Dispersal propensity and settling preferences of European corn borers in maize field borders

Richard I. Bailey, Denis D. Bourguet, Anne-Hélène Le Pallec, Sergine Ponsard

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R I C H A R D   I.   B A I L E Y,*†‡ D E N I S   B O U R G U E T†,
A N N E - H E L E N E   L E   P A L L E C‡ and S E R G I N E   P O N S A R D‡

†*Centre de Biologie et de Gestion des Populations (CBGP), UMR INRA-IRD-CIRAD-Montpellier SupAgro,
Campus International de Baillarguet CS 30016, 34988 Montferrier-sur-Lez Cedex, France; and* ‡*Laboratoire
Dynamique de la Biodiversité, UMR CNRS 5172, Université Toulouse III, 118 route de Narbonne,
31062 Toulouse Cedex 09, France*

Summary

1. *Bacillus thuringiensis* (*Bt*) crops kill pest larvae but have led to resistance evolution in several target pests. The high dose-refuge (HDR) strategy aimed at delaying *Bt* resistance evolution depends on dispersal patterns of target pests. Examination of adult dispersal of the European corn borer *Ostrinia nubilalis* (ECB), the main target of *Bt* maize, can help to improve resistance management.

2. Estimated recapture rates over 20 mark–release–recapture sessions in herbaceous field borders, where ECB adults rest during the day and mate at night, were used to examine the influence of sex, release period and site on ECB dispersal. Data from an additional 30 sessions were used to test the influence of night temperature, humidity, dew index and wind speed.

3. Average recaptures within 50 m of release were lower 12 h after night (7·7%) than 12 h after day (34·5%) releases, did not differ between sexes, and decreased during nights with higher temperatures and lower wind speed.

4. Local habitat had a major influence on dispersal. The number of unmarked adults caught initially in a given section of field border was strongly correlated with those subsequently captured in the same section, suggesting that moths flying in from the surroundings consistently settle in the same preferred spots. Moreover, recapture rates of marked adults were positively correlated with the prior density of unmarked adults in the release section.

5. The spatial distribution of recaptured moths around the release point suggests that they moved on a very local scale, while those not recaptured probably left the area by a different, long-range type of dispersal.

6. *Synthesis and applications*. A proportion of European corn borer adults typically remained within a few metres of their initial location for at least 12 h. This should favour non-random mating early in the flight season when nights are cold, population mixture is low and most individuals are unmated. Non-random mating can accelerate the evolution of resistance, but this effect may be offset by non-random oviposition. Our findings suggest that the intensity and direction of dispersal could be manipulated by field border management. Our data on the range and prevalence of short-range dispersal and the factors influencing this process, support the view that resistance evolution is multifactorial. Our results can be used to parameterize multifactorial models from which specific management recommendations can be formulated.

Key-words: *Bt* maize, habitat, *Ostrinia nubilalis*, resistance management, temperature

Correspondence: Denis Bourguet, Centre de Biologie et de Gestion des Populations (CBGP), UMR INRA-IRD-CIRAD-Montpellier SupAgro, Campus International de Baillarguet CS 30016, 34988 Montferrier-sur-Lez Cedex, France (fax +33 499 623345; e-mail bourguet@supagro.inra.fr).
*Present address, Université Rennes 1, UMR CNRS 6553 'ECOBIO', Campus Beaulieu, Bâtiment 14 A, 263 Avenue du Général Leclerc, 35042 Rennes Cedex, France.

Introduction

Management of pesticide resistance (Vila-Aiub *et al.* 2005) often requires the analysis of insect dispersal (Congdon, Lange & Clarke 1997) because of the spatial nature of resistance management systems. *Bacillus thuringiensis* (*Bt*) genes, causing crop plants to produce an insecticidal toxin, have been introduced by transgenesis into several major crops, including maize. In the USA, *Bt* maize makes up approximately 25% of the surface planted with maize (James 2004). Several *Bt* maize varieties have also been approved by the European Union and in 2004 were already present on 58 000 ha in Spain (Eizaguirre *et al.* 2006). The current strategy for preserving the efficacy of *Bt* crops against agricultural pests is the high dose-refuge (HDR) strategy (Alstad & Andow 1995), which aims to reduce the selection of resistance to *Bt* toxins in targeted pest populations by planting pest 'refuges' of non-*Bt* food plants. Such refuges are thought to delay resistance because they provide a pool of susceptible individuals to mate with resistant individuals emerging from *Bt* crops. Offspring of such crosses would be heterozygotes and hence susceptible, as *Bt* resistance is expected to be rendered functionally recessive by the high dose of toxin produced by *Bt* crops (Alstad & Andow 1995). Refuges must be close enough to *Bt* crop fields to ensure effective mixing between resistant individuals and the much more numerous susceptible individuals before they mate.

For a given *Bt* crop, the optimal spatial layout of the refuges therefore depends on the dispersal behaviour of the target pest. The early version of the HDR strategy suggested that increasing pest dispersal among *Bt* crop fields and refuges would necessarily delay resistance. However, the effect of dispersal on the evolution of resistance may not be so straightforward. Several models (Caprio 2001; Guse *et al.* 2002; Ives & Andow 2002; Onstad *et al.* 2002; Heimpel, Nauhauser & Andow 2005) have shown that, depending on the sex and mating status of dispersing individuals, and on whether they move mainly away from or into *Bt* fields, a high dispersal rate can delay, accelerate or have no influence on resistance evolution.

