

# Efficiency of pheromone traps for monitoring *Diabrotica virgifera virgifera* LeConte

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Mark–release–recapture trials with male western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) beetles were conducted to better understand capture data of pheromone traps produced in Europe during monitoring programmes. Median recapture rate in maize fields in Hungary, Italy and Switzerland was 10%. Two types of sex pheromone trap (sticky and non-sticky container traps), placed inside or outside maize fields, showed no differences in efficiency. Logit analyses of recapture data in maize showed that at distances of <1 m fewer than 20% of beetles ended up in the traps. A beetle in a 1 ha maize field would have about a 5% chance of being caught in a trap placed in the centre of the field.

## Introduction

*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae (western corn rootworm) is an important maize pest in North America and, despite its status as a quarantine organism (EPPO/CABI, 1997), has accidentally been introduced several times into Europe since the 1980s (Miller *et al.*, 2005; Ciosi *et al.*, 2008). Currently, Central and South Eastern Europe as well as Northern Italy are widely infested, and management strategies are applied to avoid economic damage (Kiss *et al.*, 2005; Boriani *et al.*, 2006). There is an ongoing spread of this species along the borders of the infested areas.

In order to eradicate both introduced and established populations of *D. v. virgifera* beetles, all of the member states have been operating monitoring programmes (Furlan *et al.*, 2002; Kiss *et al.*, 2005), as required by European Commission Decisions (EU, 2003, 2006a,b). These programmes rely on pheromone traps to capture males of this univoltine species (Kiss *et al.*, 2005). During summer, traps are placed in maize fields, the beetle's preferred habitat for food and oviposition. Trapping techniques for *Diabrotica* spp. have been intensively studied in recent decades (Hein & Tollefson, 1984; Shaw *et al.*, 1984; Hesler & Sutter, 1993; Hoffmann *et al.*, 1996; Tóth *et al.*, 2003), concentrating on comparisons of pheromones and trap types. Little is known, however, about the probability of trapping *D. v. virgifera* beetles, especially its dependence on release distance.

Because diffusion is not linear in nature (Curry & Feldman, 1987), a model was chosen that calculates the rate of recapture as a function of distance with asymptotic behaviour (Schlyter, 1992). To estimate the parameters of such a model, and to cover a broad range of environmental and agronomic conditions, a large number of mark–release–recapture trials were conducted in three European countries. Traps of the type used in the European

monitoring programmes were placed both inside and outside maize fields. *Diabrotica v. virgifera* beetles were released at various distances from a single trap. Clearly, a model based on such observations is crucial to evaluating the value of pheromone traps in *D. v. virgifera* monitoring programmes.

## Materials and methods

Mark–release–recapture trials with male *D. v. virgifera* beetles were carried out between 2004 and 2007 in three countries: Hungary (Csongrad county), Italy (Lombardy and Veneto provinces) and Switzerland (Ticino canton) (Table 1). In all three countries, trials were conducted in first-year maize fields of between 2 and 9 ha. In Hungary, trials in non-maize fields (grassland or ploughed fields) were also conducted. Trials were implemented prior to or after the peak of *D. v. virgifera* beetle emergence (mid-July) (Toepfer & Kuhlmann, 2006). This period and type of maize field were chosen to reduce orientation disruption of the released marked males by native females (Hammack, 1995). A trial consisted of a release of a known number of marked *D. v. virgifera* beetles at a known distance from a single trap. Recaptured *D. v. virgifera* beetles were counted during recurrent visits to the traps.

## Trap types

Two trap types commonly used in the monitoring programmes of European plant protection services were investigated in our study:

- *Pheromone-baited sticky sheet traps* (CsalomoN<sup>®</sup> PAL, Tóth *et al.*, 2003). Transparent sticky sheets with glue on one side are curled with the sticky side facing outward around the maize plant or a wooden stick. A bait dispenser with the female sex

**Table 1** Number of trials and total number of beetles released (in brackets) at different distances from traps during mark–release–recapture trials with pheromone-baited sticky sheet and non-sticky container traps used in maize fields in Hungary, Italy and Switzerland and in non-maize fields in Hungary

