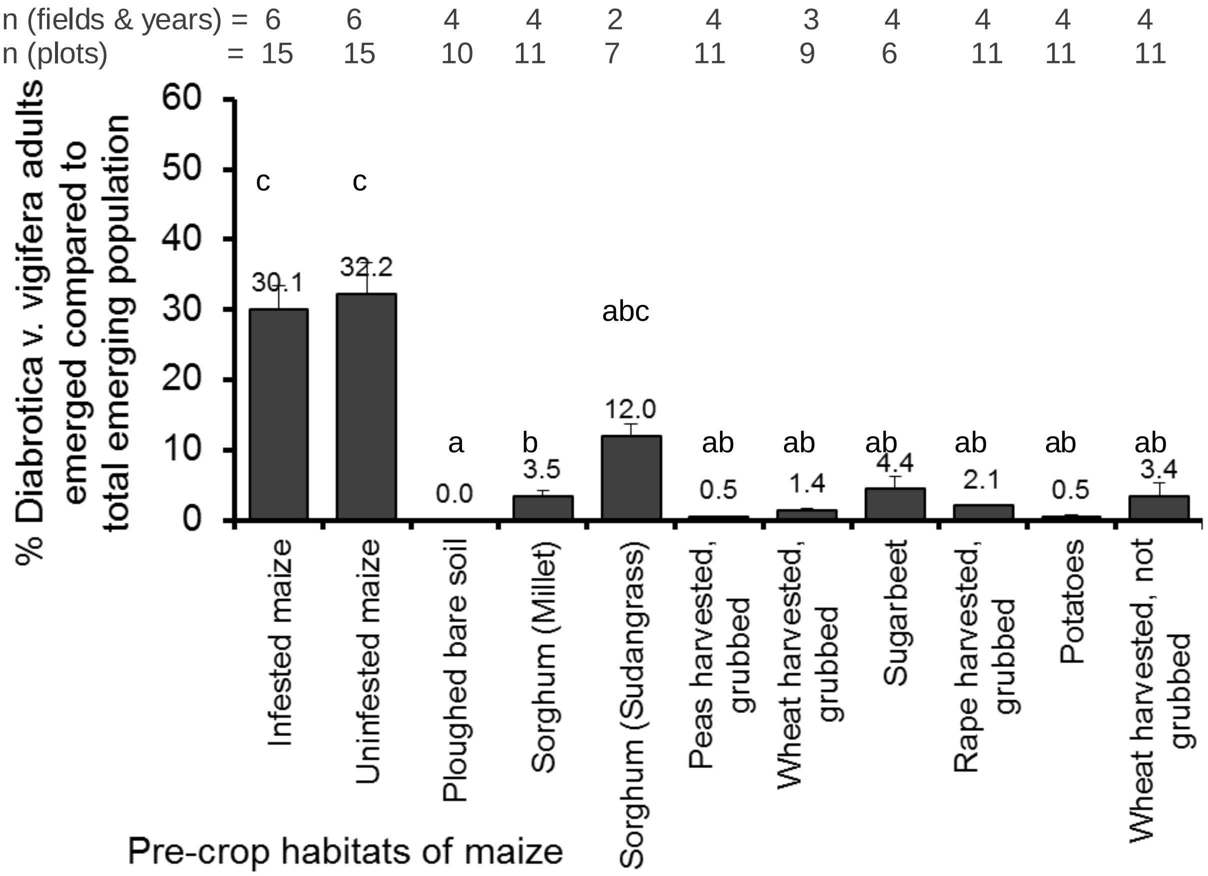


*Fig. C2* ***Average proportions of D. v. virgifera ovipositing into different pre-crops of maize,*** *measured as adults emerging from maize the following year compared to totally emerging adults in the study site. Experiments conducted in two sites from 2009 to 2010, from 2010 to 2011 and from 2011 to 2012, each with 2 to 3 plots per pre-crop habitat, field and year. Two to three emergence cages per plot. Small letters on bars indicate significant differences according to fdr-corrected Games Howell multiple pairwise comparisons at P<0.05 following GLM. Error bars = SEM.*

*Time period and year*

The size of the source population in the infested maize fields decreased with time, i.e. mainly during late July and early August. Consequently, the absolute number of dispersing *D. v. virgifera* into uninfested crop habitats decreased with time (Fig. C3) (GLM: $F_{(13, 4911)} = 7.8$, Adjusted $R^2 = 0.02$, $P < 0.001$). The temporal decrease of absolute numbers of dispersing adults was found in all crop habitats. By mid-August (from 7th week after start of emergence in a region onwards), most crop habitats no longer hosted large numbers of immigrating *D. v. virgifera* even though some habitat types still offered potential pollen sources (e.g. Sudan grass, millet) or green leaves (e.g. sugar beet, Sudan grass, millet, or re-growing winter wheat, winter rape or peas).

Despite the overall decrease of *D. v. virgifera* numbers over time, the proportion of adults dispersing from the infested maize field into the uninfested crop habitats increased with time (Fig. C3) (GLM: $F_{(8\ 2719)} = 4.4$, Adjusted $R^2 = 0.01$, $P < 0.001$). On average, between 10 and 39 % of the entire *D. v. virgifera* population dispersed more or less constantly between late July and mid-August from the infested maize field into uninfested crop habitats (Fig. C3). From mid-August onwards, the proportion of *D. v. virgifera* dispersing away from the infested maize field increased reaching up to 100% by mid-September, i.e. when the maize fields were usually harvested or dry.

In general, the study year influenced the dispersal of *D. v. virgifera* from the infested maize field into the uninfested crop habitats (e.g. for both sexes for 4 week period of major dispersal: GLM: $F_{(3, 1023)} = 9.6$, Adjusted $R^2 = 0.025$, $P < 0.001$). In 2011, proportionally more adults dispersed during the major dispersal period (14.3 ± 2.9 % SEM) than in year 2012 (5.1± 1.3 %) and 2010 (4 ± 0.9 %); and least dispersed in 2009 (2.9 ± 0.4 %).

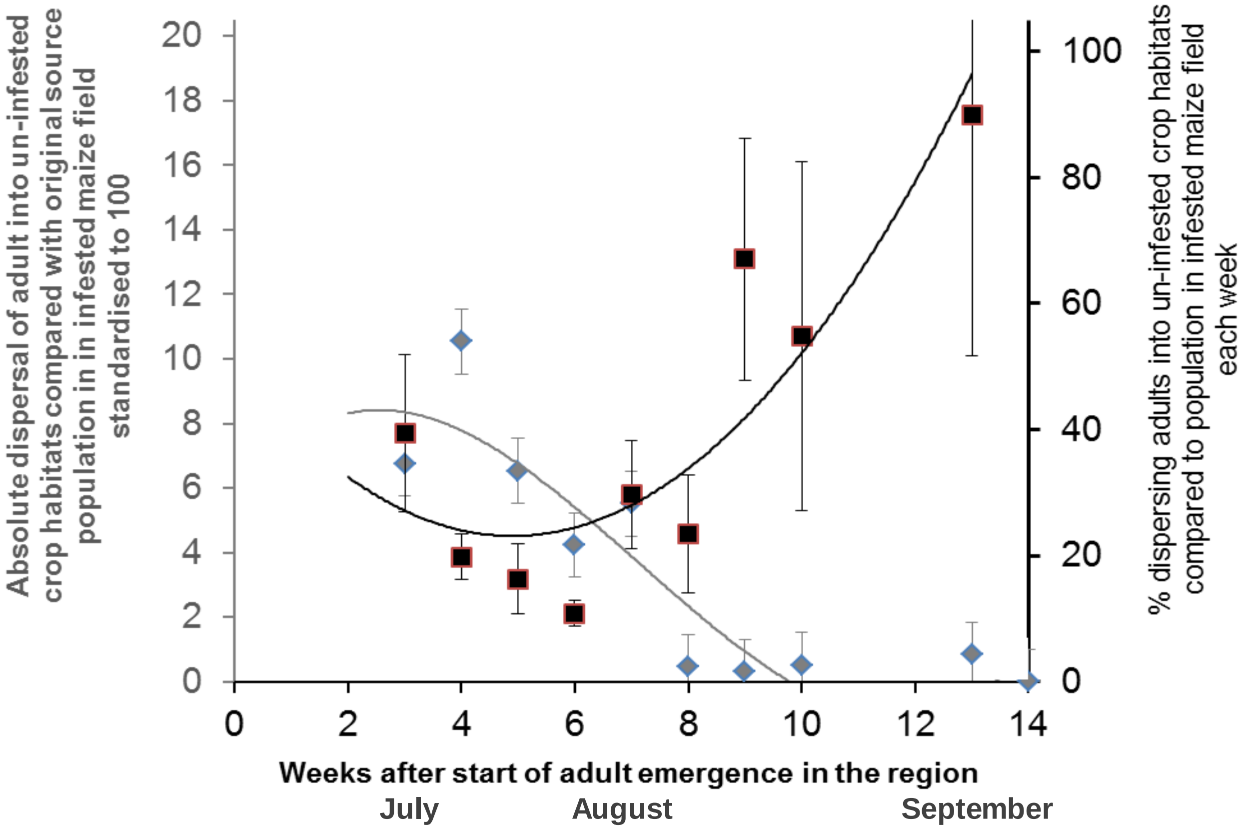


Fig. C3 **Temporal pattern of absolute and proportional dispersal** *of D. v. virgifera adults from infested maize into uninfested crop habitats. Absolute dispersal compared to the size of the original source population in the infested maize field, standardised to 100. Proportional dispersal per week compared to entire population in the study site per week. Field sites A and B as well as years 2009, 2010, 2011, 2012. Dispersal assessed through captures of adults on un-baited yellow sticky traps placed 15 cm above ground. Harvest of maize in site A took place 30 August 2009, 29 August 2010, 25 August 2011 and end August 2012. Harvest in site B took place 10 September 09, 20 September 2010, November 2011, and 7 August 2012.*

### *Distance*

Within the studied range of 0 to 60 metres, the distance did not influence the dispersal of *D. v. virgifera* adults from the infested maize field into the adjacent habitats (GLM: $F_{(10; 2719)}$ =1.2, Adjusted $R^2$ = 0.001, P =0.279) (Fig. C4). The distance slightly influenced the dispersal of males from the infested maize field into the adjacent uninfested crop habitats (GLM: $F_{(10; 2719)}$ =3.9, Adjusted R2 = 0.011, P <0.001). This distance effect was not proven for females (GLM: $F_{(10; 2719)}$ =0.7, Adjusted R2 = 0.001, P =0.764). No prediction can be made on distance effects for non-adjacent fields as well as for large scale agriculture.