When the HDR strategy became mandatory for *Bt* maize growers in the USA in 2000 (EPA 2001), the dispersal of the European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae; ECB), one of the primary target pests of *Bt* maize, was not well known (Caffrey & Worthley 1927; Showers *et al.* 1976). Hence the amount and spatial layout mandated for refuges were based on 'best guesses' rather than on a substantial corpus of data (Bourguet, Desquilbet & Lemarié 2005). Since then, three empirical studies aimed mainly at estimating ECB dispersal distances have been published.

Showers *et al.* (2001) released several batches of nearly 100 000 ECB adults each into maize fields, of which they recaptured 0–5 males release^{-1} in pheromone traps situated 0·2, 0·8, 3·2 and 9·6 km from their release point. Hunt *et al.* (2001) released several batches of 3000–7500 adults and recaptured < 0·8% in light traps < 210 m from the release point. Finally, Qureshi *et al.* (2005) released several batches of 23–24 000 adults into *Bt* maize fields and recaptured between 0·08% and 9·9% in pheromone and light traps at distances up to 670 m from the release point. These three studies confirm previous observations that ECB are capable of long-range flights (Caffrey & Worthley 1927). Hunt *et al.* (2001) and Qureshi *et al.* (2005) also concluded that a fraction of ECB adults may settle within the release field. However, the pheromone and light traps used in these studies may have changed moth dispersal behaviour by attracting them, as observed by Mo *et al.* (2003) for the diamondback moth *Plutella xylostella* L. Also, recapture was not designed to be exhaustive. Hunt *et al.* (2001) suggested that the effective attraction radius of light traps might be < 3 m. Hence in these studies the number of recaptures was an underestimate of the number of marked individuals present in the surroundings of the traps.

We have investigated further the dispersal behaviour of ECB using sweep nets for recapture, in an attempt to recapture exhaustively all released males and females present in the study sites. We were interested in the turnover and dispersal behaviour of adults once they have emerged from a maize field and moved into herbaceous borders of the cultivated fields, i.e. places in which they frequently rest during the day and mate at night (Pleasants & Bitzer 1999). For that purpose, we released ECB adults into such borders and estimated the proportion staying in the close vicinity of their point of release over 12 h. We examined the influence of a number of factors on this proportion, including habitat preferences, sex and, for an extended data set covering 3 years and five flight periods, a number of weather variables. Finally, we fitted curves to the spatial distribution of recaptured individuals to infer the dispersal behaviour of moths that were not recaptured.

Materials and methods

FIELD METHODS

Mark–recapture experiments took place in a maize-growing area about 20 km south of Toulouse, France. Adult ECB were caught in the field with sweep nets and taken to the laboratory. After *c.* 30 min at 6 °C (until they were unable to fly), they were marked on the dorsal thorax and base of the wings with a 1:1 ink:ethanol mixture. Mortality of marked adults over 12 h, the time between release and recapture (see below), was typically < 5% (unpublished observations at external temperature during the course of these experiments). A different colour was used for each release. After marking, moths were stored in cool boxes to reduce agitation that might cause damage and abnormal dispersal at the time of

release. Each experimental site consisted of two parallel herbaceous borders running along either side of a small (3–4 m wide) road and separating it from cultivated fields on one or both sides. The borders were 1–3 m wide, including a c. 0·75-m deep drainage ditch along most of their length (except for occasional field entry points) and contained naturally growing mixed vegetation (monocotyledons and dicotyledons) that remained uncut throughout the experiments. Adult ECB densities are often higher in such borders than in maize fields themselves. Temperature and other weather variables were taken from the Lherm/Sa meteorological recording station of Météo-France located < 5 km away from the experimental sites.

EXPERIMENT 1

Experiment 1 was conducted between 5 and 14 June 2003 during the first of the two annual ECB flights occurring in this area. Daily and nightly temperatures varied between 21 and 37 °C and 18 and 25 °C, respectively. Eight mark–recapture experiments (1 'day' and 1 'night' experiment in each of four sites) were conducted. Day releases were done in the early morning and night releases shortly before dusk. Sites were located 4–18 km from each other. Two sites (M1 and M2) were borders of maize fields at about the V6–V8 stage. The two other sites (S1 and S2) were borders of sunflower *Helianthus annuus* L. fields also at the V6–V8 stage.

M1 had a maize field on one side and housing on the other, vegetation was quite lush, 0·2–0·75 m high, and consisted of about 50% grasses. Site M2 had maize on one side and a non-maize crop on the other. Vegetation consisted of 80% grasses and was 0·2–0·5 m high. Sunflower fields were present on both sides of sites S1 and S2. In site S1, borders consisted of about 70% grasses and were 0·3–0·5 m high. In site S2, vegetation was lush, 0·5–1 m high, with about 50% broadleaved plants, 20% horsetails (Equisetaceae) and 30% grasses. Preliminary examination revealed that ECB densities were close to zero in all sites.

Adults were captured from various sites < 24 h before release. After marking, they were released at a study site into a 50-m long strip, in about equal proportions on both sides of the road. Between 122 and 304 adults, 33–73% males, were released on each occasion. Recaptures were performed using sweep nets 10–14 h after release and continued until we had good evidence that the strip had been cleared of any adults, i.e. until additional checking of the strip did not yield > 5 additional adults. The number of marked and unmarked adults of each sex was recorded for each site and session.

