

Adult movements of newly introduced alien *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) from non-host habitats

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Abstract

Mark–release–recapture experiments were undertaken in order to investigate the movement of adult *Diabrotica virgifera virgifera* LeConte from accidental introduction points towards suitable habitats, such as its host plant, *Zea mays* L. In Hungary in 2003 and 2004, nine mark–release–recapture experiments were carried out in a grass steppe area and lucerne field, in which two small maize fields (10 × 10 m) had been planted 300 m distant from the central release point. After each release of 5500 to 6000 marked *D. v. virgifera*, beetle recaptures were recorded three times using non-baited yellow sticky traps placed on 30, 105, 205 and 305 m radii around the release point. In seven out of 15 recapture periods (47%), beetle populations showed no directional movements, and their movements towards any particular habitat cannot be predicted. During five recapture periods (33%), beetle populations showed a uni-directional movement, and in three cases (20%) a bi-directional movement was observed. In 10 out of 15 recapture periods (67%), the released populations moved in a direction that was comparable with the mean wind direction during these periods; thus, beetle movements were slightly correlated with wind direction. On average over sites and years, beetles were not preferentially moving towards the two small maize fields (located 300 m from the release point) compared to other directions. However, beetles moved significantly more frequently in the direction of naturally-occurring maize fields within a radius of 1500 m than towards other habitats. Beetles stayed more frequently within flowering lucerne fields out to a radius of 300 and 600 m than in non-flowering lucerne or other habitats. On average, 2.8% (SD 3.2) of all recaptured beetles arrived in one of the two small maize fields located 300 m from their release point indicating that there is a high risk of a founder population establishing. Habitat management cannot be suggested as a means of preventing the beetle's initial dispersal because movement was usually non-directional, and alternative food plants were used prior to reaching maize.

Keywords: mark–release–recapture, *Diabrotica virgifera virgifera*, western corn rootworm, invasive alien species, Europe, dispersal

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Introduction

The maize pest, western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) was accidentally introduced at least three times from North America into Europe (Miller *et al.*, 2005). This invasive

chrysomelid beetle is a univoltine maize herbivore with eggs overwintering in the soil of maize fields, larvae feeding on maize roots, and adults feeding on maize leaves, silks or any pollen source (Chiang, 1973; Moeser & Hibbard, 2005). Most damage is caused by the root-feeding larvae which results in plant lodging. In Europe, the large-scale spread of *D. v. virgifera* varies greatly between years and regions, reaching up to 60–80 km per year (Edwards *et al.*, 1999; Baufeld & Enzian, 2005). Within 10 years, this invasive beetle rapidly spread throughout central Europe and south-eastern Europe (Miller *et al.*, 2005). Recently, several new isolated satellite introductions were reported around Paris (France), Basel (France and Switzerland), Amsterdam (The Netherlands) and around London (UK) (Kiss *et al.*, 2005; Miller *et al.*, 2005). This pattern of introductions and invasions raises questions about the dispersal parameters influencing the initial flight of accidentally introduced beetles over unsuitable habitats, such as airports, to colonize maize fields. Measuring factors that influence such dispersal will likely be a key to understanding the invasiveness of the alien *D. v. virgifera* (Drake *et al.*, 1989; Wittenberg, 2005).

Components of a pest introduction are usually divided into entry, the period of adaptation and establishment, and large-scale spread (Wittenberg & Cock, 2001). A successful adaptation period and establishment depends on: (i) the ability to colonize suitable habitats or hosts after entry; (ii) a low value of the minimum viable population size; (iii) climate suitability; and (iv) a high intrinsic rate of increase (as we know from many classical biological control cases in weeds (Wittenberg & Cock, 2001; Wittenberg, 2005)). The minimum viable population size of *D. v. virgifera* is expected to be small as a strong founder effect was found in the genetic variations of its European populations, due to very small starter populations (Miller & Guillemaud, 2005). Also, climatic conditions in much of Europe are suitable for the establishment of *D. v. virgifera*, as seen by its spread over large and climatically different European regions (Baufeld & Enzian, 2005). Its adaptability to different climatic conditions is also reflected by its broad distribution area from northern Mexico, throughout the USA and up to Canada (Krysan & Miller, 1986). The high intrinsic rate of increase of *D. v. virgifera* populations is a known phenomenon (Elliott & Hein, 1991) and most probably a result of its high realized fecundity in periods of favourable weather conditions during oviposition (Toepfer & Kuhlmann, 2005).

