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# Honey bee dance decoding and pollen-load analysis show limited foraging on spring-flowering oilseed rape, a potential source of neonicotinoid contamination



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## ABSTRACT

Neonicotinoid insecticides used to treat the seeds of bee-attractive crops occur in trace amounts in nectar and pollen. Possible harm to bees has resulted in the European Commission imposing a precautionary two-year moratorium on the use of neonicotinoids on bee-attractive crops from 2013. Recent laboratory and semi-field studies on colony-level effects of neonicotinoids assumed exclusive or near-exclusive levels of colony foraging on a treated crop. But is this a realistic assumption? Six honey bee (*Apis mellifera*) colonies were monitored over two springs (April–May 2011/12) in two neighbouring locations (urban and rural) in and near Brighton, UK, to quantify foraging on oilseed rape, the most widespread bee-attractive crop in the UK, by decoding waggle dances and trapping pollen. The study area was representative of the UK agricultural landscape in that the percentage area cover of the blooming oilseed rape fields around the rural location was similar to the national average (3.3–3.9% vs 3.1%). The amount of foraging on oilseed rape fields, as indicated by dance decoding, was variable, but low, 0–0.02% for the urban and 2–26% for the rural location. Almost all foraging, 91–99%, was within 2 km, even though honey bees can forage at distances of over 10 km. Pollen trapping in 2012 supported the dance decoding results, with oilseed rape pollen comprising 14% of pollen pellets collected by foragers from rural and 4% from urban hives. The results of this study have implications for policy as they cast doubt on the generality of some previous studies on colony-level effects on social bees conducted in laboratory and semi-field settings.

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## 1. Introduction

Oilseed rape (*Brassica napus*) is a major crop, with 35 million ha grown worldwide in 2012/13, yielding 61 million tons of oil (USDA, 2013). In the UK, oilseed rape acreage has trebled in the past 30 years to 756,000 ha in 2012 (3.1% of land area) and is now the third most important arable crop after wheat and barley (DEFRA, 2012). Oilseed rape is cultivated in dense monocultures, which at flowering become an important potential resource to honey bees, bumble bees and other flower-visiting insects that feed on nectar and pollen (Fussell and Corbet, 1991; Howlett et al., 2009; Rollin et al., 2013). For example, flowering oilseed rape improves bumble bee early colony growth (Westphal et al., 2009) and increases worker density at a landscape scale (Westphal et al., 2003). Oilseed rape is associated with greater species richness of solitary cavity-nesting bees and wasps (Diekötter et al., 2014) and generally

enhances wild bee density in the long term (over 1 year, Riedinger et al., 2015). In this respect, it appears advantageous as an additional source of forage. However, to combat herbivorous insects, almost all oilseed rape (e.g. 98% in the UK, Garthwaite et al., 2012) is treated with systemic neonicotinoid insecticides applied as a seed dressing, with residues at potentially harmful concentrations present in nectar and pollen (Table 6 in EFSA, 2012). Oilseed rape is a prime route by which bees may ingest these chemicals in the UK, where it is the most widespread bee-attractive crop, and is also important in other countries, given that the UK acreage is only 2% of the world total.

There is currently much debate over the effects that neonicotinoid residues in nectar and pollen have on bees (Blacquière et al., 2012; Goulson, 2013; van der Sluijs et al., 2013). Although the effects on individual bees resulting from a single feeding event at an environmentally-realistic dose are not lethal (Suchail et al., 2000), there is a concern that chemical concentrations may bioaccumulate (Byrne et al., 2014; Rortais et al., 2005) and/or cause sub-lethal effects, such as impaired learning or foraging ability (Cresswell, 2011; Mommaerts et al., 2010; Schneider et al., 2012),

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which may in principle translate into adverse colony-level and, ultimately, population-level effects.

In May 2013 the European Commission imposed a precautionary 2 year moratorium on the use of three neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) on bee-attractive crops in the European Union (European Commission, 2013). This is intended to allow time for further research developments before the next review of scientific evidence in 2015. However, there remain important gaps in our knowledge (Cresswell et al., 2012). Most recent key studies investigating colony-level effects in social bees were done under laboratory or semi-field conditions. One challenge in these studies is to choose doses that are relevant to field exposure, including the concentration, duration of exposure, and choice (reviewed in Carreck and Ratnieks, 2014). For example, Henry et al. (2012) administered 1.34 ng of thiamethoxam in a single feeding to individual honey bees – an amount that a bee would consume during a whole week of foraging (Carreck and Ratnieks, 2014). Other studies, this time on bumble bees, assumed great or even exclusive foraging on a treated crop either by providing no alternative choice during the treatment phase (Whitehorn et al., 2012), or by using amounts of treated sucrose solution equivalent to about half of the daily colony intake (Gill et al., 2012). However, under field conditions, the bees often have a large choice of nectar and pollen sources. Treated crops may also often be out of foraging range of bees, especially in solitary bees with short maximum foraging ranges (generally up to c. 1.0–1.5 km, Zurbuchen et al., 2010). This is less likely to be a factor in bumble bees (up to c. 1.5–3.0 km, Goulson and Osborne, 2010; Zurbuchen et al., 2010) and honey bees (up to c. 10–12 km, Beekman and Ratnieks, 2000) as these bees have greater foraging ranges. However, bees rarely forage at or near their maximum range and typically forage at much shorter distances (Couvillon et al., 2014; Zurbuchen et al., 2010). Furthermore, there is the possibility that insecticides may repel bees from treated crops (Easton and Goulson, 2013; Eiri and Nieh, 2012). In a statement evaluating the recent evidence for the impact of neonicotinoids on bees, the European Food Safety Authority concluded that it remains uncertain to what extent such exposure regimes are representative of field conditions (EFSA, 2012).