### *Study site*

The site location did not influence the weekly captures of *D. v. virgifera* in the adjacent habitats during the 4 and 8 week period considered (e.g. for both sexes for 4 week period of major dispersal: GLM $F_{(1, 2719)}$ =0.4, Adjusted $R^2$ = 0.0001, P = 0.516). The site location did not influence the dispersing proportion of the *D. v. virgifera* population (e.g. for both sexes for the 4 week period of major dispersal: GLM $F_{(1, 1023)}$ =3.8, Adjusted $R^2$ = 0.003, P = 0.051).

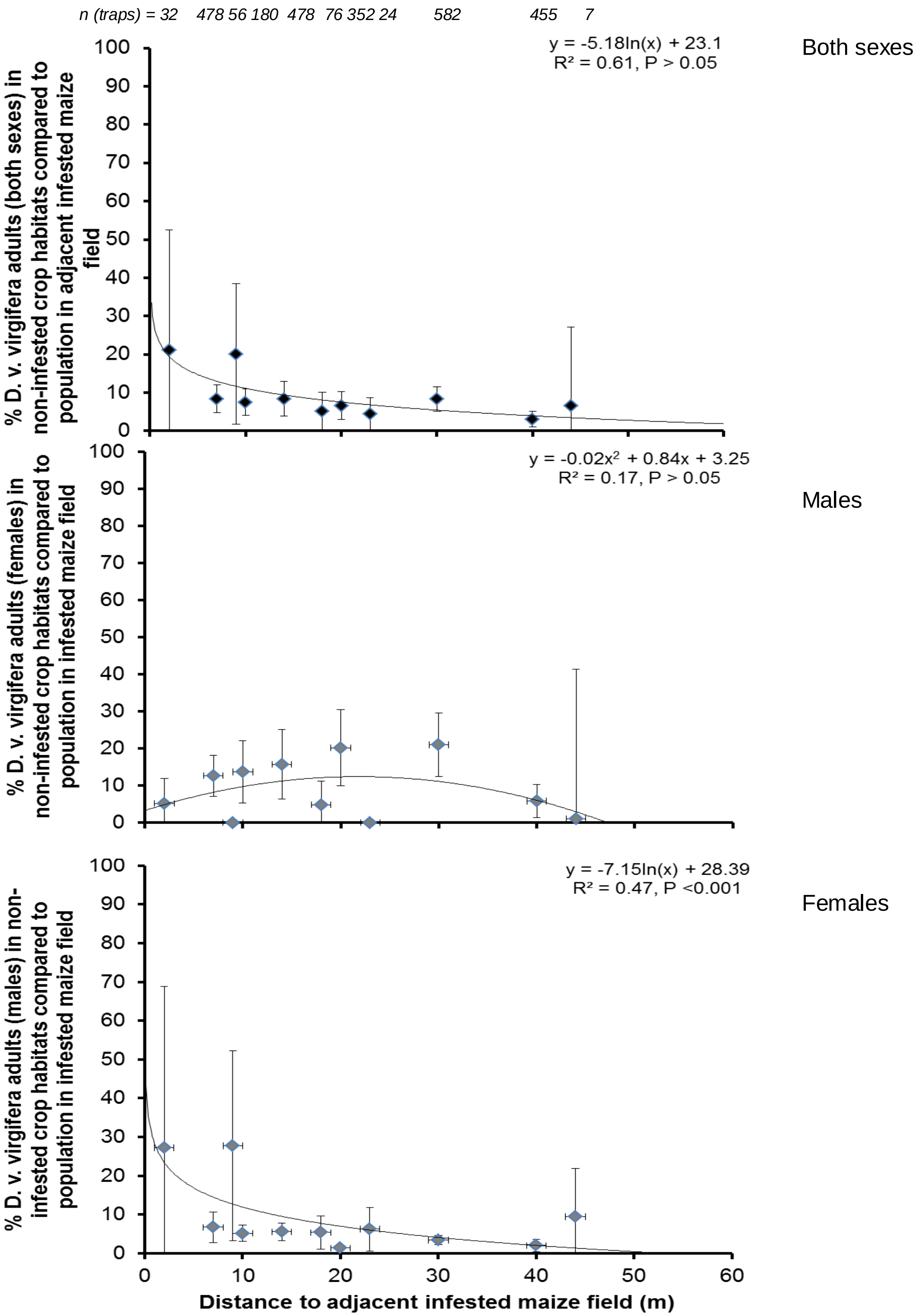


*Fig. C4* ***Dispersal distance of D. v. virgifera adults*** *from infested maize into adjacent uninfested crop habitats in study sites A and B in southern Hungary in 2009, 2010, 2011 and 2012. Dispersal shown as the average captures of adults on unbaited yellow sticky traps 15 cm above ground per week at different distances over the main oviposition period of D. v. virgifera (4th to 8th week after first detection of emergences start of D. v. virgifera in the region) in comparison to entire population of study site each week. Data of all tested crop habitats pooled. P values according to GLM.*

### *Oviposition of D. v. virgifera in uninfested habitats*

Some *D. v. virgifera* emerged from maize in 2010, 2011 and 2012 as a result of oviposition of dispersing *D. v. virgifera* into uninfested crop habitats in the corresponding previous year. On average across study sites and years, about **6 ± 3.1% SEM** of the entire *D. v. virgifera* population in the study sites emerged from maize after different uninfested pre-crop habitats of the previous year (Fig. C2). It appeared that the *D. v. virgifera* population from infested-maize fields had dispersed and oviposited over the entire maize area, regardless of whether the maize was their natal field (the infested field) or not (the uninfested field) (Table C1, Fig. C2). When not including uninfested maize in the analyses, then only about **3.1± 3.9** % of the entire *D. v. virgifera* population in the study sites emerged from maize as a result of oviposition in uninfested non-maize crop habitats.

### *Factors influencing the oviposition of D. v. virgifera into uninfested habitats*

#### *Habitat type*

The type of the pre- crop habitat influenced the oviposition into the pre-crop and consequently the emergence from maize the following year (GLM: $F_{(10,243)} = 24.9$, Adjusted $R^2 = 0.38$, $P < 0.001$).

Most adults emerged in comparable numbers from all plots of the pre-crop habitat maize (Fig. C2). This means *D. v. virgifera* adults dispersed equally over maize fields for oviposition, regardless of whether the maize field was their natal, i.e. the infested maize field or not. Relatively high proportions of adults also emerged from maize plots following the pre-crop habitat millet (Table C1, Fig. C2). To a lesser extent, adults also emerged from maize plots after most other pre-crop habitats, wherein a potential larger extent of oviposition into Sudangrass was statistically not proven due to limited replicate number (Fig. C2). No adults were observed to emerge from maize in habitats that were previously ploughed, bare soil.

In general, crops still not harvested or at least not grubbed after harvest during the *D. v. virgifera* oviposition period such as millet, Sudan grass sugar beet and harvested but not grubbed winterwheat appeared more attractive for oviposition (together 5.8 ± 1.3% SEM oviposition) than crop habitats that had experienced soil preparations, such as the ploughed bare soil, harvested and grubbed peas, harvested and grubbed winterwheat or harvested and grubbed winter rape (together 1 ± 0.3% oviposition).

The proportional amount of emerging adults from maize after different pre-crop habitats was only slightly related to the proportion of dispersing adults to these habitats in the previous year (particularly true for maize, millet, rape, see Fig. C1 versus Fig C2). In contrast, the dispersal over ploughed bare soil, harvested and grubbed peas, as well as harvested and grubbed winterwheat or potatoes did not relate to a subsequent level of oviposition. In contrast, the dispersal over Sudangrass, sugar beet and harvested non-grubbed winterwheat seemed to have led to more oviposition than expected.

None of the crop habitat related factors had any detectable effect on the proportional oviposition, i.e. plant phenology of crops, % of green foliage per crop, % dried non-green plants of crop, % crop cover, the flower ratio as well as the green plant ratio between infested maize fields and the uninfested crop habitats, weed species numbers, weed coverage and the most abundant weeds species

#### *Year*

The year did not influence the proportional oviposition into the pre-crop and consequently the emergence from maize the following year (GLM: $F_{(2,261)} = 1.8$, $P = 0.19$).