EXPERIMENT 2

Experiment 2 was conducted between 2 and 17 August 2003, during the second annual ECB flight. Daily and nightly temperatures varied between 30 and 45 °C and

19 and 28 °C, respectively. Twelve mark–recapture experiments (2 day and 2 night experiments per site) were conducted at three different sites (B, O and S) in borders of maize fields (R4–R5 reproductive stages) where large numbers of unmarked adults were present. Sites were located 2–5 km from each other. Site B consisted of two maize field borders. Vegetation was lush, c. 50 cm high, and dominated by grasses and *Convolvulus arvensis* L. The 10-m release section was dominated by a bushy area of *C. arvensis* 4 m wide and up to 1 m high. Site O had maize on the release side and wheat on the other side. Vegetation consisted of < 50% grasses and was typically 0·2–1 m high. *Urtica dioica* L. predominated in both the site and the release section. Site S had maize on both sides. Lush grass, 0·3–1 m high, predominated in the whole site, including the release section. Between 294 and 547 adults, 30–60% males, were released on each occasion.

Less than 12 h before release, a 110-m long strip (along both sides of the road) was cleared of ECB, making every effort to remove all adults and recording the number caught in each 10-m section. More adults, if needed, were caught > 3 km away from the site. After marking, adults were released into the central 10-m section of the strip, on one side of the road only. Day releases were done at 06:00 and night releases at 20:00. Recaptures, using sweep nets, took place over the 110-m strip on both sides of the road. In experiment 1, repeated scanning of the first crop rows along the release section rarely yielded any captures, suggesting adults that were not recaptured moved out of the study area either by small-range dispersal, moving along the border parallel to the road, or by another type of dispersal. In experiment 2, releasing adults into a more limited section of the border (10 m instead of 2 × 50 m) and recapturing over a larger section (2 × 110 m) allowed estimation of the extent of lateral small-range dispersal (< 55 m from the release point) along the border. Some dispersal into the adjacent maize field may have occurred, although, as in experiment 1, occasional scanning of the crop rows adjacent to the release section yielded few recaptures. Such dispersal would cause the recapture rate in the border to be an underestimate of the proportion of individuals still present locally. Recaptures started 12 h after release and went on for c. 3 h, until we had good evidence that all adults in the strip had been caught, as in experiment 1. The number of marked and unmarked adults of each sex was recorded per 10 m section and per side of the road.

ADULT DISPERSAL PROPENSITY

For any release j, the proportion of recaptures is $P_j = Nrec_j/Nrel_j$, where $Nrel_j$ is the number of marked adults released on that occasion, and $Nrec_j$ is the number of marked adults recaptured in the 2 × 50-m study section in experiment 1 and the 2 × 110-m study section in experiment 2.

For experiment 1, we tested for a possible effect of adjacent crop (maize or sunflower), site (a factor nested within adjacent crop), release period (day or night) and sex (male or female) on P_j. For experiment 2, we tested the same variables (except adjacent crop, which was always maize) and, using numbers of unmarked adults caught in the 10-m release section prior to release (estimated as $NU_{ij}before$; see habitat preference below), habitat preference (Pleasants & Bitzer 1999).

Because recapture is a binary trait, the error term for P_j was assumed to follow a binomial distribution and analyses of deviance were performed on logit-transformed data (Crawley 1993) using the GENMOD procedure of SAS (SAS Institute Inc. 1993). Over-dispersion in the data was corrected for by the square root of the ratio of deviance over the degree of freedom ratio when the latter ratio was > 2. F-statistics from SAS type 3 analyses estimated the significance of each factor. Models were [adjacent crop + site(adjacent crop) + sex + release period] for experiment 1 and (site + sex + release period + habitat preference) for experiment 2. Because of the relatively limited number of data points compared with the number of factors tested, we did not test for interactions.

As neither experiment 1 nor experiment 2 revealed any significant influence of sex on P_j (see the Results, adult moth dispersal turnover), marked adults of both sexes were pooled within a given session to estimate a common P_j value for each of the 12 sessions in experiment 2. These values were then used to calculate the probability for an adult to still be present within 55 m of its release point after 12, 24, 36 and 48 h. Dispersal could begin during the day or night, so, for instance, the probability of still being present after 36 h starting during the day would be $p_{day}^2 \cdot p_{night}$. The confidence interval around these values was ±1·96 times their standard error estimated by bootstrap (10 000 values calculated by resampling values for p_{day} and p_{night} among the 6 day and 6 night values observed for P_j).

INFLUENCE OF WEATHER

In order to provide indicators of expected variation in recaptures depending on weather variability over and above that found in summer 2003, data from three further mark–release–recapture experiments performed in summers 2004 and 2005 were used for further analysis. These experiments (3, 4 and 5), briefly described below and in more detail in Dalecky et al. (2006), yielded additional P_j values for night releases. These values were combined with those of night releases only from experiments 1 and 2 to test for a possible effect on P_j of temperature (°C), wind speed (m s^{-1}) and humidity, all 1 h after sunset, and dew index (actual temperature (°C) – temperature (°C) at dew point) 1 h before sunrise in the morning following the release. Higher dew index values indicate a lower probability of dew formation. These variables are thought to affect ECB activity

(Showers et al. 1976; DeRozari, Showers & Shaw 1977; Webster & Cardé 1982; Royer & McNeil 1993).

