Spatial scale of insect-mediated pollen dispersal in oilseed rape in an open agricultural landscape

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Summary

1. Interest in pollen-borne gene dispersal has grown with the cultivation of genetically modified plants. To date, both experimental data and models of oilseed rape (OSR) *Brassica napus* pollen movement at the landscape scale do not clearly differentiate between wind- and insect-mediated dispersal. Estimations of insect-mediated gene dispersal would be valuable for managing potential escapes of transgenes.

2. To quantify the intensity and spatial scale of pollen dispersal by insect pollinators in an agricultural landscape, bait points made of flowering male-sterile OSR that attract OSR pollinators were located at six distances (10–1100 m) from the closest OSR fields and feral populations. Flower-visiting insects were caught by net on these male-sterile flowers and were rubbed onto the stigmas of male-sterile OSR plants grown in a pollen-proof greenhouse to do a manual pollination of their flowers. In this way we were able to assess the insects' OSR pollen load and seed production at each of the six distances.

3. A large diversity of insects carried OSR pollen and contributed to seed production, but not pollen beetles *Meligethes aeneus*. Logistic regression analyses of the seed-set success from the manual pollination demonstrated that seed set significantly increased with the proximity of OSR fields, the size of the pollinating insect, and the main daily temperature. Seed set was not affected by the pollinating insect's order or genus. Seed set, both observed and predicted by the model, was above zero for flowers pollinated with large bees caught at > 1100 m from the nearest OSR field.

4. *Synthesis and applications.* Our study provides clear evidence that a large variety of insect species can transfer viable pollen between OSR plants over considerable distances. However, only 39·4% of the insects caught on male-sterile flowers carried OSR pollen. Our results provide valuable data to improve models of pollen dispersal for entomophilous crops at the landscape scale. These models are essential to help land-managers reduce pollen-borne gene dispersal from genetically modified plants to wild and cultivated relatives.

Key-words: apiformes, bees, *Brassica napus*, diptera, insect diversity, insect size, pollen dispersal, pollen flow, seed-set success, Syrphidae

Introduction

Gene flow has been studied extensively in the context of the evolution and conservation genetics of flowering plants (Sork *et al.* 1999). Historical rates of gene flow are frequently inferred indirectly from population genetic structure (Raybould *et al.* 2002). Contemporary gene flow may be estimated directly by measuring the distances moved by pollinators between flower visits, or by tracking the movement of pollen or seeds (Sork *et al.* 1999).

Interest in pollen-borne gene dispersal has grown with the cultivation of genetically modified (GM) plants. The world area planted in GM oilseed rape (OSR) *Brassica napus* (L.)

was 6·4 million hectares in 2009 (James 2009), and its cultivation could have a significant impact on the environment through unregulated gene flow (Dale, Clarke & Fontes 2002; Sanvido, Romeis & Bigler 2007). This could lead to (i) hybrids and backcrosses between OSR and weedy relatives being released into the environment (e.g. Chèvre *et al.* 2007) and (ii) hybridization and persistent seed banks causing problems for coexistence between GM and conventional OSR. Currently, practical measures such as isolation distances, voluntary clustering of fields, and delayed sowing dates are used to maximize the reproductive isolation of non-GM crops (e.g. Jank, Rath & Gaugitsch 2006).

Oilseed rape is a partially self-pollinating species. The selfing rate depends both on the cultivar and the environment but is usually > 50% (Becker, Damgaard & Karlsson 1992). Studies measuring gene flow via pollen from a single OSR field produced an L-shaped pollen dispersal curve: most dispersal events occur near the pollen source at distances up to ca. 50 m (Manasse 1992; Lavigne *et al.* 1998; Cai *et al.* 2008), and the frequency of dispersal events decreased slowly thereafter (Klein *et al.* 2006). Studies of a larger number of OSR fields at the landscape scale reported long-distance dispersal: 13% of sampled seeds had their nearest compatible site more than 1000 m away (Devaux *et al.* 2005) and up to 0·05% or 0·1% gene flow via pollen was reported between OSR fields separated by 2500 m (Rieger *et al.* 2002). Furthermore, a fat-tailed model best explained bumblebee, *Bombus* spp., pollen dispersal (Cresswell, Osborne & Bell 2002).

Insect and airborne pollen dispersal are probably not independent from each other (Pierre *et al.* 2010). OSR pollen is dispersed both by wind and insects, although their relative contributions to gene-flow are still debated, especially over long distances (Mesquida & Renard 1982; Ramsay, Thompson & Squire 2003; Cresswell 2008). It has been suggested that wind pollination is important when insect pollinators are scarce (Hoyle, Hayter & Cresswell 2007). The wind component of pollen dispersal has been modelled at both local and landscape scales (Walklate *et al.* 2004; Hoyle & Cresswell 2009). One such model predicts that the minimum distance between GM and conventional *B. napus* fields should be 1000 m to ensure a level of transgene adventitious presence in conventional fields below the EU 0·9% threshold (Hoyle & Cresswell 2009). This is consistent with landscape scale gene-flow data (Cresswell, Osborne & Bell 2002; Ramsay, Thompson & Squire 2003; Devaux *et al.* 2005; Cai *et al.* 2008) and Timmons *et al.* (1995) report of airborne OSR pollen grains 1·5 km away from source fields in numbers high enough to permit the production of seeds on emasculated plants with petals removed. Pierre *et al.* (1996) criticized this work, suggesting that the use of apetalous flowers does not rule out insect pollination.