*Distance*

Within the studied range of 0 to 60 metres, the distance to the adjacent infested maize field did not statistically influence the proportional oviposition into the pre-crop and consequently the emergence from maize the following year (GLM: F $_{(40,243)}$ =1.4, Adjusted R square = 0.06, P =0.09). However, at distances > 40 metres hardly any oviposition was detected.

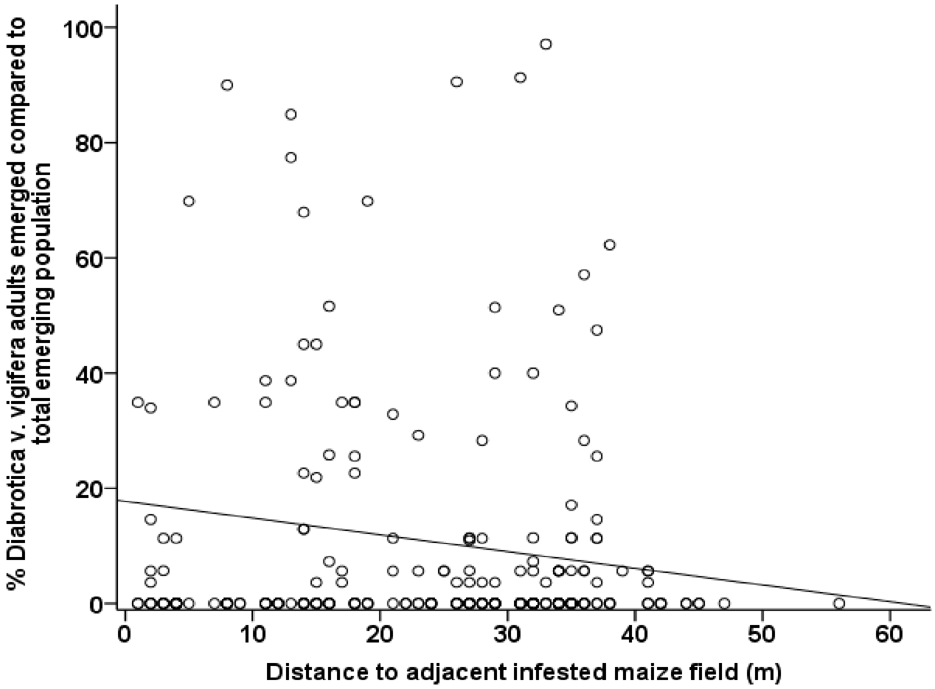


*Fig. C5* ***Oviposition distance of D. v. virgifera adults*** *from infested maize into adjacent uninfested crop habitats measured as adults emerging from maize after pre-crops compared to totally emerging adults in the study site. Experiments conducted in two sites from 2009 to 2010, from 2010 to 2011 and from 2011 to 2012, each with 2 to 3 plots per pre-crop habitat, field and year. Two to three emergence cages per plot. Linear regression of R square = 0.021 not significant according to P value of 0.09 of GLM.*

*Study site*

The study site did not influence the proportional oviposition into the pre-crop and consequently the emergence from maize the following year (GLM: F $_{(1,261)}$ =4.1, P =0.06).

## Discussion

The here reported mass-release recapture experiments of *D. v. virgifera* adult populations in crop rotation situations, revealed that only a relatively small but significant proportion of adults fly from source maize fields into other crop habitats and also lays eggs there. When considering the dispersal studies of Toepfer et al. (2005, 2006) and Carrasco et al. (2010), then significant dispersal only occurs within a 3 km radius, and the majority of dispersal only occurs close to the source, such as assumed in the here-presented study. Long distance dispersal as reported by Ciosi et al. (2011) is only relevant for the large scale spread of the species, and in situations where eradication is the aim. But it has little relevance for in-field or regional pest management. In any case, eggs layed in crop habitats that were previously uninfested in the previous year will lead to larvae that can survive and develop if maize is grown at the location in the following year (Kiss et al.2005; Spencer et al. 2005).

However, the here reported average dispersal of 5.7 ± 2.9% from infested maize fields into adjacent crop habitats, as well as the 6 ± 3.1% oviposition are, due to the set-up of the study, only valid for adjacent field situations as well as for a small scale landscape, i.e. with field sizes of about 1 ha or less. Thus for small scale agriculture, such as in northern Italy or Croatia, the here presented results are relevant. As for small scale agriculture of Croatia, already Barcic et al. (2007) have shown that significant egg laying in fields adjacent to highly infested maize fields might occur. More generally, a study by Szalai et al. (2010) in a 20 km agricultural area with different small and large field sizes showed that the infestation in continuous maize fields accounted for more than 60% of the variation in the adult *D. v. virgifera* captures in the adjacent first-year maize fields. This clearly indicated that adjacent maize fields are the major source of dispersal into first-year maize and not, or to a lesser extent, the area-wide infestation levels as for example reported by Beckler et al (2004) for the USA corn belt. Taking the above mentioned studies into account, then we may suggest that oviposition into previously un-infested crop habitats and potential development of larvae in maize due to crop rotation the following year is negligible for non-adjacent field situations as well as for agriculture with average field sizes above 1 ha.

For small scale agriculture, the question rises whether the here-reported oviposition may lead to populations above threshold the following year. When only considering maize fields, our study showed that the majority of the *D. v. virgifera* population from infested maize fields had dispersed and oviposited over the entire maize area, regardless of whether the maize was their natal field (the infested field) or not (the uninfested field). This means for cases that an infested maize field is adjacent to a similar size non-infested maize field that the population would be diluted by about half. This also means, that in case of high source populations, i.e. of a size close to the economic threshold, then despite the dilution effect the economic threshold could be reached the following year in the original continuous and the second year maize field. This is due to the fact that the average generational growth rate of *D. v. virgifera* was reported to be factor 4 (Szalai et al. 2010). However, in situations where more maize fields or larger maize fields or more distant maize fields are situated around or in the area of an infested source maize field, then the percentage of dispersal and egg laying will be much less. In this situation, the risk of experiencing *D. v. virgifera* population in second year maize fields is minimal. The same is true, for all other crops than maize, despite the here reported about 2.9 ± 1.2 % dispersal and 3.1± 3.9 % oviposition. Even in rare cases of a large initial *D. v. virgifera* population in the natal maize field, combined with an optimal year of generational growth (max is about 13x Szalai et al. 2010), and in small scale farming, damage is unlikely in the first year maize grown due to crop rotation the following year.

Dispersal decreased over time in all crop habitats. Most crop habitats no longer hosted large numbers of immigrating *D. v. virgifera* by mid-August (from 7th week after start of emergence in a region onwards), even though some crop habitats still offered potential pollen sources (e.g. Sudan grass, Millet) or green leaves (e.g. Sugar beet, Sudan grass, Millet, or re-growing winter wheat, winter rape and peas). Therefore, the small number of adults captured was not due to decreasing attractiveness of certain crops but rather a general decrease in the *D. v. virgifera* population in the experimental areas due to natural mortality over time. There is no information on how attractive the different crop habitats would be during end June to early July (no traps were placed during this period because the mass release was on-going). However, dispersal during this period is assumed to be of minor importance with regard to oviposition because the majority of adults do not finish the pre-oviposition period until mid-July.

As for oviposition, the situation was comparable to the results from the dispersal analyses, in terms of preference for maize. In general, crops still not harvested or at least not grubbed after harvest during *D. v. virgifera's* oviposition period such as millet, Sudan grass sugar beet and harvested but not grubbed winterwheat appeared more attractive for oviposition than crop habitats that had experienced soil preparations, such as the ploughed bare soil, harvested and grubbed peas, harvested and grubbed winterwheat or harvested and grubbed winter rape. This is in line with the reports on egg laying of *D. v. virgifera* in soybean in the USA (Spencer et al. 2005) and in Croatia (Barcic et al. 2007). In the USA corn belt, some *D. v. virgifera* populations have evolved to lay eggs more frequently in non-maize crops where maize is likely to grow the following year, which then allows larval development. This was called the rotation tolerant variant of this pest species, and is one of the few examples in insects where behavioural resistance to a pest management option seems to have evolved (Onstad et al., 2003, Knolhoff et al., 2006). As for Europe, it had never been proven, whether specimens of this particular population had been introduced during any of the multiple introduction events (Ciosi et al. 2008). But the here-reported dispersal and oviposition of much less than 10% indicate that the genetic population of Central South Eastern Europe examined in this study is unlikely to belong the rotation tolerant variant.

Based on the relatively minor dispersal and oviposition in non-maize fields as well as the a generational growth rate of 4 of this species, it can generally be concluded that, according to the current state of knowledge, any crop can be rotated with maize to manage this invasive alien maize pest in Europe.

# Survival analysis of adult *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)

## Abstract

In order to develop models on the population dynamics of the maize pest *Diabrotica virgifera virgifera* (Western corn rootworm, Coleoptera: Chrysomelidae), survival rates at each stage of development (eggs, the three larval instars, pupae and adults) need to be known. In contrast to the already studied and reported survival rates of the immature stages of this pest from the field, the survival of *D. v. virgifera* adults under field conditions has not been established. Particularly the understanding of the likely main period for oviposition of *D. v. virgifera* populations in the field, i.e. after a pre-oviposition period, would help to better time interventions by direct control measures against the adults. Therefore, the survival of *D. v. virgifera* adults was studied in two field sites in Hungary between 2009 and 2011. Between 9 and 23 large 9 $m^2$ gauze cages were placed into each of the two study fields, and 50 to 75 newly emerged female and 50 to 75 male adults were released in each cage each year. Each cage covered maize and two out of 11other crop habitats. Survival was recorded weekly until no adults were found any more. The populations of adult *D. v. virgifera* rapidly decreased with time following an exponential-like curve. Male and females had comparable survival patterns. After 11 to 13 days, about 50 % of the adults had already died = $LT_{50}$. After 14 days, about 55 % of the adults had already died, thus having no chance to lay eggs because of the pre-oviposition period required. Maize appeared to be the most appropriate crop for adult survival. Combining maize with other crops that could potentially act as alternative food sources did not enhance survival. Survival equations are provided to support modelling the population dynamics of this important maize pest.