Each additional experiment (3–5) was designed to answer a different, specific question related to ECB dispersal; hence their protocols differed from those of experiments 1 and 2 and from each other (Dalecky et al. 2006) but were consistent within each experiment. All involved mark–release–recapture of ECB adults conducted in the same maize-growing area as experiments 1 and 2. Experiment 3 (experiment 1 in Dalecky et al. 2006), conducted during the first ECB annual flight of 2004, investigated dispersal behaviour of young virgin males and females and non-random mating between resident and immigrant adults. Experiments 4 and 5 (both experiment 3 in Dalecky et al. 2006), performed during the first and second flights of 2005, respectively, investigated the proportion of young virgin females mating during a given night within 50 m of a maize stalk on which they had been placed the evening before. The time elapsed between release and recapture was 2 nights and 1 day in 2004, and 1 night during both flights of 2005. Only females were released in experiments 4 and 5. Recaptures were conducted within a 100-m long strip of border and maize in experiment 3 and within 50 m of all releases in experiments 4 and 5 (this did not include individuals recorded as 'mated locally' in Dalecky et al. 2006). Adults were released seven to eight rows (running parallel with the nearest field border) into the maize field in experiments 3–5, in the central 40-m portion of the parallel recapture area in experiment 3 and into an area determined by the number released in experiments 4 and 5. Experiments 3–5 all used laboratory-reared virgin adults, fed on a wheat rather than maize diet in experiments 3 and 5, and released < 24 h after emergence in all cases. Experiments 3 and 4 used individuals from an outbred laboratory mass-reared strain, while experiment 5 was performed on offspring of wild, field-collected individuals. Adults were not colour-marked in experiment 5. Experiments 1–5 were combined to examine the possible effect of weather variables on P_j after night releases by including the categorical variable experiment in the multiple regression to control for variation in experimental design. We used the GENMOD procedure of SAS as described for experiments 1 and 2 and considered the continuous variables temperature, dew index, humidity and wind speed and the factor experiment as explanatory variables.

DISPERSAL BEHAVIOUR

We estimated typical dispersal distance along the borders by assuming one-dimensional diffusion and adjusting a Normal distribution curve $(P_j(x) = [1/(2 \cdot \pi \cdot \sigma_j^2)^{1/2}] \cdot e^{-(x/2 \cdot \sigma_j^2)})$ to the data obtained in experiment 2. Adjustments were done by minimizing $\sum_i (P_{ij}$ predicted $- P_{ij}$ observed$)^2$, where P_{ij} observed $= Nrec_{ij}/Nrel_j$, and $Nrec_{ij}$ and $Nrel_j$ are the number of (marked) adults recaptured in section i and the total number of (marked) adults

released, respectively, during release j. Recaptures in both sections at equal distances to the left and right from the release section were pooled. The predicted proportion of recaptures was P_{ij} predicted $= e_j \cdot [F(dOUT_i) - F(dIN_i)]$, where e_j is the proportion of released individuals that made up the predicted distribution (including those outside the 110 m where the recapture took place) and $dOUT_i$ and dIN_i are the distances between the centre of the release section and the outer and inner edges of section i, respectively. F is the primitive of the fitted function. Two additional distributions classically used in dispersal studies (Turchin 1998; Levin *et al.* 2003) were adjusted to our data to ensure that the conclusions reached with the Normal curve were robust: the negative exponential ($P_j(x) = \lambda_j \cdot e^{-\lambda_j \cdot x}$) and the inverse power (modified as $P_j(x) = a_j \cdot (b_j - 1)/(1 + a_j \cdot x)^{b_j}$, to avoid $P(x) \to +\infty$ when $x \to 0$ and to scale $[F(0) - F(x)]$ to 1 when $x \to +\infty$). Adjustments were performed with the 'solver' facility in an EXCEL spreadsheet; e_j, σ_j, λ_j, a_j and b_j were fitted parameters.

HABITAT PREFERENCE

In experiment 2, the proportion of unmarked adults captured in the ith 10m-section of a border during the clearing before the jth release was calculated as U_{ij} before $= NU_{ij}$ before$/\sum_i NU_{ij}$ before, where NU_{ij} before is the number of unmarked adults captured in section i before the jth release. Similarly, U_{ij} after $= NU_{ij}$ after$/\sum_i NU_{ij}$ after was calculated on the number of unmarked adults caught during the jth recapture session. To determine whether unmarked adults were located randomly in the border or consistently tended to choose the same 10-m sections, we calculated Pearson's correlation coefficient between U_{ij} before and U_{ij} after.

Results

ADULT MOTH DISPERSAL PROPENSITY

In experiment 1, a total of 1940 adults was released and 209 recaptured (Table 1). The percentage of recapture, P_j, pooled for both sexes ranged from 0% to 29·9%. The logistic regression analysis revealed a significant effect of adjacent crop ($F_{1,10} = 5·45$, $P = 0·042$), site(adjacent crop) ($F_{2,10} = 4·64$, $P = 0·038$) and release period ($F_{1,10} = 16·59$, $P = 0·002$) on P_j. Recapture rates were lower in field borders close to a maize than a sunflower field, and lower after night than day releases. Sex had no significant effect ($F_{1,10} = 0·29$, $P = 0·603$).