Few studies have addressed the insect component of pollen dispersal and have empirically determined its spatial extent. Using molecular techniques on pollen trapped at hive entrances, Ramsay *et al.* (1999) showed that honeybee *Apis mellifera* L. colonies foraged up to 2 km, and in some circumstances up to 4 km. In addition they used honeybees from a hive near a field of GM OSR to hand-pollinate male-sterile (MS) OSR flowers. Pollen found on the body hair of these honeybees came from both GM and non-GM OSR, indicating either recent switching between these crops, or bee-to-bee contact within the hive as major means of effective pollen dispersal throughout the foraging area of the colony.

Several studies have measured the distances travelled by pollinators between successive flower visits to indirectly quantify pollen flow, especially with bees (Osborne *et al.* 1999; Beil, Horn & Schwabe 2008; Pasquet *et al.* 2008). The foraging range of bees (Apiformes) varies considerably among species as well as between individual workers in social species like bumblebees (*Bombus* spp.; Walther-Hellwig & Frankl 2000). In particular the flight range is linked to the body size of bees both among species (e.g. Zurbuchen *et al.* 2010) and within species in social bees (Kuhn-Neto *et al.* 2009).

Different experimental methods have been used to assess the maximum foraging range of insect pollinators and provide information on the spatial scale of insect-borne pollen flow (harmonic radar, Osborne *et al.* 1999; analyses on pollen loads, Beil, Horn & Schwabe 2008; radio-tracking of individual bees, Pasquet *et al.* 2008; experimental transect setting, Wolf & Moritz 2008; feeders, Kuhn-Neto *et al.* 2009). Yet, none of these methods provides enough information to infer the spatial scale of the effective gene flow that results from this foraging because they do not consider the amount of pollen transported by the insect, the viability of this pollen, or the pollen deposition onto the stigma.

Although most studies have focused on bees, other insects could also play a role in long-distance effective pollen dispersal. Ramsay, Thompson & Squire (2003) recorded the presence of pollen beetles *Meligethes aeneus* (Fabricius) on OSR plants at 26 km from the nearest OSR field and suggested that these insects might play a role in pollen dispersal. Among dipterans, the most important family of flower visitors is the Syrphidae or hoverflies: the very common and abundant hoverfly *Episyrphus balteatus* (DeGeer) is an effective pollinator of OSR (Jauker & Wolters 2008). The foraging span of syrphid flies is mostly unknown, however.

Good estimations of the spatial scale of pollen-borne gene dispersal and a better understanding of the underlying mechanisms would be valuable for managing potential escapes of transgenes to feral plant populations and populations of wild relatives as well as for establishing recommendations for isolation distances that would minimize effective pollen flow between fields (Meagher, Belanger & Day 2003). The objectives of our study were (i) to assess the spatial scale of insect-borne pollen dispersal in an open agricultural landscape, and (ii) to determine the factors that affect it using OSR as a model. In particular, we hypothesized that pollen dispersal would be affected by the type of pollinating insect (taxonomic status) and the environment (weather and OSR flowering stage), and that it would increase with insect size and decrease with distance from the pollen source.

Materials and methods

STUDY SITE AND EXPERIMENTAL DESIGN

The experiment was conducted in 2008 around the village of Selommes (47°45′1″ N lat., 1°11′8″ E long., 120 m altitude; Pessel *et al.* 2001) in a 10 × 10 km study site within a large area of open agricultural landscape planted with conventional winter-sown cultivars of *B. napus*. All 98 OSR fields were mapped in January. At the onset of OSR flowering we set up bait points consisting of four flowering MS OSR plants at different distances from the nearest OSR fields. Six such points were set up following a roughly exponential scale of increasing distance from the nearest OSR field (10, 20, 55, 150, 400 and 1100 m; Fig. 1) using ArcView (Version 9·1; ESRI, Redlands, CA, USA). When possible, we favoured points that were at the same distance from several near-by fields. All OSR feral populations along roads and dirt tracks were also mapped twice during the season, once in mid-April during rape flowering and once in June (method in Pivard *et al.* 2008).

INSECT CAPTURE AND MANUAL INSECT POLLINATION

Insects were caught with a bug net during their visit to MS OSR bait plants, which ensured a dietary preference for OSR, but prevented the accumulation of further OSR pollen. The insects were anaesthetized with CO_2 and preserved individually at 6 °C in a glass tube (see Appendix S1 in Supporting Information). At each bait point we caught all insects visiting flowers during periods of 30 min chosen randomly between 10·00 and 17·00 h. Sampling was only undertaken when the weather was favourable for bee activity (temperature > 12 °C, wind speed ≤5 m s^{-1}, no precipitation and dry vegetation; Westphal *et al.* 2008). We caught insects at all bait points on five separate days from the beginning to the peak of flowering of most fields (3, 11, 15, 25 April and 6 May) and we tried to catch the same number of insects at all bait points. Rainfall, mean daily temperature and wind speed were available from a national weather station located at Selommes. The level of flowering of OSR fields was characterized at four dates (4, 16, 26 April and 7 May). We randomly selected and tagged 24 plants in 16 randomly selected fields and recorded the number of open flowers.