Diabrotica v. virgifera is an active flyer (Coats *et al.*, 1986; Isard *et al.*, 2000), and was observed to fly up to 24 km on flight mills in the laboratory (Coats *et al.*, 1986). However, nothing is known about its ability to colonize suitable habitats, e.g. maize fields, after introduction (entry) in a non-maize area, such as on an airport. Therefore, mark-release-recapture experiments were conducted to follow the movements of *D. v. virgifera* adults from non-maize areas, where they were introduced, towards small maize fields. The experiments were conducted in an agricultural region in southern Hungary in 2003 and 2004. Key factors behind such initial movements were expected to be: (i) the wind direction and speed as known from wind tunnel experiments with *D. v. virgifera* (VanWoerkom *et al.*, 1983; Spencer *et al.*, 1999) as well as from small-scale release-recapture studies in maize growing areas in the USA (VanWoerkom *et al.*, 1983; Naranjo, 1994; Spencer *et al.*, 2005), and from the patterns of large-scale spread of *D. v. virgifera* in North America (Grant & Seevers, 1989; Onstad *et al.*, 1999); and (ii) the attraction

and long distance recognition of maize (Prystupa *et al.*, 1988; Spencer *et al.*, 1999, 2005). However, to allow correlations of as many factors as possible with the movement direction of released beetle populations, other habitats were recorded in this study, such as non-maize crops (potential alternative food sources (Prokopy & Owens, 1983; Moeser & Hibbard, 2005)) and vertical vegetation structures (such as forests that could influence visual orientation (Rowe & Potter, 1996)).

Finally, the probability of beetle arrival in a maize field at a defined distance was calculated in order to estimate the risk of colonization of maize fields by small numbers of newly introduced *D. v. virgifera* in agricultural regions of Europe.

Materials and methods

Source of *Diabrotica v. virgifera*

Adult *D. v. virgifera* were mass-collected from highly infested maize fields in southern Hungary using a plastic funnel with a gauze bag attached. Maize plants infested with *D. v. virgifera* were shaken, so that adults fell through the funnel into the gauze bag. Adults were maintained in cages (300 × 300 × 500 mm) at 24 to 26°C during the daytime and 18 to 22°C at night. Soft maize kernels and water were provided (Branson *et al.*, 1975). Sex ratios were determined by dissecting sub-samples of 20 beetles before each experiment.

Experimental sites

The release-recapture study was carried out in two non-maize areas, referred to as site A and B, in a flat agricultural region in Csongrad County in southern Hungary in 2003 and 2004. Site A was an 80 ha grass steppe that was drying out in June and was cut once a year in late June. Site A served as an example of an unfavourable introduction area for *D. v. virgifera* because no food sources were available. Site B was a 60 ha lucerne field (*Medicago sativa*), approximately 25 km south of site A. Site B consisted of one section that served as forage crop and was cut at four-week intervals, and one section that remained uncut for seed production. The position of those sections alternated between 2003 and 2004. This site served as an example of a non-maize area where introduced beetles would have an alternative food source, i.e. flower pollen.

At each site, two maize plots of 10 × 10 m (var. Borbala, Cereal Research Institute, Szeged, Hungary) were established 300 m away from a central release point (fig. 1). The position of the two maize plots, as well as of more distant maize fields and other habitats, i.e. sunflower, lucerne, wheat, grasslands, forest residues and wind-breaking tree-bush strips, were geo-referenced in terms of distance and direction in a radius of 2500 m from the release point (GPS, Garmin, USA). Once every fortnight, the phenological stage of maize and other crops were recorded as follows: flowering, non-flowering or senescent maize, as well as flowering or non-flowering sunflower, and ripe or harvested winter wheat, and flowering or non-flowering lucerne. Maize fields finished flowering in mid July in 2003 and late July in 2004. Sunflower finished flowering in late July in 2003 and early August in 2004. Winter wheat was harvested from early to mid July in both years.

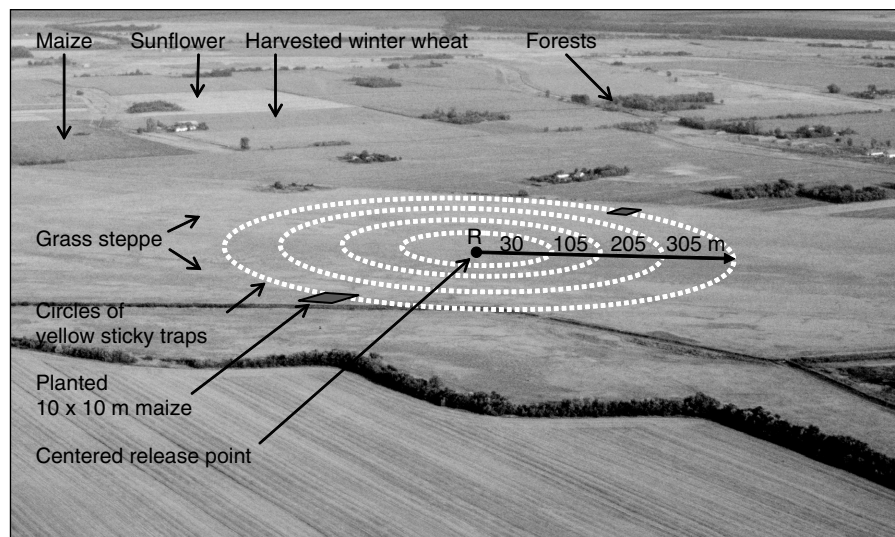


Fig. 1. Mark–release–recapture experiments with *Diabrotica v. virgifera* in non-host habitats in southern Hungary (photograph of site A in 2003).