Country	Hungary				Italy		Switzerland
	Maize		Non-maize		Maize		Maize
Trap type	Sticky sheet	Non-sticky container	Sticky sheet	Non-sticky container	Sticky sheet	Non-sticky container	Sticky sheet
Distance (m)	Trials (released beetles)						
2					4 (106)	2 (40)	
5	10 (200)	16 (320)	17 (340)	11 (220)	9 (188)	4 (80)	1 (80)
10					6 (98)	2 (40)	
15	10 (200)	16 (320)	17 (340)	12 (240)	1 (20)	2 (40)	
20					6 (98)		1 (80)
25	10 (200)	16 (320)	17 (340)	11 (220)	1 (20)	2 (40)	
50							1 (215)
60					4 (62)		
Total	30 (600)	48 (960)	51 (1020)	34 (680)	31 (592)	12 (240)	3 (375)

pheromone is positioned on the top edge. Male *D. v. virgifera* beetles are attracted by the pheromone and captured on the sticky sheet.

- *Pheromone-baited non-sticky container traps* (Csalomón® VARs, Tóth *et al.*, 2003). Two transparent containers are placed on a horizontal green plastic surface and a bait dispenser is placed in the upper container. Organophosphate insecticide inside the containers prevents *D. v. virgifera* beetles from escaping. Male *D. v. virgifera* beetles are attracted by the pheromone and caught inside either container. The supplier has since replaced this trap type with Csalomón® KLP (Tóth *et al.*, 2006).

All traps were fixed at heights of between 1.2 and 1.5 m.

#### Marking of *D. v. virgifera* beetles

Different colours were used to distinguish distinct release trials (different release dates or release distances) conducted in a field. Marking also allowed recaptured *D. v. virgifera* beetles to be distinguished from other native *D. v. virgifera* co-captured in the trap. Colours (yellow, green, blue, orange) were dry fluorescent powders not soluble in fat or water (Toepfer *et al.*, 2005).

*Diabrotica v. virgifera* beetles were collected in highly infested maize fields using pheromone-baited, non-sticky, insecticide-free container traps. It was assumed that no females were caught by these traps. Captures were transferred to appropriate bottles containing coloured powder, and immediately shaken (Krysan & Miller, 1986; Naranjo, 1990). *Diabrotica v. virgifera* beetles were released about 1 h later, directly from the bottle. Captured *D. v. virgifera* were checked below a UV lamp in a dark room for colour residues (Toepfer *et al.*, 2005).

#### Release and recapture of *D. v. virgifera* beetles

In order to limit the effect of variable wind conditions on recapture rates, trials were conducted only during days without wind from a dominant quarter, and the direction from release points to traps was varied between trials. A total of 4467 *D. v. virgifera*

beetles were released during 209 trials (Table 1). The distance of release points from traps ranged from 2 to 60 m. Trials lasted until recapture was no longer observed, usually a period of about a week. Traps were visited every day. Total recapture was considered as the sum of all marked *D. v. virgifera* beetles counted during all trap visits for the complete trial duration.

#### Data analysis

Our dependent variable was a proportion and data contained many zero values. Therefore, the common assumptions of linear regression, such as normality of the error terms, and constant variance did not hold. Standard linear regression and analysis of variance methods could not be applied. To avoid this problem, a generalized linear model was employed with the logit link function and (quasi-)binomial distributed errors, as implemented in R software (R Development Core Team, 2009). The model for data from maize fields contained the categorical predictor variables ‘trap type’ and ‘country’, and the continuous predictors ‘distance’ (between release point and trap), ‘field size’ and ‘number of unmarked *D. v. virgifera* beetles captured’. The latter predictor may serve as a proxy for the density of native females in the trap neighbourhood (Kuhar & Youngman, 1995), which is thought to reduce the recapture of released male *D. v. virgifera* beetles (Hammack, 1995). The country was included in the model to take account of regional maize production practices and eventual differences in mark–release–recapture procedures applied by experimenters. Recapture rate was weighted in the models by the number of released *D. v. virgifera* beetles to take into account that not all releases consisted of the same number of *D. v. virgifera* beetles. The significance of predictors was evaluated by comparing the full model, using a likelihood-ratio test, with a model in which the target predictor was dropped. Models were simplified by eliminating non-significant ( $P > 0.1$ ) predictors resulting in a final model for maize fields.

Because the model never reaches zero recapture probability, the half-distance (analogous to half-life in exponential decay) was used as a measure of the decline of recapture rate due to

distance. This half-distance was calculated as the distance where recapture rate is half the recapture rate at zero distance.