To estimate pollinating success of captured insects, each one was rubbed onto the stigma (manual insect pollination) of a male-sterile OSR plant (cultivar Yudal). These plants were grown in a pollen-proof greenhouse at Université Paris-Sud (Orsay, Essonne, France) ca. 150 km away from the bait points. Manual insect pollination took place 3–6 h after the last insect capture on each day. For each insect at least five MS flowers were pollinated and pollination continued until all the pollen on the insect body visible under a magnifying lens had disappeared. Pollen beetles were an exception: due to their very small size only a single flower was pollinated with each individual. We hand pollinated a single flower per plant on each date to maximize seed set. Flowers were bagged individually after pollination and tagged with the insect code. Pods were collected at maturity and seeds were counted.

Each insect was identified to the species level (except for two sphecid wasps) and the distance between its tegulae (points of wing insertion) was measured with a micrometer. This distance referred to as the inter-tegular span (IT span) was used as a measure of insect size because it is well correlated with the flight range for bees (Cane 1987).

STATISTICAL ANALYSIS

Seed-set success is a binary variable stating whether an insect produced any seed over all the manual pollinations conducted using pollen from its body. Seed-set success could be limited by (i) the quantity of pollen transported by the insect, (ii) the number of OSR flowers visited by the insect (possibly none), (iii) the availability of pollen in each field, (iv) the number of flowers of other species visited by the insect since its last OSR floral visit, and (v) the viability of pollen on the body of the insect. The variables tested in the models were therefore both qualitative variables (insect order, genus and species, sam-
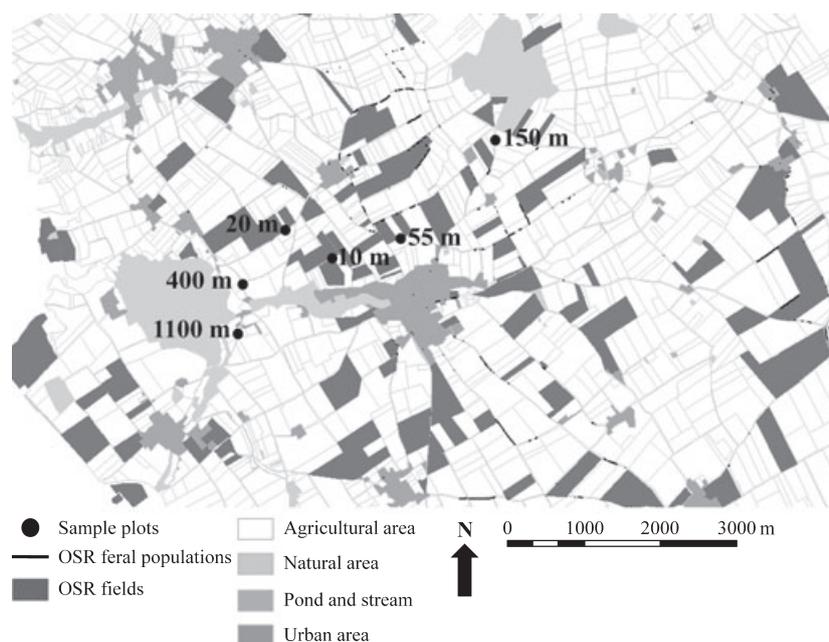
Fig. 1. Distribution of winter oilseed rape (OSR) fields within the 10 × 10 km study area and OSR feral populations along the paved and dirt road network in 2008. The six baits points were chosen following a roughly exponential scale of increasing distance to the OSR field. The map was drawn using ARCGIS ver. 9.1 from GPS data.

pling date) and quantitative variables (distance to the nearest OSR field, IT span, daily temperature, flowering level, hours of sunshine)

Seed-set success was analysed through a logistic regression using a binomial distribution and a logit link function (proc GENMOD, SAS 9·1; SAS Institute, Cary, NC, USA). The effects of the variables were computed with a type III approach using likelihood ratio tests. We report here the final logistic model where we kept the three significant variables (distance to the nearest OSR field, IT span and temperature).

We analysed the total number of seeds produced (Log-transformed) by insects that produced at least one seed, using a linear model with distance to the nearest OSR field, IT span, temperature and insect order as explanatory variables.

Results

ENVIRONMENT DURING THE STUDY

The mean daily temperature over the five sampling dates ranged from 7·5 to 15 °C and the lowest value was on the third date (15 April). Sampling over the first three dates took place at the onset of flowering (ca. 2 flowers/plant), while the last two sampling dates were during full bloom (114 and 230 flowers/plant; see Fig. S1 in Supporting Information).

The April survey of OSR feral populations found only two feral plants at the rosette stage closer to a bait point than its nearest field (1100-m point), but these plants were no longer present in June. One feral plant not recorded in April was observed bearing seeds in June at 140 m from the 150-m point.

INSECTS VISITING OSR FLOWERS

We collected 71 insects belonging to three orders (Coleoptera, Diptera and Hymenoptera), 9 families and 26 species. Hymenopterans consisted of a majority of bees (Apiformes; 50 specimens), but also four sawflies (Symphyta) and two sphecid wasps. Dipterans were represented by syrphid flies. Pollen beetles *Meligethes aeneus* were collected at each point (13 specimens). We collected only a single honeybee *Apis mellifera* and no beehives were located in the study area (Table 1).

The average size of the insects sampled at the different points was similar, except for the 150-m point where larger insects were caught ($F_{5,65}$ = 3·13, P = 0·014).