## Introduction

*Diabrotica virgifera* ssp. *virgifera* LeConte (Western corn rootworm, Coleoptera: Chrysomelidae) is a maize pest in North America and Europe. It is hypothesized to have originated in Mexico, where several pestiferous *Diabrotica* species occur (Branson and Krysan 1981; Krysan and Smith 1987). With the expansion of maize growing areas in the 20th century, *D. v. virgifera* became a major pest of maize, *Zea mays* (L.), in North America (Krysan and Miller 1986, Levine and Oloumi 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). It is now a threat to maize production in many European countries, particularly Austria, Hungary, Serbia, Romania, Slovakia, and Italy (Kiss *et al*. 2005b). It recently arrived in southern Germany, France, Poland and Belorussia (Ciosi *et al*. 2008) and is expected to become a threat to maize production areas in these countries as well.

*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 and Hibbard 2005). Feeding damage 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 and Kuhlmann 2006), and can occasionally further reduce crop yields through intensive silk feeding, which interferes with maize pollination (Chiang 1973).

To develop models on the population dynamics of *Diabrotica v. virgifera,* survival rates of its eggs, three larval instars, pupae and adults need to be known. Survival rates for the immature stages have already been reported from laboratory and field studies, but the survival rate of *D. v. virgifera* adults has not been firmly established under field conditions. Moreover, the contribution to population survival of other crops as alternative food sources has not previously been determined under field conditions.

To address these knowledge gaps, the survival of *D. v. virgifera* adults was studied in two field sites in southern Hungary between 2009 and 2011 by releasing young female and male adults in large gauze cages and recording their weekly survival. Survival analyses were applied, and these analyses can be used when modelling the population dynamics of this important maize pest

## Material and methods

### *Experimental setup*

The survival of *D. v. virgifera* adults was studied in field cages at two field sites, referred to as site A and B, in southern Hungary in 2009, 2010 and 2011 (Site A: Szekkutas, N46° 31 00.0, E20° 30 25.8, 87 m above sea level; Site B: Hodmezovasarhely, N 46° 25.998, E 20° 20.348, 83m). Both field sites were situated within agricultural land. The sites were divided into two sections: one half with maize field and the other half with plots of several crops that were rotated from year to year.

At both sites, large gauze field cages of 2 x 4.5 x 2 m in size, i.e. covering 9 $m^2$, were used to create enclosures to assess *D. v. virgifera* adult survival. The field cages were set up across the intersections of each field plot so that they covered plants from the maize crop and from two adjacent crops. Consequently, half of each of these large cages covered maize (ca. 4 $m^2$), and two other quarters covered two other crop habitats (each ca. 2 $m^2$). Crop combinations were assessed in 2 to 6 replicates per site and/or year. In site A, 23 field gauze cages were placed from 22 July until 28 August 2009, 20 cages from 22 July until 23 September 2010 as well as 19 cages from 13 July until 21 September 2011. In site B, 7 field gauze cages were placed from 22 July until 19 August 2009, 9 cages from 16 July until 24 September 2010 as well as from 9 July until 22 September 2011.

Habitats were as follows: ploughed bare soil (present in 13 cages), potatoes (*Solanum tuberosum;* 18 cages), sugar beet (*Beta vulgaris var. altissima*; 4 cages), millet (*Sorghum bicolor*; 24 cages), Sudangrass (*Sorghum Sudanese;* 8 cages); as well as peas (*Pisum sativum (partim; )* 21 cages), winter rape (*Brassica napus (partim*); 23 cages) winter wheat (19 cages) that had been harvested and grubbed, and finally winter wheat (*Triticum aestivum var. lutescens*; 24 cages) that had been only harvested, but not grubbed. In total over the sites and across the years, 100 cages contained maize (*Zea mays*) (= all cages), and 6 of them only contained maize and no other crop.

In 2009 and 2011, 100 young *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 / $m^2$ of cage or 10 adults per maize plant). They were released 22 July 2009 or 8 July 2011 in site A, and 17 July 2009 or 13 July 2011 in site B. In 2010, 150 *D. v. virgifera* adults (75 females and 75 males) were released onto the maize plants in each gauze cage to simulate a high adult density (equals approximately 17 adults / $m^2$ cage or 16 adults per maize plant). They were released 22 July 2010 in site A, and 16 July 2010 in site B. The approximate start of natural emergence of *D. v. virgifera* in the region was around 20.6.2009, 29.6.2010, and 20.6.2011. The timing of emergence in 2009 and 2011 was normal whereas it was delayed in 2010 (Toepfer et al. 2006).

### *Assessing the survival of adult D. v. virgifera*

All released adults were of a defined, young age because they were collected when they freshly emerged from field cages of other experiments, or they were collected from highly infested fields in the first week of start of emergence in the region. After release in the cage, live female and male adults were visually searched for and counted in the cages weekly during the above mentioned period, i.e. until no adults were found any more, i.e. until mid or end of September. Weekly survival rates were calculated for each crop combination. Moreover, total survival rates were calculated over time. Regression curves were fitted to the temporal pattern of survival.

### *Assessing factors influencing the survival of adult D. v. virgifera*

In mid-July and mid-August, the following crop related factors were recorded: (1) plant phenology of all crops (% germination, plant height, number of leaves, % green plants, % flowering plants, % dry plants), (4) vegetation cover of the crops, (5) weed density, weed species, weed flowering status, and weed coverage. The most common weeds were *Abutilon theophrasti, Amaranthus blitoides, Amaranthus retroflexus, Ambrosia artemisifolia, Chenopodium album, Cirsium arvense, Convolvulus arvensis, Datura stramonium, Hibiscus trionum, Malva sp., Lamium sp., Setaria glauca, Sonchus arvensis, Sorghum halepense, Xanthium orientale*.

Moreover, maximum, minimum and mean daily air temperature at 1.5 m height, and rainfall was recorded hourly from July to September of each study year (Davis Instruments Corp., Hayward, CA, USA). In 2009, mean temperature in July was 24 C (max 36, min 13) and the sum of rainfall 6 mm. In 2010, mean temperature in July was 24 C in 2009 (max 33, min 17) and the sum of rainfall 25 mm. In 2011, mean temperature in July was 19 C in 2009 (max 36, min 13) and the sum of rainfall 17 mm. In 2009, mean temperature in August was 24 C (max 33, min 15) and the sum of rainfall 8 mm. In 2010, mean temperature in August was 23 C in 2009 (max 33, min 14) and the sum of rainfall 5 mm. In 2011, mean temperature in August was 22 C in 2009 (max 33, min 14) and total rainfall of 4 mm.

Independent-samples Kruskal-Wallis H tests (nonparametric analogue of one-way ANOVA) were used to detect independent factors influencing the survival of adults (dependent factor).

## Results

### *Overall survival curve of adult D. v. virgifera*

The populations of adult *D. v. virgifera* rapidly decreased with time following an *exponential*-like curve (Fig. S1, S2). After 11 to 13 days, about 50 % of the adults had already died = $LT_{50}$. After 14 days, about 55 % of the adults had already died, thus having no chance to lay eggs (because of approx. 14 d pre-oviposition period required). After 24 to 26 days, about 80 % of the adults had already died = $LT_{80}$.

### *Assessing factors influencing survival curve of adult D. v. virgifera*

There is certain variability in the survival of adults between fields and years. However, the survival curves remained largely comparable (Fig. S3). The combination of other crops, and thus alternative food sources, with maize had little influence on the adult survival (e.g. pooled sexes Kruskal-Wallis H test $Chi2_{(8; 88)} = 135$, $P = 0.04$; Fig. S3).

*Influence of site location, year, and sex*

There was certain variability in survival of adults between field sites and years; however, the survival curves remain largely comparable. Only in site B in 2011, the survival appeared less good than in other years or site locations. Male and females had similar survival patterns (Fig. S2).

*Influence of certain crop type combinations and crop vegetation cover*

The combination of other crops with maize had little influence on the adult survival. Exclusive access only to maize appeared sufficient for adult survival, and it seemed to be the most suitable crop habitat. Only the combination of potatoes with maize was less suitable than the other crop habitat combinations.

*Influence of max, mean, min temperature and rain on survival*

The weekly mortality of adults increased slightly with increasing weekly air temperature (Pearson Correlation $r = 0.17$, $P < 0.001$, $n = 512$; ANOVA $F_{1;510} = 15.1$, $P = 0.0001$; $y = 19.6+1.13x$). Such relationships were not evident when maximum or minimum daily temperatures were considered.

The weekly mortality of adults decreased slightly with increasing weekly rainfall (Pearson Correlation $r = -0.17$, $P < 0.001$, $n = 512$; ANOVA $F_{1; 510} = 14.6$, $P = 0.0001$; $y = 48.3-0.36x$ or $y = 43+1.47x^3-0.09x$).