Mark–release–recaptures

Beetles were marked with different fluorescent powder for each release (Orange T1-0Y6612 and Yellow T1-CH6620 Magruder Colour, Elizabeth, New Jersey, USA; Pink R17/M3115 Radiant Colour, Houthalen, Belgium) (Toepfer *et al.*, 2005). Three tea spoons, i.e. about 5 g, of fluorescent powder were thrown into a rearing cage with about 3000 beetles. Then the beetles had 3 to 5 h to mark themselves and the cages were shaken just before release.

In total, nine releases of 5500–6000 beetles each were carried out, that is, two releases at site A and B in 2003, three releases at site A in 2004 and two releases at site B in 2004. Beetles were released in the centre of the experimental area (fig. 1) at around 0700–0800 h.

To recapture beetles, 416 and 528 non-baited yellow sticky traps (Attractiveness < 1 m; Pherocon AM, Trece Inc., USA) were used per release in 2003 and 2004, respectively. Those traps were fixed on 1.50 m long wooden sticks and placed in four circles around the release points, i.e. at radii of 30, 105, 205 and 305 m. In 2003, 32 traps were placed on the 30 m distant circle, this is 0.17 traps per metre circumference of this circle ($32/2\pi * 30$ m). In total, 63 traps were placed on the 105 m distant circle, 128 on 205 m and 192 on 305 m at c. 0.1 traps per metre (fig. 1). In 2004, 48 traps were placed on the 30 m distant circle; this is 0.25 traps per m. In total, 88 traps were placed on the 105 m distant circle, 160 on 205 m, and 240 on 305 m at c. 0.12 traps per metre. The probability of beetles being recaptured in the inner circle was higher than in the outer circles because of the higher trap density. Therefore, all recaptures in the outer circles, i.e. at 105 m, 205 m and 305 m distance, were weighted by factor 1.7 in 2003 and by 2.1 in 2004 (Krebs, 1999). Beetles were recaptured from the traps in the morning on three occasions each two days apart. For each recaptured beetle, vectors of movement were recorded according to the position of the trap, i.e. distance and direction to the release point (geo-referenced by GPS, Garmin, USA). Recaptured beetles were taken to the laboratory and the marking was checked under UV light. Sex was determined by dissection.

Weather conditions, in particular wind speed and direction, were recorded every 10 min by a field weather station and arithmetic averages were calculated (Davis Instruments Corp., Hayward, California, USA).

Type of movements

Movement directions of recaptured beetles were plotted in circular histograms (figs 2–4) in order to visually evaluate if the movement of the released beetle population had been uni-directional, bi-directional or uniform (non-directional). In the case of bi-directional movements, the directions of the recaptured beetles were transformed to axial data before further analyses, i.e. by doubling each angle and reducing the multiples modulo 360° (Batschelet, 1981).

The mean movement direction of the recaptured beetles (n) from the release point, i.e. the mean vectors of movement (μ) and their circular standard deviation (SD) were calculated using circular statistics (Batschelet, 1981; Zar, 1998; Services, 2004). The mean vectors of movement were considered to represent a preferred direction (uni- or bi-directional) when data were not distributed uniformly according to the one sample Rayleigh test at $P < 0.05$ (Batschelet, 1981) (table 1). The length of the mean vector (r) and the Rayleigh Z-value ($r n^2$) were calculated as measures of concentration. A length (r) close to 1 and a high Z-value indicated high levels of concentration.

The mean vectors of movements of recaptured beetles were initially calculated separately for the first and second recapture period after each of the nine releases, when more than five beetles were recaptured in such a period. This resulted in 15 out of 18 theoretically possible beetle movement vectors (table 1). The mean vectors were then calculated for each of the nine releases, that is, with pooled recapture data from the first and second recapture period. This resulted in nine mean beetle movement vectors (table 2). Data from the third recapture period were not used to calculate mean vectors of movements, because most beetles had already left release boxes at time of third recaptures and