SEED SET FOLLOWING MANUAL INSECT POLLINATION

We conducted 451 manual pollinations by rubbing the 71 insect specimens onto the stigmas of MS flowers. We harvested 4047 seeds from 227 pods resulting from pollination with 28 insects among the 71 specimens collected.

Pollination using the 13 pollen beetles *M. aeneus* did not produce seeds. Pollination with one Diptera (*Episyrphus balteatus*), three sawflies (two *Dolerus haematodes* Schrank and one *Mesoneura opaca* Fabricius) and one sphecid wasp produced seeds (Table 1). For bees, pollination with 23 out of 50 specimens produced seeds, two of which were collected at the 1100 m point (*Xylocopa violacea* L. and *Andrena strohmella* Stoeckhert).

The proportion of insects for which pollination produced seeds was roughly similar at the first four distances (58·8% for

all insects sampled at ≤150 m; G = 2·145; df = 3; P = 0·542). However, at distances further from the fields the proportion was significantly smaller (7·7% for all insects at ≥400 m and the values at 400 and 1100 m were similar with P = 0·206 based on Fisher's exact test; Fig. 2).

The lowest seed-set success was recorded on the third date (15 April; 18% of insects produced seeds) and highest on the last date (6 May; 50% of the insects produced seeds, see Fig. S2 in Supporting Information).

Bees of the family Andrenidae and *B. terrestris* produced the largest numbers of seeds (> 100 seeds) (Table 1). The honeybee produced 62 seeds from five manual insect pollinations. None of the tested variables had a significant effect on seed production, when considering only insects that produced seeds.

MODELLING POLLEN DISPERSAL BY INSECTS

The seed-set success (S) of all 71 specimens was best explained by a logistic model that took into account the distance to the nearest OSR field (D), the IT span and the mean daily temperature (θ) (Table 2). The probability that an insect produce any seed was estimated as:

$$S = \frac{\exp^{-7\cdot32-0\cdot004D+1\cdot02IT+0\cdot41\theta}}{1 + \exp^{-7\cdot32-0\cdot004D+1\cdot02IT+0\cdot41\theta}} \qquad \text{eqn 1}$$

Thus, seed-set success was affected negatively by distance to the nearest OSR fields (P = 0·0005; Table 2, Fig. 3) and positively by insect size (IT span) (P = 0·0103; Table 2). This relationship remained true when considering only the bees (P = 0·042). The Generalized Rsquared (Nagelkerke 1991) was equal to 0·60. For the largest IT span (7·5 mm) at mean temperature (11·2 °C), eqn 1 predicted seed-set success <1% at distances ≥2230 m from the nearest OSR field, and for the smallest IT span (0·8 mm) a seed-set success < 1% at ≥670 m (Fig. 3). The interaction between distance to the nearest field and insect size was not significant, nor was any other interaction.

Differences between sampling dates were explained by the positive effect of the mean daily temperature (P < 0·0001). Seed-set success was lower on the coldest day (7·5 °C) and higher on the warmer day (15 °C; Fig. 3).

In addition to these three significant effects, none of the other investigated variables appeared as significant (flowering level, insect order, genus and species, sampling date and hours of sunshine).

Discussion

We observed a diversity of insects on MS OSR flowers that carried viable pollen at distances up to 1100 m, including bees, syrphid flies, sawflies, and sphecid wasps. Our method enabled us to focus on insect-borne pollen at the landscape scale contrary to previous studies using open pollinated plants for which air-borne and insect-borne pollen could not be separated (e.g. apetalous plants in Timmons *et al.* 1995; MS plants in Ramsay *et al.* 1999 and Devaux *et al.* 2005; other cultivars in Cai *et al.* 2008). Because we collected insects during their floral

Table 1. Seed-set success and number of seeds produced by the 71 insects caught on flowers of MS OSR

Order	Family	Species	IT span (mm)	Distance to the nearest field					
				10 m	20 m	55 m	150 m	400 m	1100 m
Hymenoptera	Andrenidae	*Andrena cineraria*	3·6	1/1 3(14)		1/1 4(7)		0/1	
		Andrena distinguenda	1·7			0/2			0/1
		Andrena flavipes	2·2	1/1 5(26)				0/3	0/1
		Andrena gravida	3·1				1/2 31(759)	0/1	
		Andrena haemorrhoa	2·9	1/1 20(504)		1/1 2(2)		0/1	0/1
		Andrena nigroaenea	3·2	1/1 1(8)		1/1 5(16)	0/1		0/1
		Andrena nitida	3·5		1/1 1(1)	1/1 80(1898)	0/1		
		Andrena pusilla	1·7						0/1
		Andrena strohmella	1·6					0/3	1/1 3(3)
		Andrena subopaca	1·6						0/1
	Apidae	*Anthophora plumipes*	4·7	1/1 2(11)					
		Apis mellifera	3·7			1/1 5(62)			
		Bombus hypnorum	4				0/1		
		Bombus lapidarius	6·6			2/2 5·5(35·5)	3/3 3(47·7)		
		Bombus terrestris	7·1				2/2 5·5(108·5)		
		Nomada flavoguttata	1·6						0/1
		Xylocopa violacea	7·1						1/1 1(1)
	Halictidae	*Lasioglossum calceatum*	2·4					0/1	0/1
		Lasioglossum malachurum	2				1/1 5(25)		0/1
		Lasioglossum xanthopus	2·5		1/1 2(2)				
		Sphecodes ruficrus	2·5					0/1	
	Megachilidae	*Osmia cornuta*	4·7						0/1
	Sphecidae		1			1/2 5(19)			
	Tenthredinidae	*Dolerus haematodes*	2·5	1/1 5(64)	0/1		1/1 5(54)		
		Mesoneura opaca	1·7		1/1 6(138)				
Diptera	Syrphidae	*Episyrphus balteatus*	2·5			1/1 5(8)			
		Syrphus vitripennis	3·6	0/1					
Coleoptera	Nitidulidae	*Meligethes aeneus*	0·5	0/2	0/4	0/2	0/1	0/3	0/1