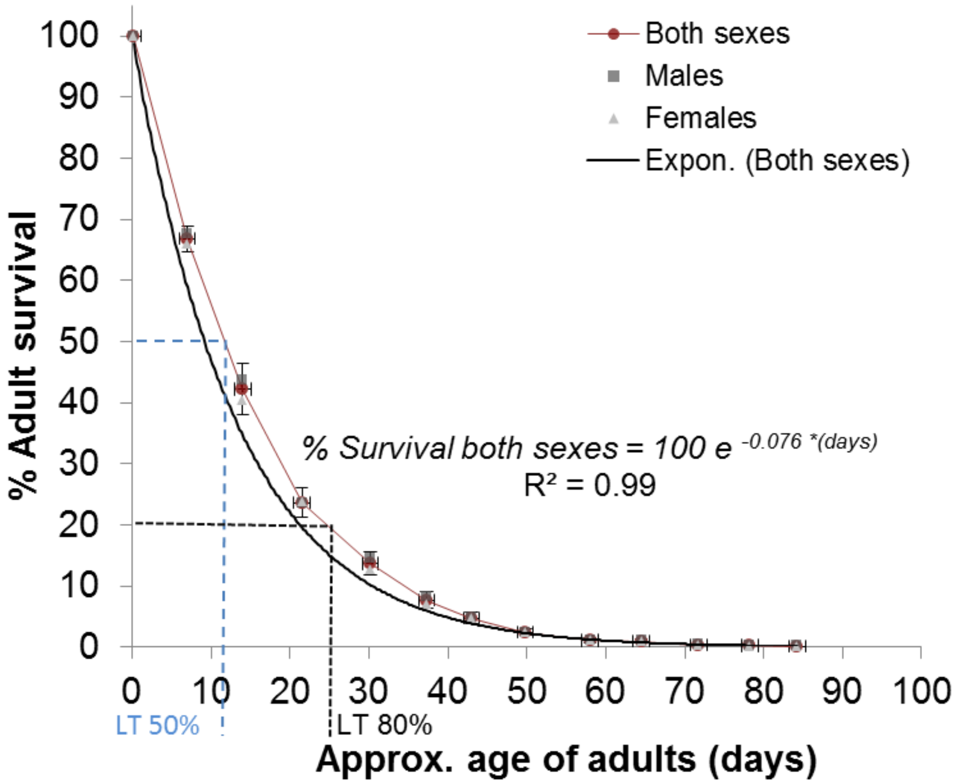
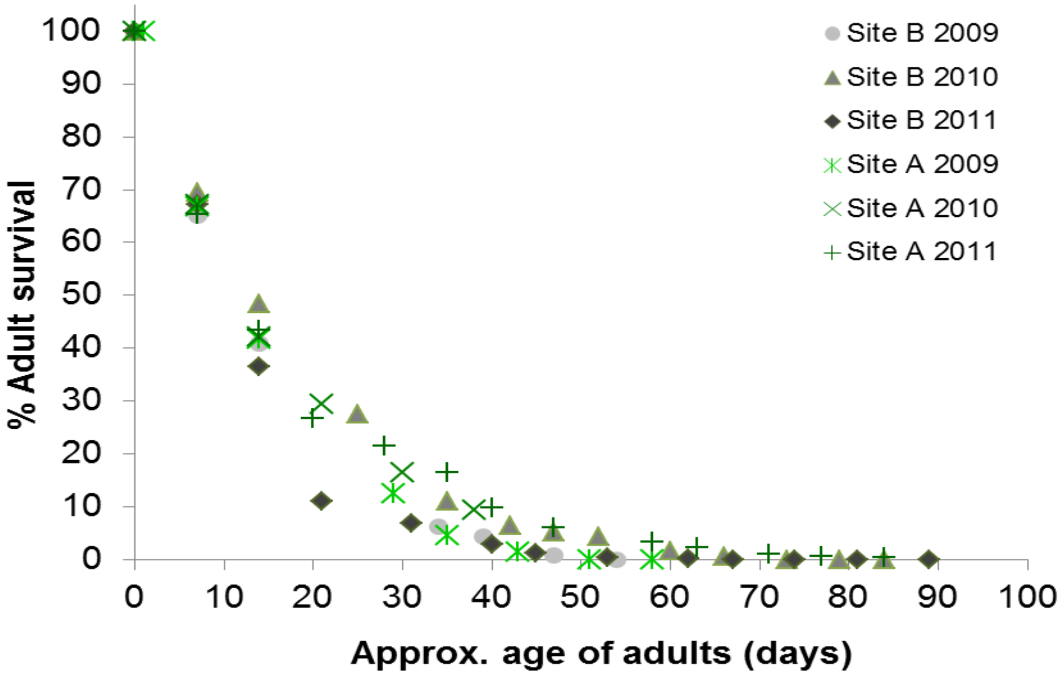


*Fig. S1* ***Average survival of adult D. v. virgifera over time*** *in large field gauze cages covering maize and different other crops in two field sites in southern Hungary in 2009, 2010, and 2011. CIRCLES = both sexes, SQUARES = males, TRIANGLES = females. Between 9 and 23 large gauze cages (ca. 4.50 x 2 x 2 m) per study site, and 50 to 75 young female and 50 to 75 young male adults released in each cage. 11 to 13 days of time until 50 % of adults have died = LT* $_{50}$*, and about 24 to 26 days until 80 % of adults have died = LT* $_{80}$*.*

Sites and years



Crop habitat combinations

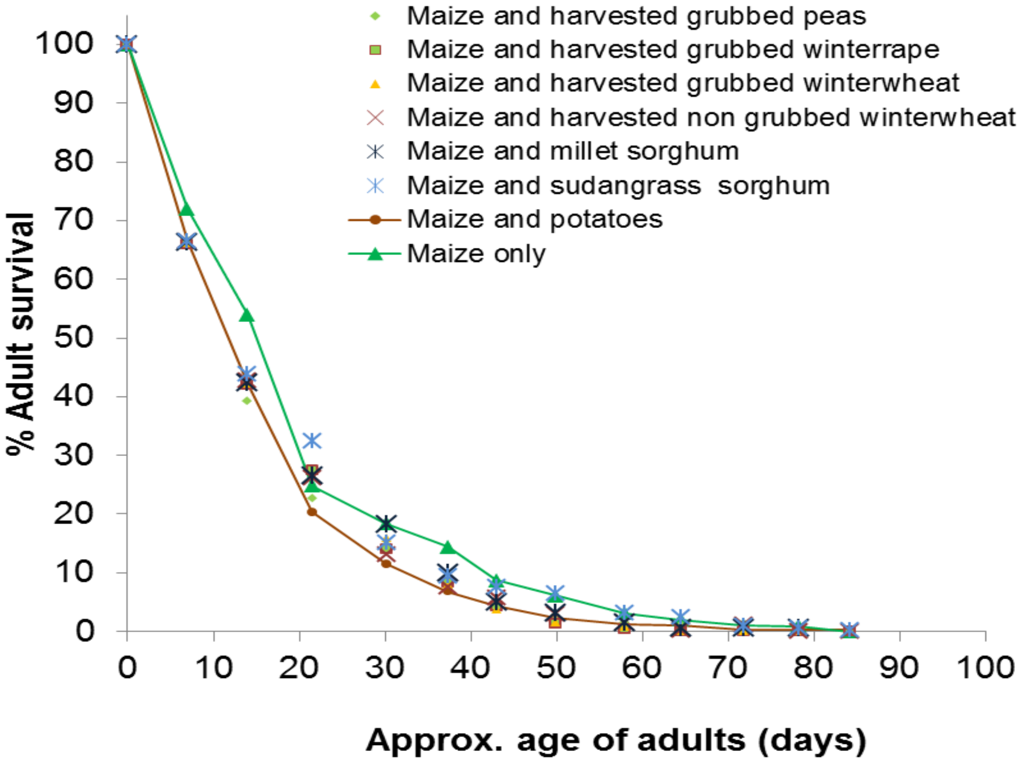


*Fig. S3* ***Average survival of adult D. v. virgifera over time depending on site location and year as well as depending on crop habitats****. Large gauze cages covering maize and different other crops in two field sites (field A = Szekkutas, B = Hodmezovasarhely) in southern Hungary in 2009, 2010, and 2011. Between 9 and 23 large gauze cages (ca. 4.50 x 2 x 2 m) per study field, and 50 to 75 young female and 50 to 75 young male adults released in each cage. Each crop was present in 2 to 6 cages per field and year.*