In experiment 2, a total of 4578 adults was released and 978 recaptured (Table 1). P_j varied between 0·6% and 44·8%. As in experiment 1, P_j values were significantly lower after night than day releases ($F_{1,18} = 82·25$, $P < 10^{-4}$). In addition, P_j was significantly greater for releases into border sections preferentially chosen by wild adults ($F_{1,18} = 5·85$, $P = 0·026$). As in experiment 1, sex had no detectable effect on P_j ($F_{1,18} = 0·36$, $P = 0·558$)
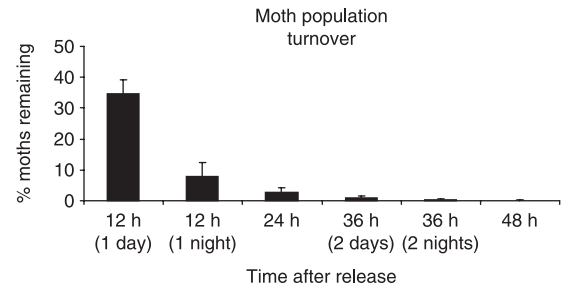

Fig. 1. Average ($\pm$ 95% confidence intervals) bootstrap estimates of the probability that an adult stays in a maize field border within < 55 m of its release point for 12–48 h.

but, in contrast to experiment 1, P_j was not significantly influenced by site ($F_{2,18} = 1·72$, $P = 0·207$).

The bootstrap averages and 95% confidence intervals for the percentage of adults remaining within 55 m of their release point (i.e. within the 110-m section of a ditch where we recaptured) after 12–48 h are shown in Fig. 1. They were 34·5% (30·0–39·1%) after 12 h day, 7·7% (3–12·4%) after 12 h night, 2·7% (1–4·2%) after 24 h, < 1% after 36 h and < 0·2% after 48 h (Fig. 1).

INFLUENCE OF WEATHER

Experiments 1–5 yielded 4, 6, 17, 5 and 8 P_j values for night releases (sexes combined) for examination of weather effects (Table 2). As crop had a significant effect on P_j, only the two June 2003 sessions conducted in maize field borders were used in the present analysis. Dew index was not available for 12 August 2003, decreasing the number of data points to 37. Humidity and dew index had no significant impact on P_j ($F_{1,28} = 0·03$, $P = 0·865$ and $F_{1,28} = 1·42$, $P = 0·244$, respectively). Once the significant ($F_{4,28} = 9·10$, $P < 10^{-4}$) differences in P_j between experiments had been accounted for, a significant effect of temperature ($F_{1,28} = 6·07$, $P = 0·020$; P_j decreased when night temperature increased) and wind speed ($F_{1,28} = 4·50$, $P = 0·043$; P_j increased with increasing wind speed) was detected. These conclusions held when humidity and dew index were removed from the analysis and the 12 August 2003 data were included (experiment, $F_{1,31} = 12·29$, $P < 10^{-4}$; temperature, $F_{1,31} = 20·77$, $P < 10^{-4}$; wind speed, $F_{1,31} = 4·96$, $P = 0·033$)

DISPERSAL BEHAVIOUR

In experiment 2, most recaptures occurred either in the release section itself or the adjacent sections, while other sections were virtually empty of adults (Fig. 2). Estimates for e_j were very close to P_j (Table 1; adjustments of both other dispersal curves, negative exponential and inverse power, yielded very similar values; data not shown), suggesting that a fraction $\approx P_j$ of the released adults moved along the ditch at a very local scale and formed the distribution from which we

Table 1. Figures for mark–release–recapture experiments performed in experiments 1 and 2 during the two annual ECB flights and parameters of a Normal distribution curve fitted to the spatial distribution of recaptured individuals (see the Materials and Methods: dispersal behaviour)

Flight	Site	Period	Sex*	n released	% recaptured	% under the fitted curve (e)	σ (m/12 h$^{1/2}$)
First	S1	Day	M	176	27·8	–	–
			F	108	20·4	–	–
		Night	M	152	3·9	–	–
			F	152	3·9	–	–
	S2	Day	M	68	14·7	–	–
			F	54	9·3	–	–
		Night	M	48	10·4	–	–
			F	167	10·8	–	–
	M1	Day	M	155	1·9	–	–
			F	130	3·1	–	–
		Night	M	108	1·9	–	–
			F	84	3·6	–	–
	M2	Day	M	134	29·9	–	–
			F	159	22·6	–	–
		Night	M	75	0·0	–	–
			F	170	0·0	–	–
Second	B	Day 1	M	144	44·4	43·7	3·14
			F	251	41·8	41·8	2·28
		Day 2	M	89	34·8	34·8	3·57
			F	205	40·5	40·0	2·22
		Night 1	M	210	21·9	20·5	3·78
			F	297	16·8	15·5	4·46
		Night 2	M	133	18·0	16·5	3·36
			F	206	4·4	3·9	3·26
	O	Day 1	M	160	30·0	27·5	2·96
			F	154	24·0	23·4	3·14
		Day 2	M	194	26·3	25·8	4·84
			F	175	36·0	36·0	3·41
		Night 1	M	243	5·8	5·9	32·35
			F	304	10·2	9·6	23·70
		Night 2	M	153	6·5	4·1	7·54
			F	160	3·8	1·3	2·00
	S	Day 1	M	114	28·1	24·6	2·38
			F	183	44·8	43·7	1·37
		Day 2	M	133	22·6	21·1	3·10
			F	232	33·2	32·8	2·58
		Night 1	M	170	0·6	0·0	2·27
			F	113	2·7	2·7	1·5E^{-15}
		Night 2	M	47	4·3	4·3	1·75
			F	57	1·8	1·8	1·86

*M, male; F, female.

recaptured (almost exhaustively), while the remaining fraction ($\approx 1 - P_j$) was too large to be accounted for merely by the parts of the adjusted curve that fell outside the study section. This fraction must have left by another type of dispersal.