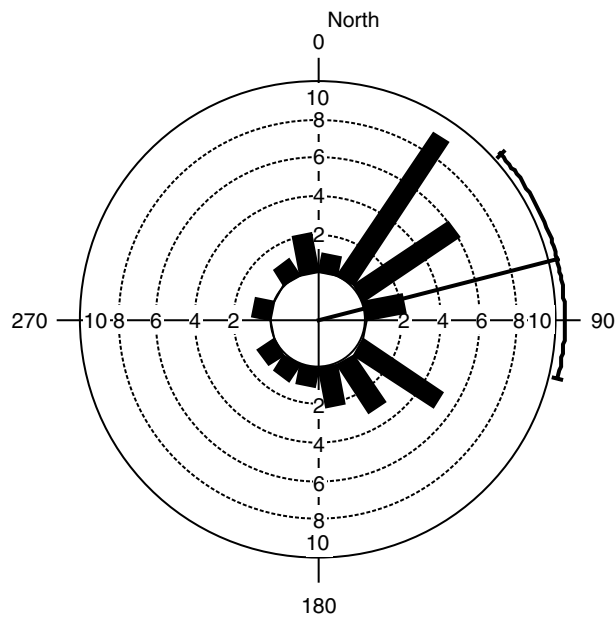


Fig. 2. Example of a uni-directional movement. Recaptures after the first release in site A in southern Hungary in 2004 (mean vector 76° SD 72° , $P=0.0005$ Rayleigh test; 0° = North, 90° = East, 180° = South, 270° = West, small numbers indicate frequency of beetles caught along each a 22.5° section of direction).

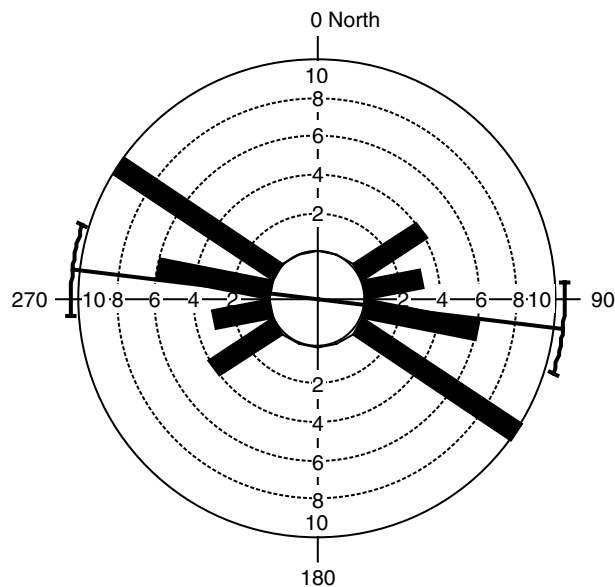


Fig. 3. Example of a bi-directional movement. Recaptures after the second release in site B in southern Hungary in 2003 (mean vector 133° and 313° SD 30° , $P=0.005$ Rayleigh test; 0° = North, 90° = East, 180° = South, 270° = West, small numbers indicate frequency of beetles caught along each a 22.5° section of direction).

may have arrived on the traps from another direction than from the release point.

To determine differences between mean vectors, i.e. directions of movements, the parametric multisample

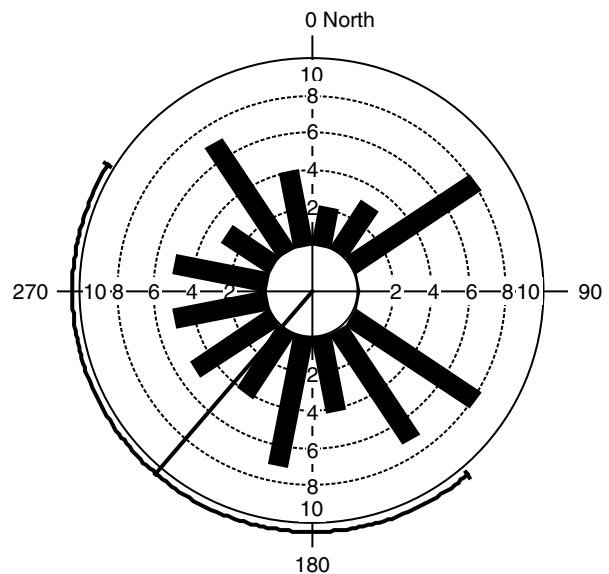


Fig. 4. Example of a uniformly distributed, i.e. non-directional movement. Recaptures after the first release in site A in southern Hungary in 2003 (mean vector 220° SD 119° , $P=0.38$ Rayleigh Test; 0° = North, 90° = East, 180° = South, 270° = West, small numbers indicate frequency of beetles caught along each a 22.5° section of direction).

Watson-Williams F-test (Fisher, 1993) was applied when at least five recaptures lead to each of the vectors to be compared.

Direction of movements related to wind

The 15 beetle movement vectors, i.e. for the first and second recapture period of each release, were correlated with the mean wind direction of the same periods during (a) day and night, (b) daytime only, (c) night only, and (e) for the morning and evening peaks of beetle flight activity (Chiang, 1973; Isard *et al.*, 2000), i.e. from 0500 to 1000 h and from 1700 to 2200 h; using circular-circular correlations (Fisher & Lee, 1983; Mardia & Jupp, 2000) (Rayleigh test, $P<0.05$). This correlation used the Fisher & Lee (1983) method, which is comparable to the Pearson product-moment correlation. The significance of the correlation was tested by using the jack-knife method (Mardia & Jupp, 2000).