For each species and bait point distance we provide: (top line) the number of individuals that gave seeds/the total number of individuals captured and (bottom line) the mean number of fruits per individual (the mean number of seeds per individual). The inter-tegular span (IT span) provides a references measure for insect size.

visits, we were able to link foraging behaviour and pollen dispersal for a single plant species. This new approach complements existing approaches that focus on flight behaviour (harmonic radar, Osborne *et al.* 1999; radio tracking, Pasquet *et al.* 2008; or with a feeder, Kuhn-Neto *et al.* 2009) by providing information on seed-set success, and thus gene flow, as a function of insect and flight characteristics.

Studies on pollination distances usually focus on bumble-bees (*Bombus* spp.: Osborne *et al.* 1999; Cresswell *et al.* 1995; Cresswell, Osborne & Bell 2002) or honeybees (*Apis mellifera*: Ramsay *et al.* 1999; Pierre *et al.* 2010). Our approach allowed us to consider the diversity of naturally occurring pollinators, including small insects, and directly assess their potential contribution to effective pollen dispersal. A relatively high
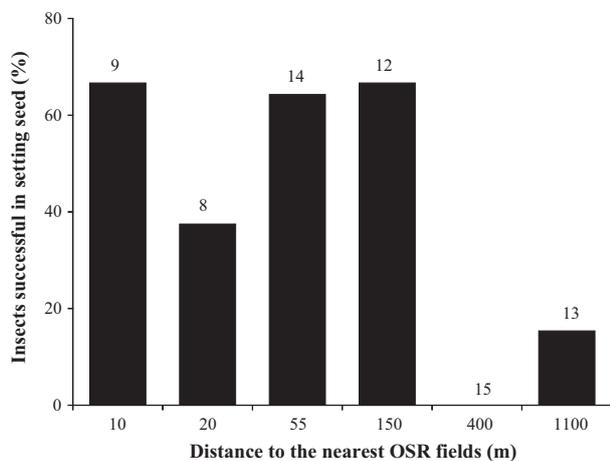
Fig. 2. Effect of distance to the nearest fields of oilseed rape (OSR) on pollen dispersal by insects, measured as the percentage of captured insects successfully setting seed when used to manually pollinate male-sterile plants.

Table 2. Effect of distance to the nearest oilseed rape (OSR) field, inter-tegular span and temperature on the probability of seed-set success

Effects	d.f.	χ^2	P
Distance to the nearest OSR field	1	12·02	0·0005
Main daily temperature	1	23·04	< 0·0001
IT span	1	6·58	0·0103

We provide the results of the logistic model including these three quantitative covariates analysed using type III likelihood ratio tests.
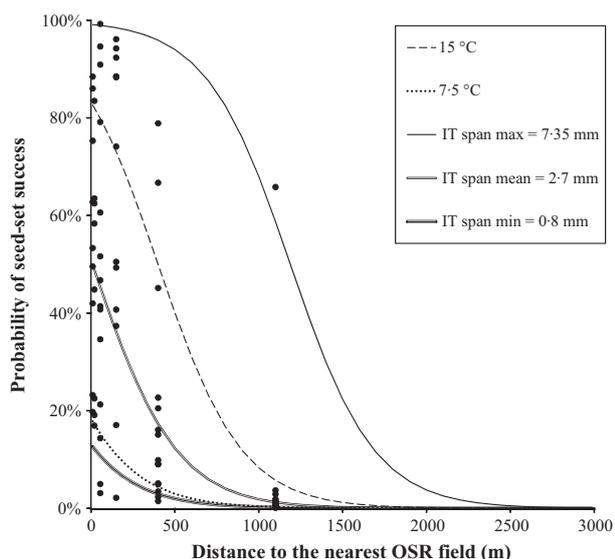
Fig. 3. Probability of seed-set success (*S*) in relation to the distance to the nearest oilseed rape (OSR) field, the insect size measured by the inter-tegular span (IT span, mean temperature = 11·2 °C) and the five mean daily temperatures (mean IT span = 2·7 mm) during which the foraging insects were caught. Each dot represents an insect caught foraging on male-sterile flowers.

proportion (39·4%) and diversity of insects visiting isolated OSR plants carried some viable OSR pollen in their body hairs. A majority of bees carried viable pollen (14 species, 82·1%), as expected because they are the main pollinators of OSR (Klein *et al.* 2007). *Episyrphus balteatus* (Syrphidae) also carried viable OSR pollen, consistent with the work of Jauker & Wolters (2008). More surprisingly, sawflies (Tenthrenidae) also visited flowers and carried viable OSR pollen, which, to our knowledge, has not been reported previously. None of the pollinations using the 13 *M. aeneus* produced a seed, contradicting their hypothesized role in long-distance pollen transfer (Ramsay, Thompson & Squire 2003). In fact, insect size was the only significant factor pertaining to the insects that explained the seed-set success. This size effect could be explained by the general ability of larger insects to carry more pollen grains for longer periods of time or to have larger foraging ranges. However, some particular species have specific adaptations to enable them to carry large amounts of pollen despite their small size (e.g. *Andrena nitida* with an IT span of 3·5 mm carried enough pollen to produce 1898 seeds) (Thorp 1979). Overall, the diversity of species we found carrying OSR pollen is consistent with the fact that its flowers are relatively unspecialized. Such diversity should not be neglected in assessments of pollen flow in agricultural landscapes. Furthermore, our finding that, after accounting for body size, taxa did not differ significantly in seed-set success should not be interpreted as a functional equivalence of all pollinator species. Indeed, the small number of specimens per species and even genus yielded a low statistical power for comparisons between them.