HABITAT PREFERENCE

In experiment 2, there was a positive, highly significant correlation between U_{ij}before and U_{ij}after, the percentage of unmarked adults captured in a given section within a given study site before and after release, respectively ($r = 0.465$, $P < 10^{-4}$, $n = 264$; Fig. 3). This result held when males and females or night and day releases were considered separately (details not shown). Hence, when moving to the herbaceous ditches, ECB

adults consistently preferred to settle in some sections rather than others.

Discussion

ADULT MOTH DISPERSAL PROPENSITY

Our results from seven different sites show that a small but detectable fraction of ECB adults located in a given border portion alongside a cultivated field are still present after 12 h. Indeed, average recapture rates after 12-h nights and days were 7·7% and 34·5%, respectively (and up to 44·8%; Table 1). Only once in 20 sessions was no adult recaptured. This result held for first and second annual flights, males and females and a range of adult densities (11–547 adults/10-m ditch section)

Table 2. Weather variables and recapture rates within 55 m of release location for 'night' releases, Experiments 1–5. Differences in protocol between experiments are described in the Materials and Methods: influence of weather. Day releases were excluded from these analyses because they only occurred in experiments 1 and 2 and the release period significantly affected recapture proportions

Experiment	Year	Annual flight period	Number of sites	Number of recapture sessions per site	Temperature (°C)*	Wind speed (m s⁻¹)*	Dew index*†	Humidity*	Number of released adults*	% adults recaptured*
1	2003	First	4	1	25·9 (20·1–30·8, 4·4)	1·8 (1·0–2·0, 0·5)	1·4 (0·5–2·9, 1·1)	72 (5–88, 15)	239 (192–304, 49)	4·3 (0–10·7, 4·6)
2		Second	3	2	34·7 (31·5–36·3, 1·9)	3·7 (1·0–5·0, 1·8)	4·7 (1·8–10·2, 3·2)	40 (28–65, 13)	349 (104–547, 161)	7·7 (1·4–42·7, 6·3)
3	2004	First	4	4–5	21·9 (15·9–26·1, 3·1)	3·6 (1·0–6·0, 1·6)	3·0 (0·9–9·7, 2·5)	69 (40–93, 14)	517 (340–600, 62)	4·3 (0·2–26·9, 6·5)
4	2005	First	1	5	20·9 (17·1–23·0, 2·4)	2·8 (1·0–4·0, 1·3)	1·2 (0·3–3·1, 1·1)	78 (66–95, 11)	40 (20–67, 18)	36·7 (16·6–62·1, 19·2)
5		Second	1	8	25·2 (19·1–27·6, 2·4)	3·9 (2·0–6·0, 1·9)	3·5 (0·7–15·9, 5·1)	59 (26–95, 19)	69 (36–145, 35)	15·6 (7·6–27·8, 6·7)

*Average over all sessions followed by (in parentheses) minimum – maximum and SD.
†Dew index = absolute temperature: temperature at dew point 1 h before dawn; higher values mean lower probability of dew formation.

and sex ratios (30–73% males) within realistic field values (S. Ponsard, D. Bourguet and R. Bailey, personal observations).

During experiment 1, and in particular experiment 2, our study area experienced an exceptional heat wave compared with 2004 and 2005 (Table 2). Although these conditions were not abnormal for parts of the ECB's broad geographical range, they may have produced unusual recapture rates; therefore we tested the influence of a number of weather variables on recaptures over 3 years and five flight seasons. Increased recaptures with greater wind speed would be expected because wind reduces moth activity (D. Bourguet, S. Ponsard and R. Bailey, personal observations). Temperature during the exceptionally warm summer of 2003 could have decreased the propensity of adults to move, hence resulting in unusually high recapture rates. However, during 30 further mark–release–recapture sessions performed in 2004 and 2005, a fraction of the released adults, varying from 0·2% to 62·1%, was recaptured in the close vicinity (< 55 m) of the release point or release section (Table 2). Average recapture rates found in these experiments are not directly comparable with those obtained in experiments 1 and 2 because of differences in vegetation type, individual characteristics of released adults and experimental design. However, our global analysis over the five experiments shows that increasing night temperature consistently increases the propensity of adults to engage in long-range dispersal within any given experiment. Hence recapture rates were probably lower, not higher, in June and August 2003 as a result of the temperatures being higher than they would have been during a more typical year in the same site and experimental setting. Nevertheless, recapture rates obtained in experiments 1 and 2 remained within the global range obtained in our study region (Table 2). This suggests that more adults leave the herbaceous ditches during warm rather than cold nights. Congruent relationships between temperature and flight activity have been recorded for other insects and spiders (Walters & Dixon 1984; Isaacs & Byrne 1998; Thomas, Brain & Jepson 2003).