Direction of movements related to habitats

In order to relate beetle movements to different habitats, the circle around a release point was divided into 16 directions of 22.5° . All habitats (see above) in each of the 16 directions were recorded from the release point up to a 300 m radius, as well as up to radii of 600, 900, 1200, 1500, 1700 and 2000 m. In order to standardize the recapture data, the number of recaptured beetles into each of the 16 directions and per each of the nine releases was divided by the total number of recaptures from each of the nine releases (Krebs, 1999). The mean relative number of beetles recaptured in the directions of a certain habitat, such as maize, were compared to the mean relative number of beetles

Table 1. Recapture numbers and movement characteristics of released marked *Diabrotica v. virgifera* per recapture period and per release in site A and B in southern Hungary in 2003 and 2004 (9 releases and 26 recapture periods).

Release	Recapture period	Site	Year	<i>n</i>	Movement type	Mean vector concentration					Diff.
						$\mu(^{\circ})$	SD	length <i>r</i>	<i>P</i> +++	<i>Z</i>	
1 (2 July 03)	1	A	2003	65	Not directional	235	112	0.15	0.26	1.4	
	2	A	2003	7	Not directional	112	82	0.36	0.42	0.9	
	3	A	2003	4	++						
2 (14 July 03)	1	A	2003	11	Uni-directional	205	48	0.7	0.002	5.4	c
	2	A	2003	2	+						
	3	A	2003	2	++						
3 (17 July 03)	1	B	2003	15	Bi-directional	133 & 313	30	0.57	0.005	4.9	a
	2	B	2003	0	+						
	3	B	2003	0	++						
4 (30 July 03)	1	B	2003	12	Bi-directional	103 & 283	28	0.61	0.008	4.6	b
	2	B	2003	11	Bi-directional	90 & 270	22	0.75	0.0001	6.1	b
	3	B	2003	3	++						
5 (14 July 04)	1	A	2004	21	Uni-directional	87	62	0.63	0.0001	6.6	d
	2	A	2004	14	Not directional	48	84	0.35	0.19	1.7	
	3	A	2004	3	++						
6 (23 July 04)	1	A	2004	27	Not directional	185	106	0.18	0.43	0.9	
	2	A	2004	4	+						
	3	A	2004	2	++						
7 (11 Aug 04)	1	A	2004	78	Not directional	350	29	0.16	0.14	1.9	
	2	A	2004	16	Uni-directional	11	21	0.45	0.038	3.2	e
	3	A	2004	10	++						
8 (19 July 04)	1	B	2004	32	Uni-directional	74	15	0.44	0.002	6.2	f
	2	B	2004	15	Not directional	150	64	0.24	0.43	0.9	
	3	B	2004	4	++						
9 (31 July 04)	1	B	2004	27	Uni-directional	229	21	0.37	0.026	3.6	cd
	2	B	2004	17	Not directional	261	33	0.29	0.23	1.5	

n, number of recaptured marked beetles; SD, circular standard deviation; a high *Z* value and an *r* close to 1 indicate a high concentration of the mean vector.

+, Not enough data to calculate a mean vector; ++, data from the third recapture period were not used to calculate mean vectors, because beetles had already left release boxes at time of third recaptures and may have arrived on the traps from another direction than from the release point; +++, concentrated vectors, i.e. with a preferred direction, at $P < 0.05$ (one sample Rayleigh test); Diff., letters in last column indicate significant differences between vectors at $P < 0.05$ (multisample Watson – Williams F-test).

Table 2. Averaged movement characteristics of released marked *Diabrotica v. virgifera* per release in site A and B in southern Hungary in 2003 and 2004 (9 releases).

Release	Site	Year	<i>n</i>	Movement type	Mean vector concentration					Diff.
					$\mu(^{\circ})$	SD	length <i>r</i>	<i>P</i> +++	<i>Z</i>	
1	A	2003	72	Not directional	220	119	0.12	0.38	0.9	
2	A	2003	13	Uni-directional	216	50	0.68	0.001	6.1	d
3	B	2003	15	Bi-directional	133 & 313	30	0.58	0.005	4.9	a
4	B	2003	23	Bi-directional	97 & 277	26	0.66	0.0001	10	b
5	A	2004	35	Uni-directional	76	72	0.45	0.0005	7.2	e
6	A	2004	31	Bi-directional	60 & 240	110	0.34	0.027	3.6	c
7	A	2004	94	Uni-directional	357	102	0.2	0.02	3.9	f
8	B	2004	47	Uni-directional	87	86	0.33	0.006	5	g
9	B	2004	44	Uni-directional	240	86	0.33	0.009	4.7	h
1–2	A	2003	89	Uni-directional	220	101	0.21	0.017	4.1	a
3–4	A	2004	38	Uni-directional	37	112	0.14	0.036	3.3	b
5–7	B	2003	174	Bi-directional	109 & 289	33	0.52	0.0002	10	c
8–9	B	2004	91	Not directional	155	129	0.08	0.57	0.6	

n, number of recaptured marked beetles; SD, circular standard deviation; a high *Z* value and an *r* close to 1 indicate a high concentration of the mean vector.