Our results provide evidence that insects play a role in long-distance OSR pollination and may induce gene escape from GM fields, even when the insect population density in a field is relatively low (Cresswell 2008). Some seeds were obtained from insects caught at 1·1 km from the closest pollen source. It is unlikely that this distance is overestimated as our intensive survey during the experiment provided no evidence of any flowering feral plants between each bait point and the closest field. The decrease in seed-set success at large distances most probably results from the small proportion of OSR among flowering plants in the vicinity of bait plants (Goodell *et al.* 1997). A decrease in effective pollen dispersal with distance to the nearest pollen source has been reported in numerous studies (Cresswell *et al.* 1995; Timmons *et al.* 1995; Ramsay, Thompson & Squire 2003; Devaux *et al.* 2005; Cai *et al.* 2008; Hoyle & Cresswell 2009) but these studies were not based exclusively on insect-borne pollen. Furthermore, we observed a major drop in pollen dispersal only beyond 150 m from the pollen source. This distance is further than the distance of ca. 50 m reported by previous authors who considered both airborne and insect-borne pollen (Devaux *et al.* 2005; Klein *et al.* 2006; Cai *et al.* 2008). Considering the decrease in the probability of seed-set success with distance, we found an exponential decrease at a rate of 0·004 per metre for long distances (eqn 1), which is consistent with the rate of decrease between fields reported by Damgaard & Kjellsson (2005) ranging from 0·003 to 0·006.

Seed-set success also varied with mean daily temperature. This is consistent with studies that have shown the negative impact of lower temperature on the foraging activity of bees (Szabo & Smith 1972) and pollen dispersal (Hirao *et al.* 2006), and more generally the impact of weather conditions on insect foraging activity (Eastham & Sweet 2002).

Two possible caveats for our method are (i) we may have underestimated the potential pollen transfer because the seed-set observed here represents only a fraction of the effective pollen that was present on the insect body hair at the time of its capture and it is possible that some pollen on the insects was lost during transport and manual insect pollination. Alternatively, (ii) we may have overestimated the potential pollen transfer of the insects by rubbing their body onto virgin MS stigmas until all visible pollen from their body hair had disappeared. However Pierre *et al.* (1996) found that insects foraging on OSR flowers used a large array of intra-floral behaviour to access nectar and pollen, thus pollen from most body parts is likely to be deposited onto the stigma of OSR flowers (but see Cresswell 2000 for the sensitivity of pollen deposition to stigma length). Importantly, our method of killing the insects (CO_2) and transporting them at 6 °C maintained pollen viability at around 50% after 12 h, which is probably close to OSR pollen viability when insect visit OSR plants *in natura* (Mesquida & Renard 1989).

Finally, the cross-pollination rate, i.e. the ratio of seeds produced from incoming allo-pollen vs. self-pollination, is the variable of interest for managing the coexistence of GM and conventional crops as it determines the GM adventitious presence in conventional fields and feral populations. Assuming that (i) cross-pollination represents a small fraction of the total seeds, and (ii) self-pollination is similar at every distance (subject only to density of pollinating insects and independent of distance to the closest field), then the decrease in seed-set success with distance would be similar to that of the cross-pollination rate if the number of self-fertilized seeds was independent of distance. However, although the trend was not significant, we observed that the number of seeds per pod was smaller at larger distances (18·13 seeds per pod up to 150 m and 1 seed per pod at greater distances) suggesting that the decrease in cross-pollination rate in male fertile plants would be faster than the decrease in seed-set success observed here.

The bait points of MS plants in our experiment can be compared to small OSR feral populations. Our data and model indicate that these plants could be pollinated by fields in the vicinity, but also from fields at distances of over 1·1 km. Resulting seeds could then remain in the seed bank for several years (D'Hertefeldt, Jørgensen & Pettersson 2008), and subsequently produce OSR feral populations contributing to gene flow at a landscape scale. Our results further indicate that in this open agricultural landscape planted abundantly with OSR, on average only about 39·4% of the insects actually carried viable OSR pollen. This value estimates the *E* parameter (fraction of pollinators delivering extrinsic pollen to a sink population) in the model for incoming gene flow in conventional OSR fields of Cresswell, Osborne & Bell (2002). *E* had been set at 1 until now because of a lack of empirical data. Our estimation is the first for this parameter, and we found that it varied between 0 and

66·7% depending on the distance to the closest OSR field. Even if fertilization success differs between natural pollination of plants *in natura* and manual insect pollination after CO_2 exposure and transport of insect to the greenhouse, we believe our estimates reflect reasonably the dynamics of pollination by insects in an open agricultural landscape.