FACTORS INFLUENCING ADULT POPULATION TURNOVER

Turnover, the rate of change of adult moth individuals in a location, varies with changing propensity to disperse and propensity of dispersers to settle. The magnitude of the proportion recaptured in experiments 1 and 2 was, as in experiments 3, 4 and 5, somewhat variable, and at least two factors appeared to influence it. Dispersal was higher at night than during the day. This is not surprising, as ECB are virtually inactive during the day, while mating and mating-related movements occur at night: females search for suitable places to emit pheromones, and males follow pheromone plumes towards receptive females. In addition, mated females visit maize fields to oviposit, and presumably move
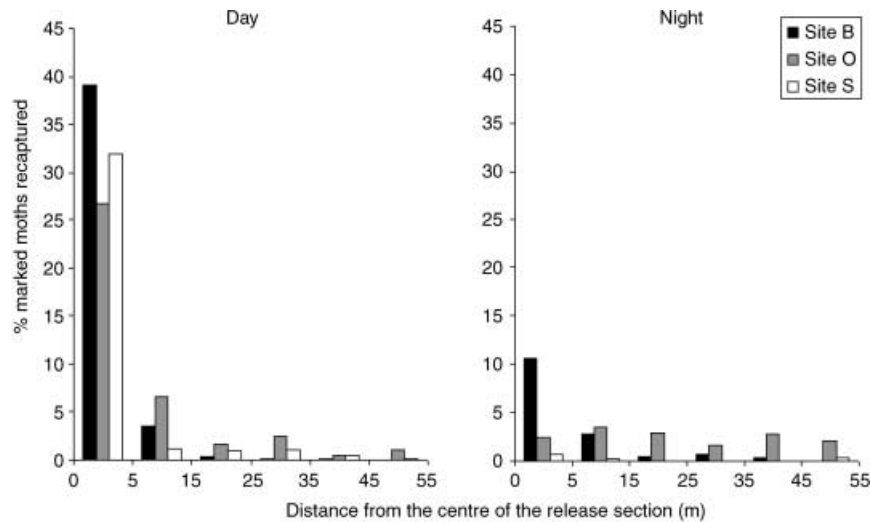
Fig. 2. Distribution of movement distances of marked adults recaptured 12 h after a 'day' or 'night' release. Sexes, and recaptures on either side of the release section, were pooled for all mark–recapture sessions of the second annual ECB flight.
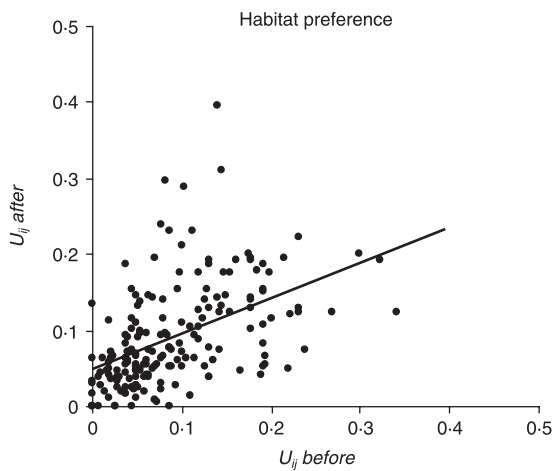

Fig. 3. Correlation between U_{ij}*before* and U_{ij}*after*: percentage of unmarked adults captured in a given 10-m section within a given study site before and after release, respectively ($r = 0.465$, $P < 10^{-4}$, $n = 264$).

back to herbaceous ditches afterwards. Interestingly, these different motivations to move did not result in any detectable differences in dispersiveness between the sexes. Wang *et al*. (1995) and Shirai (1998) also found no difference between male and female dispersal in a related species, *Ostrinia furnacalis* Guenée. For ECB, Qureshi *et al*.'s (2005) results were inconclusive while Dalecky *et al*. (2006) found no difference between the sexes. This suggests that sex-related differences, if any, are limited.

Two results from experiment 2 suggest that habitat strongly influences ECB dispersal. First, there was a strong correlation between the proportion of unmarked adults found in each 10-m section within a study site during initial clearing and the unmarked adults subsequently captured in the same section during recapture, suggesting that adults flying in from the surrounding

areas consistently settle in the same sections. Unfortunately, we did not monitor habitat variables of the different sections but these results suggest that it would be important to do so. Secondly, habitat preference, estimated by previous unmarked adult density in the release section, had a positive effect on the percentage of recapture, i.e. a negative influence on ECB dispersal. Notably, the significant site effect in experiment 1 may result from the fact that, unlike in the analysis of experiment 2, site and habitat preference could not be distinguished (sites being initially empty, no surrogate for habitat preference was available).

Other studies support the observation that habitat variation can substantially affect ECB dispersal. Sappington (2005) showed that, during the first ECB flight, the presence or absence of adjacent maize was the predominant factor affecting the distribution of ECB adults among herbaceous ditches. Hunt *et al*. (2001) also found that ECB tend to leave irrigated maize fields less than non-irrigated ones, and Hellmich, Pingel & Hansen (1998) and Pleasants & Bitzer (1999) observed that ECB were preferentially located in cooler and more humid vegetation. The fact that dense, tall grass, high humidity and the presence of dew at night are important for the ECB's sexual activity (Showers *et al*. 1976; DeRozari, Showers & Shaw 1977; Webster & Cardé 1982; Royer & McNeil 1993) may partly explain these trends.