+++ , Concentrated vectors, i.e. with a preferred direction, at $P < 0.05$ (one sample Rayleigh test); Diff., letters in last column indicate significant differences between vectors at $P < 0.05$ (multisample Watson-Williams F-test).

moving into the remaining directions with other habitats using the independent sample T-test after testing equality of variance by Levine's test (Kinnear & Gray, 2000). This test

was first carried out using the relative recapture data from all nine releases, as this is the most powerful measure because directions of crop habitats changed from year to

year in both sites. The test was also carried out for sites A and B separately. All tests were carried out for both sexes of beetles, and for males and females separately.

Probability of beetle arrival in maize fields

The recapture data of all three recapture periods of each of the nine releases was weighted to account for the capture probability of traps as described above. The probability of beetle arrival in a 10 × 10 m maize plot at over a 300 m distance was calculated as the mean percentage of recaptured beetles in each of the maize plots per release in comparison to the totally recaptured beetles from each release. This probability was calculated as an overall mean over sites and years, as well as for site A and site B separately.

Results

Recaptures

On average, 0.9% (SD 0.5) of the 5500–6000 released beetles in each of the nine releases was recaptured. This represents a mean recapture rate of 0.002% (SD 0.001) per trap per release. In total, 289 marked beetles were recaptured. After weighting according to their recapture probability (see above), a beetle number of 398 was used for analyses leading to the results presented in the next sections. The sex ratio of the released beetles was 63% males to 37% females (SD 16%, $n=9$), and of the recaptured beetles 41% males to 59% females (SD 39%, $n=9$).

Type of movements

Diabrotica v. virgifera adults spread over more than 80 ha of non-maize areas. In five out of 15 recapture periods, released beetle populations showed a uni-directional movement (33%) (fig. 2), in three cases beetles showed a bi-directional movement (20%) (fig. 3), and in 7 cases no directional movements (47%) (fig. 4, table 1). The directions, i.e. the mean vectors, differed in most cases among each other ($P < 0.05$, Watson-Williams F-test, table 1).

When beetle movements per release were pooled, five out of nine released populations showed a uni-directional movement, three showed a bi-directional movement, and only one released population showed a non-directional movement, i.e. release one in site A in 2003 (table 2). When beetle movements over all releases per site and year were pooled, the beetles in site A in 2003 and in 2004 showed a uni-directional movement, however, their mean vectors differed significantly. The beetles in site B in 2003 showed a bi-directional movement and the beetles in site B in 2004 did not perform a directional movement (table 2).

Considering the movements of male and female beetles separately, some male and some female populations showed directional movements and others did not, i.e. three out of eight released male populations; and three out of four female populations (only releases with more than five recaptures per sex were analysed). No statistical differences were found between directional movement of males and females ($P > 0.5$, Watson-Williams F-test) when using the mean vectors of their directional and non-directional movements. There was, however, little comparative data for males and females

Table 3. Correlations between the direction of beetle movement and wind direction for sites A and B in 2003 and 2004.

Wind during	Beetles		
	R	P	n
Day and night	0.36	<0.05	15
Daytime 0500–2200 h	0.55	<0.05	13
Night 2100–0600 h	0.41	<0.05	11
0500–1000 & 1700–2200 h	0.47	<0.05	13

Circular-circular correlation coefficient (R); significance of R tested by jack-knife method at $P < 0.05$.

because they were rarely found directionally moving during the same period.

Direction of movements related to wind

During the 2003 and 2004 experimental periods, the wind blew mainly from NW or WNW (mean 303° , SD 44°) with a mean speed of 0.54 m s^{-1} (SD 0.36). Wind speed was 0.73 m s^{-1} (SD 0.4) during the experimental period in 2003 and 0.41 m s^{-1} (SD 0.2) in 2004. In 10 out of 15 recapture periods (67%), the released populations moved in a direction comparable to the mean wind direction. The mean wind direction was significantly correlated with the mean direction of movements of released beetle populations (table 3). This correlation coefficient was higher for the relationship between the direction of beetle movement and wind direction during daytime, than for wind during the activity peak of the beetles in the morning and evening, or for the wind during the night only or for the wind during day and night (table 3).

The concentration of beetle movements, i.e. the mean vector lengths (r), were not correlated with the concentration of the wind directions (Pearson correlations with daytime wind, $R = 0.14$, $P > 0.05$, $n = 15$), nor with the wind speed (day and night, daytime, night, morning and evening: Pearson correlation $R < 0.35$, $P > 0.05$, $n = 15$).