Finally, manual insect pollination with captured insects produced an average of 7·26 fruits, which is more than the 1·22 fruits estimated by Cresswell, Osborne & Bell (2002). This difference is possibly explained by the absence of selfing and lower pollen competition. Our results thus provide quantitative data to improve the modelling of gene flow by pollen dispersal from entomophilous crops at the landscape scale, an essential step in managing the co-existence of GM and conventional crops.

To conclude, an isolation distance of 1·1 km between GM and conventional OSR fields is not sufficient to fully avoid transgene escape into conventional production, and OSR feral populations should be rigorously managed to eliminate relay points for pollen dispersal at the landscape scale.

Acknowledgements

References

Becker, H.C., Damgaard, C. & Karlsson, B. (1992) Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). *Theoretical and Applied Genetics*, **84**, 303–306.

Beil, M., Horn, H. & Schwabe, A. (2008) Analysis of pollen loads in a wild bee community (Hymenoptera: Apidae) – a method for elucidating habitat use and foraging distances. *Apidologie*, **39**, 456.

Cai, L., Zhou, B., Guo, X., Dong, C., Hu, X., Hou, M. & Liu, S. (2008) Pollen-mediated gene flow in Chinese commercial fields of glufosinate-resistant canola (*Brassica napus*). *Chinese Science Bulletin*, **53**, 2333–2341.

Cane, J.H. (1987) Estimation of bee size using intertegular span (Apoidea). *Journal of the Kansas Entomological Society*, **60**, 145–147.

Chèvre, A., Adamczyk, K., Eber, F., Huteau, V., Coriton, O., Letanneur, J., Laredo, C., Jenczewski, E. & Monod, H. (2007) Modelling gene flow between oilseed rape and wild radish. I. Evolution of chromosome structure. *Theoretical and Applied Genetics*, **114**, 209–221.

Cresswell, J.E. (2000) Manipulation of female architecture in flowers reveals a narrow optimum for pollen deposition. *Ecology*, **81**, 3244–3249.

Cresswell, J.E. (2008) Reply by Dr James Cresswell, School of Biosciences, University of Exeter. *Weather*, **63**, 369–370.

Cresswell, J.E., Osborne, J.L. & Bell, S.A. (2002) A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. *Oikos*, **98**, 375–384.

Cresswell, J.E., Bassom, A.P., Bell, S.A., Collins, S.J. & Kelly, T.B. (1995) Predicted pollen dispersal by honey-bees and three species of bumble-bees foraging on oil-seed rape: a comparison of three models. *Functional Ecology*, **9**, 829–841.

Dale, P.J., Clarke, B. & Fontes, E.M. (2002) Potential for the environmental impact of transgenic crops. *Nature Biotechonoly*, **20**, 567–574.

Damgaard, C. & Kjellsson, G. (2005) Gene flow of oilseed rape (*Brassica napus*) according to isolation distance and buffer zone. *Agriculture, Ecosystems & Environment*, **108**, 291–301.

Devaux, C., Lavigne, C., Falentin-Guyomarc'h, H., Vautrin, J., Lecomte, J. & Klein, E.K. (2005) High diversity of oilseed rape pollen clouds over an

agro-ecosystem indicates long-distance dispersal. *Molecular Ecology*, **14**, 2269–2280.

D'Hertefeldt, T., Jørgensen, R.B. & Pettersson, L.B. (2008) Long-term persistence of GM oilseed rape in the seedbank. *Biology Letters*, **4**, 314–317.

Eastham, K. & Sweet, J. (2002) *Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer*. Report No 28, Expert's Corner Series. European Environment Agency, Copenhagen, Denmark.

Goodell, K., Elam, D.R., Nason, J.D. & Ellstrand, N.C. (1997) Gene flow among small populations of self-incompatible plant: an interaction between demography and genetics. *American Journal of Botany*, **84**, 1362–1371.

Hirao, A.S., Kameyama, Y., Ohara, M., Isagi, Y. & Kudo, G. (2006) Seasonal changes in pollinator activity influence pollen dispersal and seed production of the alpine shrub *Rhododendron aureum* (Ericaceae). *Molecular Ecology*, **15**, 1165–1173.

Hoyle, M. & Cresswell, J.E. (2009) Maximum feasible distance of windborne cross-pollination in *Brassica napus*: a 'mass budget' model. *Ecological Modelling*, **220**, 1090–1097.

Hoyle, M., Hayter, K. & Cresswell, J.E. (2007) Effect of pollinator abundance on self-fertilization and gene flow: application to GM canola. *Ecological Applications*, **17**, 2123–2135.

James, C. (2009) *Global status of commercialized biotech/GM crops: 2009*. ISAAA Brief No. 41, ISAAA, Ithaca, NY.

Jank, B., Rath, J. & Gaugitsch, H. (2006) Co-existence of agricultural production systems. *Trends in Biotechnology*, **24**, 198–200.

Jauker, F. & Wolters, V. (2008) Hover flies are efficient pollinators of oilseed rape. *Oecologia*, **156**, 819–823.

Klein, E.K., Lavigne, C., Picault, H., Renard, M. & Gouyon, P. (2006) Pollen dispersal of oilseed rape: estimation of the dispersal function and effects of field dimension. *Journal of Applied Ecology*, **43**, 141–151.

Klein, A., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 303–313.