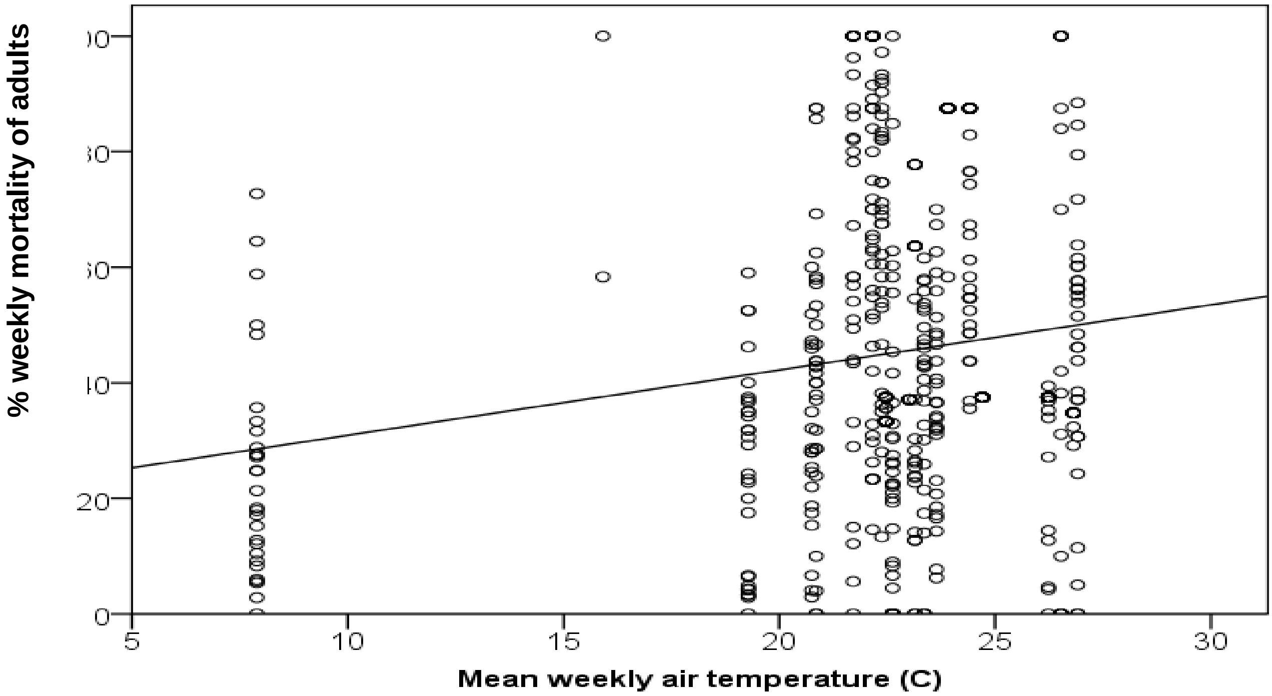


*Fig. S4 Weekly mortality of adult D. v. virgifera depending on mean weekly air temperature (Pearson Correlation r = 170, P < 0.001, n = 512; ANOVA F $_{1;\,510}$ = 15.1, P = 0.0001; y = 19.6+1.13x).*

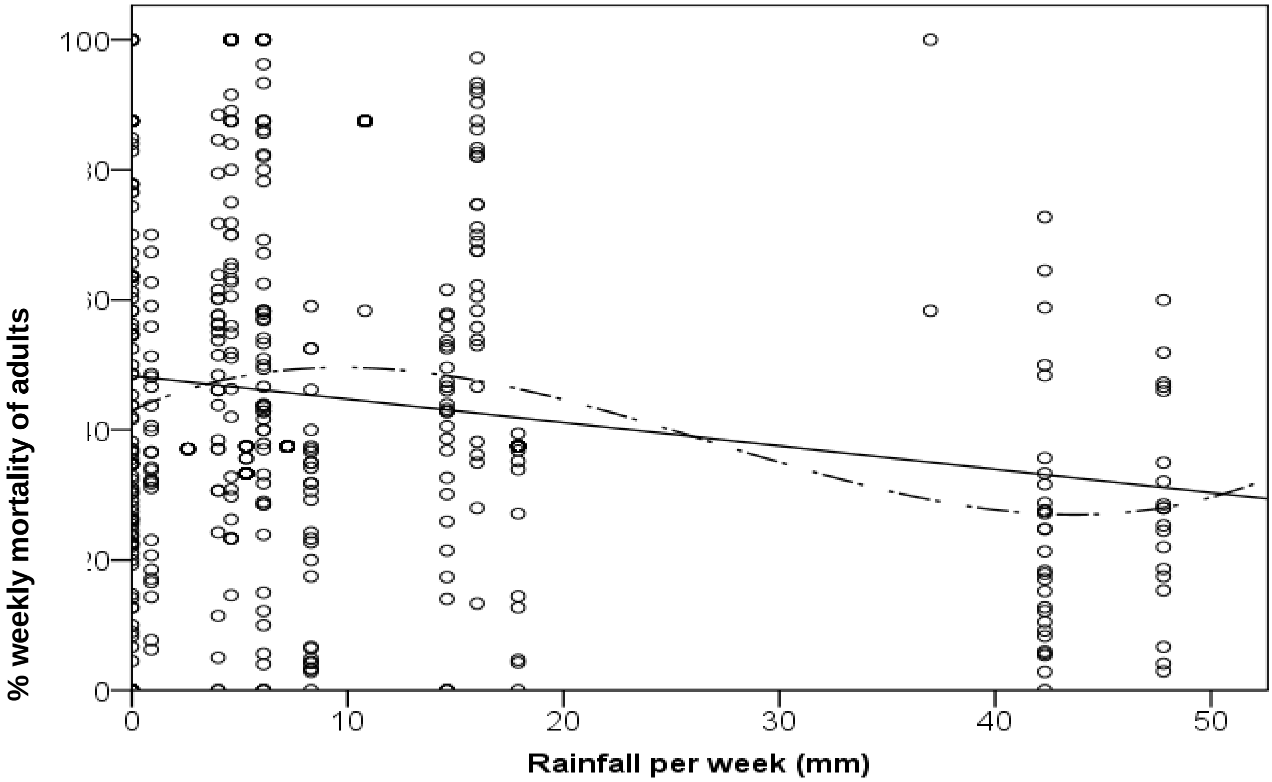


*Fig. S5 Weekly mortality of adult D. v. virgifera depending on mean rainfall (Pearson Correlation r = -0.17, P < 0.001, n = 512; ANOVA F $_{1;\,510}$ = 14.6, P = 0.0001; y = 48.3-0.36x or y = 43+1.47x3-0.09x).*

## Discussion

The populations of adult *D. v. virgifera* decreased astonishingly rapidly with time. Already after 11 to 13 days, about 50 % of the adults had died = $LT_{50}$. After 14 days, about 55 % of the adults had already died, thus having no chance to lay eggs because of the pre-oviposition period required. Still, the surviving adults are likely to be enough for producing sufficient offspring that allows population growth.

Maize appeared to be the most appropriate crop for adult survival. The combination with other crops, thus potential alternative food sources, did astonishingly not enhance survival.

The provided survival equations are suggested to be used to support modelling of the population dynamics of this important maize pest

# Oviposition preferences of *Diabrotica v. virgifera* in multiple-choice field cages

## Abstract

Adults of the maize pest *Diabrotica virgifera virgifera* (Western corn rootworm, Coleoptera: Chrysomelidae) primarily lay their eggs in the soil of maize fields. This is because the larvae of this pest are largely restricted in their feeding to maize roots. In the USA, rotation tolerant populations, however, also lay eggs in non-maize crops where maize is usually grown in the subsequent year, which then allows larval development.

To date, it is not known whether the rotation tolerant variant has been introduced to Europe. The oviposition behaviour of *D. v. virgifera* adults was studied in large multiple-choice field cages at two Hungarian field sites between 2009 and 2012. During each year of the study, between 9 and 23 large gauze cages (ca. 9 $m^2$) were placed at each study site. The cages included different combinations of three out of 10 selected habitat types. From 50 to 75 newly emerged female and male adults were released in each cage in each year. Maize was planted the following year, allowing the development of larvae and emergence of adults that were captured in small emergence cages placed over the areas of the three different crop habitats of the previous year.

Maize was found to be the most attractive for oviposition. Of medium proportional attractiveness were millet, Sudangrass, and ploughed bare soil. Third, the harvested and grubbed winter rape with regrowth, harvested and grubbed or not grubbed winterwheat with regrowth, as well as potatoes had a small attractiveness for oviposition. Least suitable were harvested and grubbed peas and soybean. Populations grew the most from year to year when the entire multiple-choice cage was planted with maize. In this case, the populations doubled on average across sites and years. When Sudan grass and millet were combined with maize in multiple-choice cages, a slight population grow appeared possible. When maize was combined with any other habitat type, the oviposition of *D. v. virgifera* was so much decreased, even in maize, so that the populations decreased from year to year. Considering the fact that only cages entirely planted with maize and no other crop reached considerable population growth, it can be assumed that all other crop habitats pose little risk that pest populations would reach threshold levels when maize is grown the following year. Consequently, crop rotation is suggested to be possible with all tested crops in order to control *D. v. virgifera* populations.

## Introduction

*Diabrotica virgifera* ssp. *virgifera* LeConte (Western corn rootworm, Coleoptera: Chrysomelidae) is a maize pest in North America and Europe. It is hypothesized to have originated in Mexico, where several pestiferous *Diabrotica* species occur (Branson and Krysan 1981; Krysan and Smith 1987). With the expansion of maize growing areas in the 20th century, *D. v. virgifera* became a major pest of maize, *Zea mays* (L.), in North America (Krysan and Miller 1986, Levine and Oloumi 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). It is now a threat to maize production in many European countries, particularly Austria, Hungary, Serbia, Romania, Slovakia, and Italy (Kiss *et al*. 2005b). It recently arrived in southern Germany, France, Poland and Belorussia (Ciosi *et al*. 2008), and is expected to become a threat to maize production areas in these countries as well.