In order to compare recaptures in maize fields with recaptures outside maize fields, a second model for maize and non-maize fields contained the categorical predictors ‘crop’ and ‘trap type’ and the continuous predictors ‘distance’ and ‘unmarked *D. v. virgifera* beetles’, in accordance with data from Hungarian maize and non-maize fields.

**Results**

The proportion of recaptured *D. v. virgifera* beetles was low in all trials. The median rate over all trials inside and outside maize fields was 6.7% (inter-quartile range: 0.0–15.0%).

During the 124 trials in maize fields of all three countries, lasting on average  $4.7 \pm 3.4$  days, a total of 2767 marked *D. v. virgifera* beetles were released at distances from traps ranging from 2 to 60 m. A total of 421 *D. v. virgifera* beetles were recaptured, resulting in a median recapture rate of 10.0%, but reaching at most 87.5% (Table 2).

The complete model taking in all predictors was fitted to data on recapture rate obtained during these trials. ‘Country’ and ‘trap type’ had no significant effect on recapture rate and therefore the model was simplified by dropping these two predictors. In the reduced model, ‘field size’ appeared to negatively influence recapture rate but was not significant, and this predictor was also omitted. The final model for maize fields contained only the significant predictors ‘distance’ and ‘unmarked *D. v. virgifera* beetles’ (Table 3). The ‘unmarked *D. v. virgifera* beetles’ predictor was, contrary to the authors’ expectation, positive. When setting the value of the predictor ‘unmarked *D. v. virgifera* beetles’ to its average value, the final model for maize fields indicated that recapture rate at zero distance was 20% and that at ‘half-distance’ it was 26 m (Fig. 1).

During the 78 trials in and 85 trials outside maize fields in Hungary, lasting on average  $4.9 \pm 1.6$  days, a total of 3260 marked *D. v. virgifera* beetles were released at distances of 5, 15 and 25 m from traps. In total, 377 *D. v. virgifera* beetles were recaptured, resulting in a median recapture rate of 10.0%, but reaching at most 65.0% (Table 2). The ‘crop’ predictor had no significant influence in the model for maize and non-maize fields.

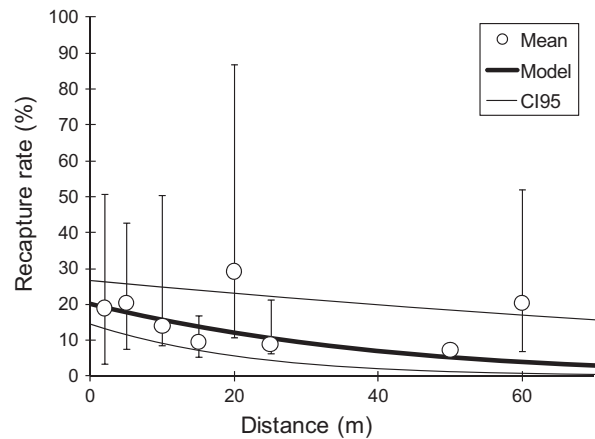
**Table 2** Proportion of trials with recaptures and descriptive statistics (%) of recapture rates observed during mark–release–recapture trials. Trials were used to develop the final model for maize fields in Hungary (HU), Italy (IT) and Switzerland (CH) and the model for maize and non-maize fields in Hungary

	Maize HU, IT, CH	Maize and non-maize HU
Trials with recaptures	75.8	74.2
Recapture rates		
Minimum	0.0	0.0
1st quartile	5.0	0.0
Median	10.0	10.0
3rd quartile	20.0	15.0
Maximum	87.5	65.0

**Table 3** Quasi-binomial Logit regression of recapture rate dependent on distance and unmarked beetles during mark–release–recapture trials in maize in Hungary, Italy and Switzerland: degrees of freedom (df), coefficient of determination ( $r^2$ ), intercept and coefficients of regression. Where appropriate: standard error (SE) and significance ( $P$ )

	Estimate	SE	$P$
df	123		
$r^2$	0.107		
Intercept	-1.523	0.194	0.000
Distance	-0.0303	0.0109	0.006
Unmarked	0.00245	0.00082	0.003

$$*r^2 = 1 - \frac{\text{residual.deviance}}{\text{null.deviance}}$$



**Fig. 1** Observed mean recapture rate ( $\pm$ SD) of western corn rootworm beetles released during several day-long observation durations in maize at different distances from sticky sheet traps and recapture rate estimated by a quasi-binomial logit model (95% confidence interval, CI95). For released beetle numbers used as weight in the regression, see Table 1 ( $N = 2767$ ).