DISPERSAL BEHAVIOUR

What happened to the marked individuals that were not recaptured in our experiments? Because recapture efficiency of the adults that did not leave the study section was probably close to 100%, and because movements into the adjacent crop field were probably low (see the Materials and Methods, experiments 1 and 2), two possibilities remain: they left the study area mostly along field borders, or mostly by long-range and probably

higher altitude flights. The fact that adults released into a 10-m section in experiment 2, if recaptured, were located mostly in the release section itself or in the two adjacent sections (note the very small values of σ_j in Table 1, experiment 2), suggests that most of the movement out of the study area must be the latter. Several insects, for example *Pectinophora gossypiella* (Saunders) (Tabashnik *et al.* 1999), *Bemisia tabaci* (Byrne *et al.* 1996) and *Diabrotica virgifera virgifera* Le Conte (Coats, Tollefson & Mutchmor 1986), display such a mixture of local and long-distance dispersal (Rankin & Burchsted 1992), and the ECB adults recaptured by Showers *et al.* (2001) in traps > 800 m from their release point may have performed long-distance dispersal. Based on recapture rates around the release points, Qureshi *et al.* (2005) also concluded that populations of ECB adults may include both long- and short-distance dispersers.

IMPLICATIONS FOR *Bt* RESISTANCE MANAGEMENT

Our results suggest that ECB adults do not necessarily engage systematically in long-range dispersal. Instead, they indicate that the typical distance covered by ECB adults recaptured after 12 h (on average 5·05 m ± 7·3 m, and up to 35 m; Table 1) may be too limited to ensure random mating between adult moths emerging from fields hundreds of meters apart. Similarly, Dalecky *et al.* (2006) found that a small fraction of newly emerged adults, males and females, actually mate at a local scale, sometimes at the very edge of their emergence field.

As proposed in Dalecky *et al.* (2006), local mating may not necessarily lead to a substantial number of resistant × resistant crosses, a feature that could reduce the efficacy of the HDR strategy, as many susceptible adults may already be present in the herbaceous ditches along *Bt* maize fields when a resistant individual emerges. However, mixing between resistant (emerged from *Bt* maize fields) and susceptible (emerged from the refuges) adults will not happen instantaneously (Sappington 2005). According to our results, it can be further delayed if nights are cold or windy. Cold nights are particularly likely to occur early in the flight season when, in addition, most individuals are still virgins. Limited dispersal thus appears particularly likely to limit random mating among resistant and susceptible individuals during that period, especially in colder regions or years.

In early models describing the HDR strategy (Alstad & Andow 1995) and assuming crop rotation, low dispersal rates systematically increased the evolution of resistance. More recent and more detailed models (Caprio 2001; Guse *et al.* 2002; Ives & Andow 2002; Onstad *et al.* 2002; Heimpel, Nauhauser & Andow 2005) suggest that this may not always be true. Indeed, Caprio (2001), Ives & Andow (2002) and Heimpel, Nauhauser & Andow (2005) showed that, provided male dispersal is high enough and crops are not rotated, limited female dispersal may decrease the selection intensity for resistance. If the same fields are used as refuges year after year, susceptible mated females emerging from these refuges would oviposit in non-*Bt* fields, reducing the exposure of their offspring to *Bt* crops. The absence of crop rotation would also increase the probability of homozygous resistant females emerging from *Bt* fields ovipositing there, rather than in refuges. If those females were mated with susceptible immigrant males, their heterozygous offspring would be counter-selected on *Bt* maize, thus reducing the frequency of *Bt* resistance alleles in the population. However, neither our results nor those of Dalecky *et al.* (2006) show any evidence for sex-related differences in dispersiveness.

Actually, the net effect of limited dispersal, causing non-random mating that can accelerate the evolution of *Bt* resistance, and non-random oviposition which may reduce the selection pressure towards resistance and hence slow resistance evolution, is difficult to predict. First, it depends on whether dispersal mainly occurs before or after mating and can be further complicated by the fact that limited dispersal may increase non-random mating but also reduce the mating success of males carrying *Bt* resistance alleles. Secondly, it depends on possible fitness costs associated with *Bt* resistance and on the effective dominance of this trait (Caprio 2001), which remains unknown for the ECB, as no resistant strain to *Bt* maize has been selected to date. Thirdly, as recently noted by Sisterson *et al.* (2005), this net effect depends on the regional abundance of *Bt* fields, the temporal distribution of *Bt* and refuge fields (i.e. on crop rotation practices), and the spatial distribution of both types of fields. In summary, resistance evolution is thought to be multifactorial. Models exploring different plausible scenarios tend to conclude that most factors, including dispersal, are not always detrimental or beneficial, but that their effects depend on the magnitude and possible influence on each other. Therefore, while it is unclear today whether the short-range dispersal revealed in the present study is good or bad for the HDR strategy, the availability of empirical values of the range and prevalence of short-range dispersal, and influences on this dispersal, can help to parameterize multifactorial models and restrict the number of cases to be considered.

Acknowledgements

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