*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 and Hibbard 2005). 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 and Kuhlmann 2006), and can occasionally further reduce yields through intensive silk feeding, which interferes with maize pollination (Chiang 1973). Adults are however, polyphagous and frequently search for alternative food sources, particularly pollen from weeds or other crops (Moeser et al. 2006, 2007). From laboratory studies, it is obvious that the diet experience and diet quality of adults influences their fecundity and fertility. Whether food uptake from non-maize sources is relevant for the oviposition of *D. v. virgifera* populations under field conditions was unknown.

Adults of *D. v. virgifera* are known to primarily lay their eggs into the soil of maize fields. This is because the larvae of this pest are largely restricted in their feeding to maize. Consequently, crop rotation is one of the most powerful management options against this pest. 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. Whether such populations had been introduced into Europe during the multiple introductions of this species is unknown. Other studies suggest that it belongs to the natural behaviour of *D. v. virgifera*, to oviposit to some extent into non-maize crops (Barcic et al. 2007, Spencer et al. 2008). This seems to be of particular importance for small scale agriculture, such as in Northern Italy, or in Croatia (Barcic, et al. 2012).

Therefore, the oviposition behaviour of *D. v. virgifera* adults was studied in large multiple-choice field cages at two field sites under European conditions between 2009 and 2011. Between 9 and 23 large gauze cages were placed into each of the two study fields, each covering three different crops. Results were aimed to help underlaying crop rotation as the major pest management tool, or to suggest adaptions in the advice for certain crop rotation schemes.

## Material 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 (Szekkutas, N46° 31 00.0, E20° 30 25.8, 87 m above sea level) and site B (Hodmezovasarhely, N 46° 25.998, E 20° 20.348, 83 m). Both field sites were situated within agricultural land. The sites were divided into a half section of a maize field and a half section with plots of several crop habitats differing in replicate numbers and spatial sequence from year to year.

At both sites, large gauze field cages of 2 x 4.5 x 2 m size, i.e. covering 9 m2, were used to create enclosures for *D. v. virgifera* to oviposit. In site A, 23 field gauze cages were placed from 22 July until 28 August 2009, 20 cages from 22 July until 23 September 2010 as well as 19 cages from 13 July until 21 September 2011. In site B, 7 field gauze cages were placed from 22 July until 19 August 2009, 9 cages from 16 July until 24 September 2010 as well as 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 and from two other adjacent crops. Consequently, half of each of these large cages covered maize (ca. 4 $m^2$), and two other quarters were covered with two other crop habitats (each ca. 2 $m^2$). Crop combinations were assessed in 2 to 6 replicates per site and/or year. The crop habitats were as follows: ploughed bare soil (present in 13 cages), potatoes (18 cages), sugar beet (4 cages), *Sorghum bicolor* millet (24 cages), *Sorghum Sudanese* (8 cages), as well as peas (21 cages), winter rape (23 cages) or winter wheat (19 cages) that had been harvested and grubbed, and finally winter wheat (24 cages) that had been only harvested, but not grubbed. All of the totally 87 cages at the sites over the course of the study years contained maize, and 6 of them contained only maize and no other habitat type.

In 2009 and 2011, 100 young *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 / $m^2$ of cage or 10 adults per maize plant). All released adults were of a defined and young age because they were collected freshly emerged from field cages of other experiments, or they were collected with an average age of 14 days compared with the emergence of adults of natural populations in the region. They were released 22 July 2009 or 8 July 2011 in site A, and 17 July 2009 or 13 July 2011 in site B. In 2010, 150 *D. v. virgifera* adults (75 females and 75 males) were released onto the maize plants in each gauze cage to simulate a high adult density (equals approximately 17 adults / m2 cage or 16 adults per maize plant). They were released 22 July 2010 in site A, and 16 July 2010 in site B. Data from these releases were standardised to 50 : 50 released adults to allow comparability between sites and years.

The approximate start of natural emergence of *D. v. virgifera* in the region was around 20 June 2009, 29 June 2010, and 20 June 2011, with 2009 and 2011 been normal years and 2010 been a delayed year (Toepfer et al. 2006).

Survival of adults in the cages was recorded weekly until no adults were found any more, i.e. usually until mid to end of September. In mid-August, the following crop related factors were recorded: maize plant density per m2, vegetation cover of the crops, weed species, weed flowering status, and weed coverage. The most common weeds were *Abutilon theophrasti, Amaranthus blitoides, Amaranthus retroflexus, Ambrosia artemisifolia, Chenopodium album, Cirsium arvense, Convolvulus arvensis, Datura stramonium, Hibiscus trionum, Malva sp., Lamium sp., Setaria glauca, Sonchus arvensis, Sorghum halepense, Xanthium orientale*. Moreover, maximum, minimum and mean daily 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). In 2009, mean temperature in July was 24 C (max 36, min 13) and the sum of rainfall 6 mm. In 2010, mean temperature in July was 24 C in 2009 (max 33, min 17) and the sum of rainfall 25 mm. In 2011, mean temperature in July was 19 C in 2009 (max 36, min 13) and the sum of rainfall 17 mm. In 2009, mean temperature in August was 24 C (max 33, min 15) and the rainfall 8 mm. In 2010, mean temperature in August was 23 C in 2009 (max 33, min 14) and the rainfall 5 mm. In 2011, mean temperature in August was 22 C in 2009 (max 33, min 14) and the rainfall 4 mm.

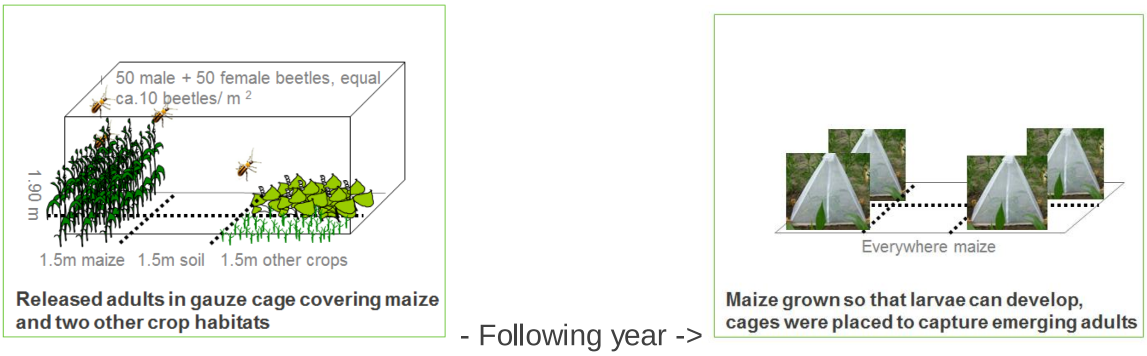


*Fig. M1 Multiple-choice gauze cages with released and ovipositing adult D. v. virgifera. Oviposition assessed by recording adult emergence from three small emergence cages placed over maize grown in the subsequent year.*

### *Assessing oviposition preferences*

Maize was planted at each location in the year following the adult release in the multiple-choice field cages. Then, three smaller gauze cages were positioned over the areas where each of the different habitat types had been established the previous year. The small cages (130 x 40 cm inner size x 120 cm height cage each covering 6 to 7 plants or 0.5 $m^2$) were used to collect and record the emerging adult *D. v. virgifera* in order to estimate oviposition in each habitat type the previous year. Cages were checked weekly (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). Emerged adult numbers were calculated as captures per crop habitat per $m^2$ and standardised to 11 adults released / $m^2$ in the cages the previous year. Overall averages of adult emergences across years and sites were weighted by the number of cages placed in a certain site and year (Tab. M1). Generational, i.e. annual growth rates of the *D. v. virgifera* populations were calculated for each multiple-choice cage and its crop combination.

### *Analysing factors influencing oviposition preference*

One-way ANOVA was conducted to detect whether 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 (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 vegetation cover, maize plant density, weed species numbers, amount of flowering weeds, weed species present and whether flowering or not, as well as max, mean, min temperature and rain during the main oviposition period, i.e. August, influenced the dependent factor of adult emergence.

## Results

*D. v. virgifera* emerged from maize as a result of oviposition into different habitats in a multiple-choice situation the previous year. The numbers of emerging adults largely differed between cages ranging from 0 to 22 adults per $m^2$, compared with an average of 11 adults released in cages per $m^2$ the previous years (Tab. M1).

The habitat type before maize significantly affected the proportional oviposition of *D. v. virgifera* (ANOVA: $F_{7;\,184}$ =50.5, P<0.0001, based on Fig. M2). Maize was found to be the most attractive for oviposition, which is reflected (i) by the 10.2 emerged adults per $m^2$ (weighted mean, SD 7.8) more than double the number of adults emerging from sites that had previously been occupied by other habitat types (Tab. M1), and (ii) by the proportionally higher percentage of emergence from maize after maize than from maize after other crops averaged across sites and years (Fig. M2).

Of medium proportional attractiveness were millet, Sudangrass, and ploughed bare soil (Fig. M2). Third, harvested and grubbed winter rape with regrowth, harvested and grubbed or not grubbed winterwheat with regrowth, as well as potatoes had a small attractiveness 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 (Tab. M1), but proportional attractiveness of this crop across site and years could not be calculated due to limited replicate numbers.