**Discussion**

The results of this study, based on a large number of mark–release–recapture trials at various locations, showed that pheromone traps captured on average only a small minority of released *D. v. virgifera* beetles, even when traps were placed at short distances from the release point. According to the statistical model, trapping probability at a theoretical distance of zero (intercept of the regression model) was estimated to be as low as 20%. However, variation among individual trials was high, resulting in large uncertainty in estimated parameters.

Recapture probability is a combination of directional trap attractiveness and random dispersal behaviour. Commonly used sampling ranges are distances from which recapture probability is not nil to reach the pheromone source in a given time period, while attraction ranges are distances over which insects direct their movement to the source (Schlyter, 1992). Several authors studied these elements for *D. v. virgifera* and other insects. Carrasco *et al.* (2010) studied dispersal distances of western corn rootworm outside maize fields. Their models suggest that half of the dispersing *D. v. virgifera* beetles travelling along a given bearing will have travelled between 117 and 425 m after 1 day,

**Table 4** A selection of attraction and daily sampling ranges of various insects cited in the literature

Attraction range (m)	Sampling range (m)	Insects	Citation
15		Summerfruit tortrix moth, <i>Adoxophyes orana</i> (Lepidoptera, Tortricidae)	van der Kraan & van Deventer (1982)
	50–100	Asiatic rice borer, <i>Chilo suppressalis</i> (Lepidoptera: Crambidae)	Kondo & Tanaka (1994)
	360	Fall webworm, <i>Hyphantria cunea</i> (Lepidoptera: Arctiidae)	Zhang & Schlyter (1996)
200	500	Pea moth, <i>Cydia nigricana</i> (Lepidoptera, Tortricidae)	Wall & Perry (1987)
50		European spruce bark beetle, <i>Ips typographicus</i> (Coleoptera, Scolytidae)	Franklin & Gregoire (2001)
	55	Sweet potato weevil, <i>Cylas formicarius</i> (Coleoptera, Curculionidae)	Sugimoto <i>et al.</i> (1994)
	280	Sweet potato weevil, <i>Cylas formicarius elegantulus</i> (Coleoptera, Curculionidae)	Mason <i>et al.</i> (1990)
	200	Douglas-fir beetle, <i>Dendroctonus pseudotsugae</i> (Coleoptera, Scolytidae)	Dodds & Ross (2002)

with 1% of the beetles travelling between 775 and 8250 m. Tóth *et al.* (2003) suggested an attraction range of <10 m for pheromone-baited sticky sheet traps for male *D. v. virgifera* in non-maize fields. Recapture rates ranged from 2.1 to 3.0%. Wamsley *et al.* (2006) estimated a recapture rate in a first-year maize field of 6.3% at 3 m, decreasing to below 1% at more than 45 m. Their recapture rates were therefore lower than that found in this study (Fig. 1). Recapture rates observed for sweet potato weevils, *Cylas formicarius* (Sugimoto *et al.*, 1994), and fall webworm, *Hyphantria cunea* (Zhang & Schlyter, 1996), were much higher at a few metres distance, and decreased more prominently with increasing distance than in the present study. The largest attraction or sampling range reported in the literature for any insect species does not exceed 500 m (Table 4). Assuming a linear relationship, half-distances based on trials of indefinite duration can be considered as about half the sampling range. The observed half-distance of 26 m in maize fields would therefore correspond to a sampling range of 52 m, which is comparable with those mentioned in the literature.

Preliminary trials were conducted to study *D. v. virgifera* behaviour over periods of both 1 h and 1 day. Observations indicated that *D. v. virgifera* beetles were not often immediately attracted to the traps. During the first hour, at a release distance of 5 m, the recapture rate was 2%, and 65% of trials in maize did not reveal any recapture. During the first day of these trials, the recapture rate was 4%, and 49% of the trials did not recapture any *D. v. virgifera* beetles. Outside maize fields, the proportion of trials without recapture was even higher and recapture rates lower. More detailed behavioural studies would be necessary to determine why so few *D. v. virgifera* beetles were directly attracted to the trap. Further studies would also be required to ascertain the effect of the presence of female *D. v. virgifera* on the effectiveness of trap attraction for male *D. v. virgifera*. The positive relationship in our trials between unmarked *D. v. virgifera* beetle capture and recapture rates of marked *D. v. virgifera* beetles probably indicated that both were favoured by good flight conditions. This relationship did not confirm that native females influence recapture rates (Hammack, 1995).