Kuhn-Neto, B., Contrera, F.A., Castro, M.S. & Nieh, J.C. (2009) Long distance foraging and recruitment by a stingless bee, *Melipona mandacaia*. *Apidologie*, **40**, 472–480.

Lavigne, C., Klein, E.K., Vallée, P., Pierre, J., Godelle, B. & Renard, M. (1998) A pollen-dispersal experiment with transgenic oilseed rape. Estimation of the average pollen dispersal of an individual plant within a field. *Theoretical and Applied Genetics*, **96**, 886–896.

Manasse, R.S. (1992) Ecological risks of transgenic plants: effect of spatial dispersion on gene flow. *Ecological Applications*, **2**, 431–438.

Meagher, T.R., Belanger, F.C. & Day, P.R. (2003) Using empirical data to model transgene dispersal. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **358**, 1157–1162.

Mesquida, J. & Renard, M. (1982) Etude de la dispersion du pollen par le vent et de l'importance de la pollinisation anémophile chez le colza (*Brassica napus* L. var. *oleifera* Metzger). *Apidologie*, **13**, 353.

Mesquida, J. & Renard, M. (1989) Etude de l'aptitude à germer *in vitro* du pollen de colza (*Brassica napus* L.) récolté par l'abeille domestique (*Apis mellifica* L.). *Apidologie*, **20**, 197.

Nagelkerke, N. (1991) A note on a general definition of the coefficient of determination. *Biometrika*, **78**, 691–692.

Osborne, J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, A.D., Reynolds, D.R. & Edwards, A.S. (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. *Journal of Applied Ecology*, **36**, 519–533.

Pasquet, R.S., Peltier, A., Hufford, M.B., Oudin, E., Saulnier, J., Paul, L., Knudsen, J.T., Herren, H.R. & Gepts, P. (2008) Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 13456–13461.

Pessel, F.D., Lecomte, J., Emeriau, V., Krouti, M., Messéan, A. & Gouyon, P.-H. (2001) Persistence of oilseed rape in natural habitats : consequence for release of transgenic crops. *Theoretical and Applied Genetics*, **102**, 841–846.

Pierre, J., Pierre, J.S., Marilleau, R., Pham-Delègue, M.H., Tanguy, X. & Renard, M. (1996) Influence of the apetalous character in rape (*Brassica napus*) on the foraging behaviour of the honey bees (*Apis mellifera*). *Plant Breeding*, **115**, 484–487.

Pierre, J., Vaissière, B., Vallée, P. & Renard, M. (2010) Efficiency of airborne pollen released by honeybee foraging on pollination in oilseed rape: a wind insect-assisted pollination. *Apidologie*, **41**, 109–115.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.-H. & Huet, S. (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. *Journal of Applied Ecology*, **45**, 476–485.

Ramsay, G., Thompson, C. & Squire, G. (2003) *Quantifying landscape-scale gene flow in oilseed rape (Defra project RG0216 final report)*. Department for Environment, Food, and Rural Affairs, London, UK.

Ramsay, G., Thompson, C.E., Neilson, S. & Mackay, G.R. (1999) Honeybees as vectors of GM oilseed rape pollen. *in Gene Flow and Agriculture: Relevance for Transgenic. CropsBCPC Symposium Proceedings*, **72**, 209–214.

Raybould, A.F., Clarke, R.T., Bond, J.M., Welters, R.E. & Gliddon, C. (2002) Inferring patterns of dispersal from allele frequency data. *Dispersal Ecology* (eds J.M. Bullock, R.E. Kenward & R. Hails), pp. 89–111. Blackwell Science, Oxford.

Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. & Roush, R.T. (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*, **296**, 2386–2388.

Sanvido, O., Romeis, J. & Bigler, F. (2007) Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. *Advances in Biochemical Engineering/Biotechnology*, **107**, 235–278.

Sork, V.L., Nason, J., Campbell, D.R. & Fernandez, J.F. (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology & Evolution*, **14**, 219–224.

Szabo, T.I. & Smith, M.V. (1972) The influence of light intensity and temperature on the activity of the alfalfa leaf-cutter bee *Megachile rotundata* under field conditions. *Journal of Apicultural Research*, **11**, 157–165.

Thorp, R.W. (1979) Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. *Annals of the Missouri Botanical Garden*, **66**, 788–812.

Timmons, A., O'Brien, E., Charters, Y., Dubbels, S. & Wilkinson, M. (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera*. *Euphytica*, **85**, 417–423.

Walklate, P.J., Hunt, J.C.R., Higson, H.L. & Sweet, J.B. (2004) A model of pollen-mediated gene flow for oilseed rape. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 441–449.

Walther-Hellwig, K. & Frankl, R. (2000) Foraging distances of *Bombus muscorum*, *Bombus lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). *Journal of Insect Behavior*, **13**, 239–246.

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G., Roberts, S.P.M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B.E., Woyciechowski, M., Biesmeijer, J.C., Kunin, W.E., Settele, J. & Steffan-Dewenter, I. (2008) Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs*, **78**, 653–671.

Wolf, S. & Moritz, R.F. (2008) Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie*, **39**, 419.

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S. & Dorn, S. (2010) Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biological Conservation*, **143**, 669–676.

Supporting Information

Additional Supporting Information may be found in the online version of this article

Fig. S1. Evolution of flowering of oilseed rape fields and of mean daily temperature.

Fig. S2. Evolution of seed-set from manual pollination over the course of our study.

Appendix S1. Detailed description of preliminary test for manual insect pollination.

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