The generational (= annual) growth rate of *D. v. virgifera* populations primarily depended on the crop type maize and to some extent on Sudangrass in the cages (maize only in multiple-choice cages: ANOVA, $F_{1;\,78}$ =17.7, P<0.0001; maize and Sudangrass, $F_{1;\,78}$ =19.8, P<0.0001). No such dependences were detected for other crop habitats. Populations most successfully grew from year to year when the entire multiple-choice cage was planted with maize. In this case, the populations doubled on average across sites and years (2.1 ± 1.99 SD) (Tab. M2). Only when Sudangrass or millet were combined with maize in multiple-choice cages, still a slight population grow appeared possible. When maize was combined with any other crop habitat, the oviposition of *D. v. virgifera* was so much decreased, even into maize that the populations decreased from year to year. Also the maize density per $m^2$ positively affected oviposition (Kruskal Wallis: Chi-square 8.45, P = 0.038). Also an increased crop coverage, regardless of 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 numbers of flowering or non-flowering weeds did not affect oviposition (Kruskal Wallis: Chi-square 4.2- 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-square 0- 5.3, all P > 0.05).

The study location did not affect the proportional oviposition of *D. v. virgifera* into the different crop habitats (ANOVA, $F_{1;\,189}$ =0.4, P=0.54). The study year did not affect the proportional oviposition of *D. v. virgifera* into different crop habitats (ANOVA, $F_{2;\,188}$ =2.7, P=0.07). Minimum, maximum, average temperatures in August as well as rainfall did not or only slightly influence oviposition.

*Tab. M1 Average adult D. v. virgifera emergence per m2 maize due to oviposition into different pre- crops in large multiple-choice gauze cages in the previous year. Each large cage had covered three of 10 different crop habitats in different combinations in two field sites in southern Hungary in 2009, 2010, and 2011. Oviposition assessed by recording adult emergence from three small emergence cages placed over the grown maize in the subsequent years, i.e. in 2010, 2011, and 2012.*

| | | Emergence of adult D. v. virgifera per m2 maize due to oviposition into different pre-crops in previous year | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Maize | | | Sorghum (Millet) | | | Sorghum (Sudangrass) | | | Ploughed bare soil | | | Peas harvested and grubbed | | | Rape harvested and grubbed | | | Wheat harvested and grubbed | | | Wheat harvested not grubbed | | | Potatoes | | | Soybean | | |
| Site | Year | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n | Mean | s.d. | n |
| A | 2009 to 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 to 2010 | 1.7 | 2.3 | 6 | | | | 2.6 | 2.2 | 3 | | | | | | | 3.2 | 2.2 | 3 | | | | | | | | | | 0.96 | 1.1 | 4 |
| A | 2010 to 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 to 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 to 2012 | 22.0 | 18.4 | 14 | 7.2 | 7.3 | 4 | 15.4 | 0.0 | 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 to 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 | 0.0 | 4 |

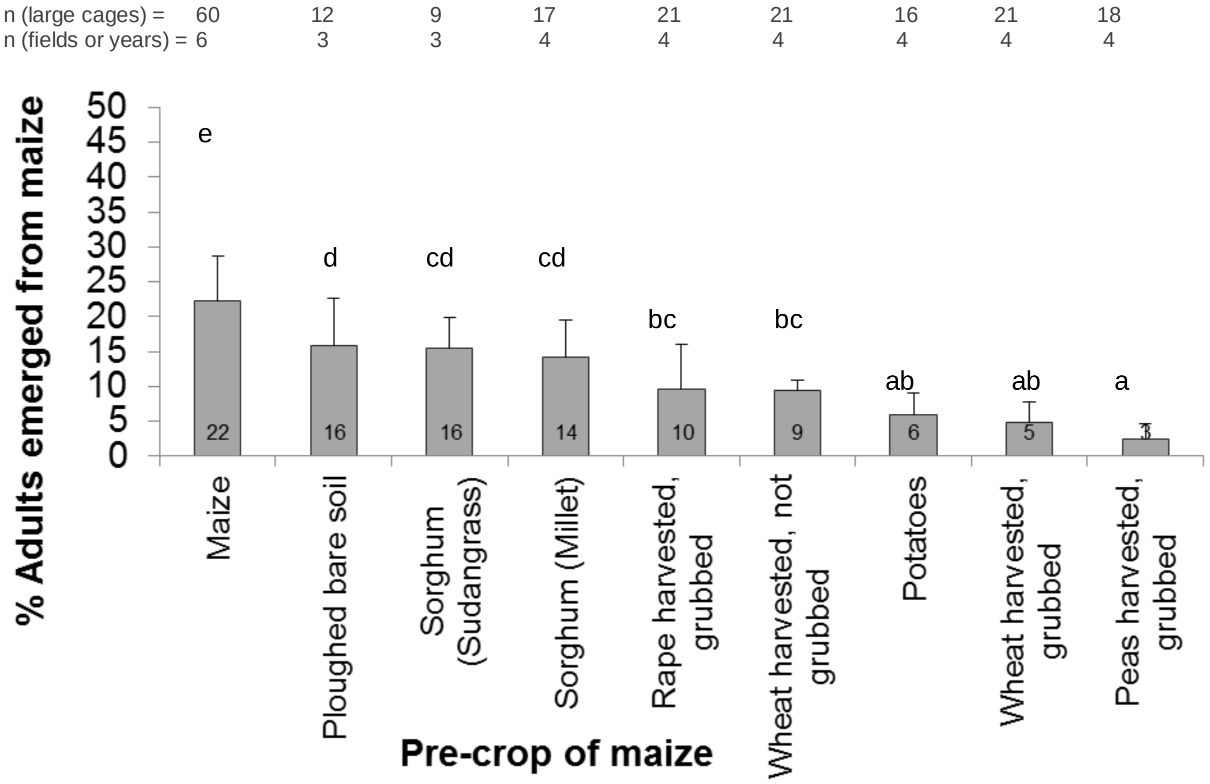


*Fig. M1 Proportional oviposition of D. v. virgifera (%) into different crops in large multiple-choice field gauze cages each covering three different crop habitats in two field sites in southern Hungary in 2009, 2010, and 2011. Oviposition assessed by recording adult emergence from maize in three small emergence cages after oviposition into different crops in multiple-choice field cages in the previous year, and calculating the % emergence in each crop habitat compared with all adults emerged from the area of the large multiple-choice cage placed the previous year. 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.*

*Table. M1. The generational (=annual) growth rate of D. v. virgifera populations in dependence of oviposition into different crop combinations in large multiple-choice field gauze cages each covering three different crop habitat in two field sites in southern Hungary in 2009, 2010, and 2011. Oviposition assessed by recording adult emergence from maize in three small emergence cages the following year, and calculating the % emergence in each crop habitat compared with all adults emerged from the area of the large multiple-choice cage placed 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. SEM = standard error of mean. n = replicates of large multiple-choice cages over sites and years*

| | **Generational growth rate** | | | |
|---|---|---|---|---|
| **Crop combination** | **Mean** | SEM | n | Diff. |
| Maize only | 2.1 | 0.9 | 5 | f |
| Maize and Sudangrass | 1.7 | 0.9 | 4 | e |
| Maize and millet | 1.4 | 0.6 | 8 | e |
| Maize, potatoes, and harvested and grubbed peas | 0.8 | 0.8 | 2 | d |
| Maize plus harvested grubbed and non-grubbed winterwheat | 0.7 | 0.1 | 9 | cd |
| Maize plus harvested grubbed winter rape and winterwheat | 0.6 | 0.2 | 4 | cd |
| Maize, potatoes, and harvested non-grubbed peas | 0.6 | 0.2 | 5 | cd |
| Maize and millet plus harvested non-grubbed winterwheat | 0.5 | 0.0 | 2 | bc |
| Maize and plus harvested grubbed peas and winterwheat | 0.5 | 0.3 | 3 | bc |
| Maize and potatoes plus harvested grubbed winter rape | 0.4 | 0.0 | 2 | bc |
| Maize and plus harvested grubbed peas and winter rape | 0.4 | 0.2 | 8 | bc |
| Maize and potatoes | 0.3 | 0.1 | 2 | bc |
| Maize, bare soil and 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 and millet | 0.2 | 0.0 | 2 | b |
| Maize, potatoes and ploughed soil | 0.1 | 0.1 | 2 | ab |
| Maize, soya | 0.1 | 0.1 | 2 | ab |
| Maize and harvested grubbed peas | 0.0 | 0.0 | 2 | a |

## Discussion

Maize was clearly the most attractive crop habitat for ovipositing *D. v. virgifera* adults, and maize density was positively correlated with adult emergence. Some adults emerged from the non-maize habitats of millet, Sudan grass, and ploughed bare soil, showing that either their vegetational characteristics or the soil conditions below certain crops were in principal suitable for adult oviposition. From multiple-choice cage conditions, however only relative comparisons can be made and the corresponding conclusions extracted. 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.

Considering the fact that only cages entirely grown with maize and no other crop reached considerable population growth, it can be assumed that all other crop habitats pose little risk that pest populations would reach threshold levels when maize is grown the following year. Consequently, crop rotation is suggested to be possible with all tested crops in order to control *D. v. virgifera* populations, despite the observed oviposition into other crops and subsequent pest development when maize is grown the following year.

## Conclusion for a crop rotation guideline

A relatively small proportion of *D. v. virgifera* adults flies from the natal maize field into other non –infested non-maize crop habitats. Most of the studied non-maize crops were visited to some extent by those dispersing *D. v. virgifera.* These *D. v. virgifera* seem also to lay eggs into the non-maize crops and larvae can develop to adulthood when maize is grown the following year.  Although the average growth rate of *D. v. virgifera* generations is reported to be at factor 4, it is unlikely that economic damage will appear in the first year maize. Therefore, it can generally be concluded that, according to the current state of knowledge, any crop can be rotated with maize to manage this invasive alien maize pest.

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