Several sources of error could not be eliminated. The employed mark–release–recapture procedures might influence recapture rates (Mattioli & Cabrera Walsh, 2008). Handling, and especially marking, may have been quite stressful for *D. v. virgifera*

beetles. Unstressed, they might be more efficient in searching for pheromone sources. Collecting male *D. v. virgifera* beetles with pheromone-baited traps might select for beetles at a specific physiological stage, or influence their behaviour during trials, and therefore lead to under- or overestimation of recapture rates. Wind certainly plays a role in the experiments. The resulting high variability in recapture rates may have made the field size, country and trap type predictors non-significant.

The findings presented in this paper have implications for interpretation of *D. v. virgifera* monitoring programmes. Although our experiments were not intended to study these predictors, they did not contradict the findings of Tóth *et al.* (2006), who did not reveal differences in the efficiencies of sticky sheet traps and non-sticky container traps. Our comparison of the recapture rates of traps placed inside maize fields with traps placed outside did not support the current practice of setting up traps inside maize fields. The main reason for placing traps inside maize fields is that *D. v. virgifera* move toward maize fields (Toepfer *et al.*, 2006). In a square field of 1 ha, the shortest distance to its centre is 50 m; at this distance, the recapture probability of one *D. v. virgifera* beetle was estimated at 5.2%. In a 10 ha field, recapture probability at the corresponding distance is reduced to 0.2%. Trap catches of a monitoring programme that accords with European Commission Decisions identify only a small portion of the population present in a maize field.

## Acknowledgements

This work was made possible thanks to the hospitality and technical support of the Plant Health Service in Hodmezovasarhely, Hungary, offered by I Zsella, J Gavallier, K Buzas, E Dormannsné and others. We thank S Canzi, G Perissinotto and P Sipos for help with fieldwork. This study was partially funded by the Jedlik Foundation of Hungary, Hungarian grant NKFP07-A3-KUKBOGMV, CABI Europe and Agroscope Changins-Wädenswil ACW.

## Efficacité des pièges à phéromones pour le suivi de *Diabrotica virgifera virgifera* LeConte

Des essais de marquage-lâcher-recapture avec des mâles de chrysome du maïs *Diabrotica virgifera virgifera* LeConte (Coleop-

tera: Chrysomelidae) ont été conduits pour mieux comprendre les données de capture des pièges à phéromones générées en Europe au cours des programmes de suivi. Le taux médian de recapture dans les champs de maïs en Hongrie, Italie et Suisse était de 10%. Les deux types de pièges à phéromones sexuelles (feuille engluée et piège en entonnoir), placés à l'intérieur ou à l'extérieur des champs de maïs, n'ont montré aucune différence d'efficacité. Une régression logistique des données de recapture dans le maïs a montré qu'à des distances inférieures à 1 m, moins de 20% des insectes finissaient dans les pièges. Un adulte dans un champ de maïs d'1 ha aurait environ 5% chance d'être capturé dans un piège placé dans le centre du champ.

### Эффективность феромонных ловушек для мониторинга *Diabrotica virgifera virgifera* LeConte

Испытания по маркированию, вынуждению и повторному отлову жуков мужских особей западного кукурузного жука *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) проводились с целью лучше понять данные отлова в феромонные ловушки, полученные в Европе во время программ мониторинга. Средний уровень повторного отлова на полях кукурузы в Венгрии, Италии и Швейцарии составлял 10%. Два типа феромонных ловушек (клеякий лист и неклеякие контейнерные ловушки), помещавшихся внутри или за пределами полей кукурузы, не показали различий в своей эффективности. Исследования Лоджита с данными о повторном отлове на кукурузе показали, что на расстояниях менее 1 м., менее 20% жуков попадали в ловушки. У жука в поле 1 га кукурузы имелся приблизительно 5%-ый шанс попасть в ловушку, помещенную в центре поля.

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