Direction of movements related to habitats

On average, for both sites and in both years, beetles did not move more frequently in the direction of the two small maize plots than in the other directions (independent sample T-test, $t = 1.4$, $df 142$, $P = 0.19$). Similar results were obtained when males and females were analysed separately. Also, no significant movements towards the two maize plots were found when analysing the data separately per site.

Movement towards maize fields within a radius of 600, 900, and 1200 m around the release points was not significantly directed in comparison to movement towards other non-maize habitats (for both sites or for sites separately; for both sexes or for males and females separately). However, beetles moved significantly more frequently in the direction of maize fields that were situated within a radius of 1500 m than towards other habitats (independent sample T-test, $t = 2.4$, $df 138$, $P = 0.015$). This significant directional movement was particularly strong for males ($t = 2.1$, $df 138$, $P = 0.032$), but was not significant for females ($t = 0.7$, $df 138$, $P = 0.5$). Significantly directed movement was found for site A, both when considering the whole experimental period and only the period when maize was

flowering, i.e. until mid July 2003 or until late July 2004. In contrast, in site B, movements to maize habitats were influenced by the presence of the flowering lucerne (see below). On average over sites and years, directional beetle movements towards maize fields were not found for longer distances within a radius of 1700 or 2000 m.

On average, for both sites and in both years, beetles did not move more frequently in the direction of sunflower fields compared to other directions, neither within radii of 300, 600, 900, 1200, 1500, 1700 or 2000 m (independent sample T-test, $P > 0.05$). Similar results were obtained when males and females were analysed separately, or when only the flowering period of sunflower was considered, i.e. until late July 2003 and until early August 2004.

In site B, beetles stayed within flowering lucerne fields out to radii of 300 and 600 m or more frequently than in non-flowering lucerne or other habitats (independent sample T-test for 300 m, $t = 3.3$, $df = 62$, $P = 0.02$), even though the position of flowering lucerne fields was changing over time within a year, and between years. This significant movement or arrestment was particularly strong for males ($t = 3.6$, $df = 62$, $P = 0.02$), but not for females ($t = 0.8$, $df = 46$, $P = 0.41$). No such relationship was found between beetle movement and the cutting of lucerne (non-cut versus regularly cut and re-growing lucerne, $t = 1.2$, $df = 62$, $P = 0.23$). No lucerne was grown at site A.

Beetles were found to move more in the direction of small forests or wind-breaking tree strips within radii of 900, 1200 or 1500 m in comparison to the movements towards other habitats (independent sample T-test; for 900 m: $t = 2.3$, $df = 142$, $P = 0.02$; for 1200 m males only: $t = 2.0$, $df = 97$, $P = 0.048$; for 1500 m: $t = 2.1$, $df = 142$, $P = 0.036$).

Probability of beetle arrival in maize fields

On average, 2.8% (SD 3.2) of all recaptured beetles arrived in one of the two small maize plots 300 m from the point of release for sites A and B in 2003 and 2004. This was about 0.03% (SD 0.03) of all released beetles. In site A the probability of beetle arrival in a maize plot was higher than in site B, i.e. 3.8% (SD 3.8) versus 1.5% (SD 1.9).

Discussion

The mark–release–recapture experiments simulated the occurrence of accidental introductions of alien *D. v. virgifera* into non-maize areas, and their subsequent dispersal within flat agricultural regions, where mainly maize, sunflower and winter wheat were grown.

The movement patterns of the introduced, i.e. released, beetles appeared to be variable. For example, *D. v. virgifera* populations often moved without a clear pattern, i.e. randomly or uniformly, sometimes they moved to, or were carried in, one major direction, and sometimes beetles moved in two major directions (tables 1 and 2, figs 2–4). Random movements of *D. v. virgifera* in maize growing areas were also described by Naranjo (1994), and it is difficult to find the reasons behind such movements. The directional movements may either be influenced by environmental conditions, such as wind, or were a result of beetle responses to stimuli, such as an attraction and long-distance recognition or orientation towards crops or other habitats (Spencer *et al.*, 1999).

Since the main direction of movements of the released beetles changed from one release–recapture period to the other (table 2), the explanation for such movements must lie in variable environmental conditions, such as wind direction. And indeed, in 10 out of 15 recapture periods, the released populations were found to move into a direction that was comparable to the mean wind direction. That wind can influence the flight of *D. v. virgifera* is known from wind tunnel experiments (VanWoerkom *et al.*, 1983; Spencer *et al.*, 1999), from small-scale release–recapture experiments in maize growing areas in the USA (VanWoerkom *et al.*, 1983; Naranjo, 1994; Spencer *et al.*, 2004), and from the patterns of large scale spread of *D. v. virgifera* in North America (Grant & Seevers, 1989; Onstad *et al.*, 1999). Those studies showed that *D. v. virgifera* take off most frequently when there is no wind, or at low wind speeds of less than 0.5 m s^{-1} (VanWoerkom *et al.*, 1983). On the other hand, *D. v. virgifera* stop initiating flight at higher wind speeds, however, large proportions of a population can be carried away by storms (Onstad *et al.*, 1999). During the experimental periods of the present study, wind speed was normally around 0.5 m s^{-1} and wind direction was found to be a significant factor on several occasions.

Diabrotica v. virgifera are by no means limited to down-wind movement. VanWoerkom *et al.* (1983) showed that *D. v. virgifera* beetles are also able to move upwind at wind speeds of less than 0.5 m s^{-1} and that high beetle proportions can move cross wind up to a wind speed of 2 m s^{-1} . This is probably the explanation for some movements of the released *D. v. virgifera* populations in this study, which were not adequately explained by the major wind direction (e.g. figs 3 and 4) or by changes of wind over the recapture period (see correlations of lengths of vectors). In site B for example, the beetle movements were strongly influenced by the flowering areas of lucerne in the release area (fig. 3). Beetles either stayed in areas with flowering lucerne (see vectors of site B in 2003, table 2), or moved to nearby flowering lucerne (see vectors of site B in 2004, table 2). Laboratory studies showed that, in addition to maize, *D. v. virgifera* adults can feed on many other alternative food sources, mainly on pollen, and to some degree on fresh leaf material of other plants (Moeser & Hibbard, 2005). Also, field observations record feeding of *D. v. virgifera* on alternative host plants, i.e. mainly on flowering weeds within and around maize fields (Moeser, 2003). This is the first field study to establish that introduced beetles in non-maize areas will make significant use of such food sources, and will not immediately fly off to reach maize fields, neither by wind drift, by random movement or by directional movements. From these findings it can be concluded that flowering lucerne and probably other flowering plants, such as sunflower, will arrest introduced beetles in a non-maize area for some time.

Apart from the influences of wind and alternative host plants on beetle movements, few movements of the released beetles were explained by orientation towards maize, which is the major food and oviposition habitat for *D. v. virgifera*. This was particularly true for site A where the dry cut grass steppe provided no alternative food sources. All maize fields within a radius of up to 1500 m slightly influenced the direction of beetle movements. The small maize plots 300 m from the release point were probably situated within the odour plume of naturally occurring maize fields located further away, and consequently directional movements could never be solely attributed to those small maize fields

during the whole study. It is known that *D. v. virgifera* adults are attracted by maize volatiles (Prystupa *et al.*, 1988; Spencer *et al.*, 1999), but such attraction and long distance recognition is difficult to prove under field conditions because: (i) the chemical and chemosensory basis of chrysomelid host plant recognition is very complex (Mitchell, 1988); and (ii) such olfactory attractions may be overlaid by stronger environmental factors, such as wind, as shown above.

Finally, vertical vegetation structures, such as trees and forests, seem to influence *D. v. virgifera* movements. This might be a result of statistical chance, as those influences were found to be inconsistent in terms of distance and direction between the two experimental sites; or of a deposition of beetles on higher structures due to the vortices created by these structures (V. Dennis, 2005, personal communication). On the other hand, Spencer *et al.* (1999) suggested that *D. v. virgifera* beetles alight on objects in their flight path more or less indiscriminately, because *D. v. virgifera* moved more frequently to taller maize stands or soybean than to smaller maize stands or soybean in his study. Moreover, the use of trees and woodland for visual orientation of beetles has also been reported for other Coleoptera, such as Japanese beetles, *Popillia japonica* Newman towards *Tilia* sp. trees (Rowe & Potter, 1996), and may be true for *D. v. virgifera* as well.

Unfortunately, there are no multifactor analyses available for circular experiments that could rank the influences of the above-described factors for *D. v. virgifera* movements. Nevertheless, the results lead to the conclusion that accidentally introduced *D. v. virgifera* beetles in non-maize areas can be influenced by wind direction and alternative food-providing habitats within the introduction area, and to a limited degree by the surrounding maize fields. However, the present results also suggest that in nearly half of the introduction events, the populations can be expected to perform a random or uniform movement; their movements towards any particular habitat cannot be predicted. Conclusively, a habitat management strategy cannot be suggested for preventing initial dispersal of beetles.

Despite the factors that influence the movement of beetle populations in this study, *D. v. virgifera* spread over more than 80 ha of non-maize areas, and a relatively high proportion arrived in the nearby 300 m distant maize fields. Nearly 3% reached such a maize field in this study and the probability of arrival would increase with the number of fields. In addition to the number of nearby fields, knowing the number of introduced individuals is essential to calculate the risk of arrival of introduced *D. v. virgifera* in their host habitat (Grevstad, 1999). In many cases it is suggested that no more than 10 beetles are introduced in a single event of introduction (Wittenberg, 2005), however, in the case of 10 nearby maize fields, three out of 10 beetles would most probably reach a maize field. Even infestation at this level would lead to a high risk of establishment of a starter population of this alien species, followed by a population build up with a high intrinsic rate of increase that may start a more widespread invasion.

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