This study quantifies honey bee foraging on spring-blooming oilseed rape by decoding waggle dances and pollen trapping from hives in neighbouring urban and rural locations in a landscape with many oilseed rape fields within foraging range. We also hypothesized that variation in the intensity of foraging among oilseed rape fields depends on their distances to the hives.

## 2. Materials and methods

Three honey bee colonies were kept in a rural location, the University of Sussex campus on the outskirts of Brighton (lat.: 50.863889, long.: –0.083830386), which was surrounded mainly by farmland, and three in a nearby urban location, Dorothy Stringer School near the centre of Brighton (lat.: 50.849370, long.: –0.14167996), 2.2 km from the countryside at its closest point and 4.5 km to the south-west of the rural location. The urban location was chosen as a contrast to the rural location. In part this was due to the increasing popularity of urban beekeeping in the UK and to provide information relevant to urban beekeepers (Alton and Ratnieks, 2013). More importantly, the urban location was further from farmland. This allowed us to explore a wider range of hive distances from study hives to oilseed rape fields. The colonies were housed indoors in glass-walled observation hives containing three medium and one deep Langstroth frames, an egg-laying queen, brood of all ages, and c. 2000–5000 workers.

Most oilseed rape (98% in 2012) in the UK is ‘winter rape’ being sown in late summer or early autumn and flowering the following spring (DEFRA, 2012). The rest, ‘spring rape’ (2% in 2012), is sown in spring to flower in summer. In 2011 and 2012, we rented a light

aircraft to conduct aerial surveys of the study area twice each spring to locate and photograph all oilseed rape fields within 6 km of the two study apiaries. These fields stand out due to the bright yellow flowers and are visible at long distances, several kilometres, when flying at c. 600–750 m altitude. The fields were then located on the Sussex Habitat Framework map, a georeferenced land type database provided by the Sussex Biodiversity Records Centre, to determine their exact positions, areas, and distances to the apiaries using Geographic Information System software (ArcGIS Desktop 10, Esri, USA). Oilseed rape was 3.33% and 3.89% of the area within 6 km of the rural apiary in 2011 and 2012, respectively, and 1.19% and 2.34% for the urban apiary, making the amount of oilseed rape in the rural area close to the national average (3.1%) (DEFRA, 2012). In addition, a cluster of adjoining fields of flowering linseed (*Linum usitatissimum*) fields (47 ha) was spotted in 2011 and mapped, as it is also a bee-attractive crop (Abrol and Kotwal, 1996). No other bee-attractive crops were seen during the study period in the study farmland area, which was otherwise dominated by wheat, barley and grazing land.

### 2.1. Waggle dance analysis

Concurrent to mapping the oilseed rape fields, honey bee dancing was monitored in each hive using video cameras. The colonies used were from a typical British stock consisting of mainly the Northern European dark bee, *A. mellifera mellifera*, with some Italian *A. mellifera ligustica*. Videos were recorded on days with good weather that resulted in high foraging and dancing activity. Using the videos in total 1646 waggle dances were decoded from the six observation hives during the main period of oilseed rape flowering (April and May) in both 2011 and 2012. Methods followed Couvillon et al. (2012), where four waggle runs per dance were decoded to obtain each dance’s mean duration, which encodes distance, and angle, which encodes direction. Distance was estimated using a Bayesian linear calibration model built for our honey bee population and landscape (Schürch et al., 2013), which takes into account the imprecision inherent in the honey bee dance. Probability distributions for distance estimates can then be combined with probability distributions representing the variability in the second vector component, the direction (Schürch et al., 2013). In this way we were able to map the foraging locations shown by the dances in a manner that includes the uncertainty in the dance vector (Schürch et al., 2013). This methodology also allowed us to determine confidence intervals for our estimates of the proportion of dancing for oilseed rape fields.

### 2.2. Pollen collection and identification

Pollen samples were collected from returning foragers during April and May 2012 by placing pollen traps (5.0 mm plastic mesh; E.H. Thorne, UK) at the entrance of each hive for 1 h at approximately weekly intervals. The mesh knocks pollen pellets from the corbiculae of returning foragers as they pass through. Pollen collection took place during high foraging activity between 10:00 and 16:00 BST and was done at the same time for hives in the same apiary, but at slightly different times between the two apiaries.

Pollen pellets were first categorized by colour. Up to five pollen pellets per colour per sample (from each hive on each data collection day) were mounted on slides and examined using a light microscope at 400× magnification (Zeiss Axiophot, Oberkochen, Germany). In a few cases when pellets of one colour in a sample were not monofloral, the number of pellets of each morphotype was estimated from its proportion among the five pellets examined. Oilseed rape pollen was identified by reference to samples collected locally from oilseed rape flowers. The remaining

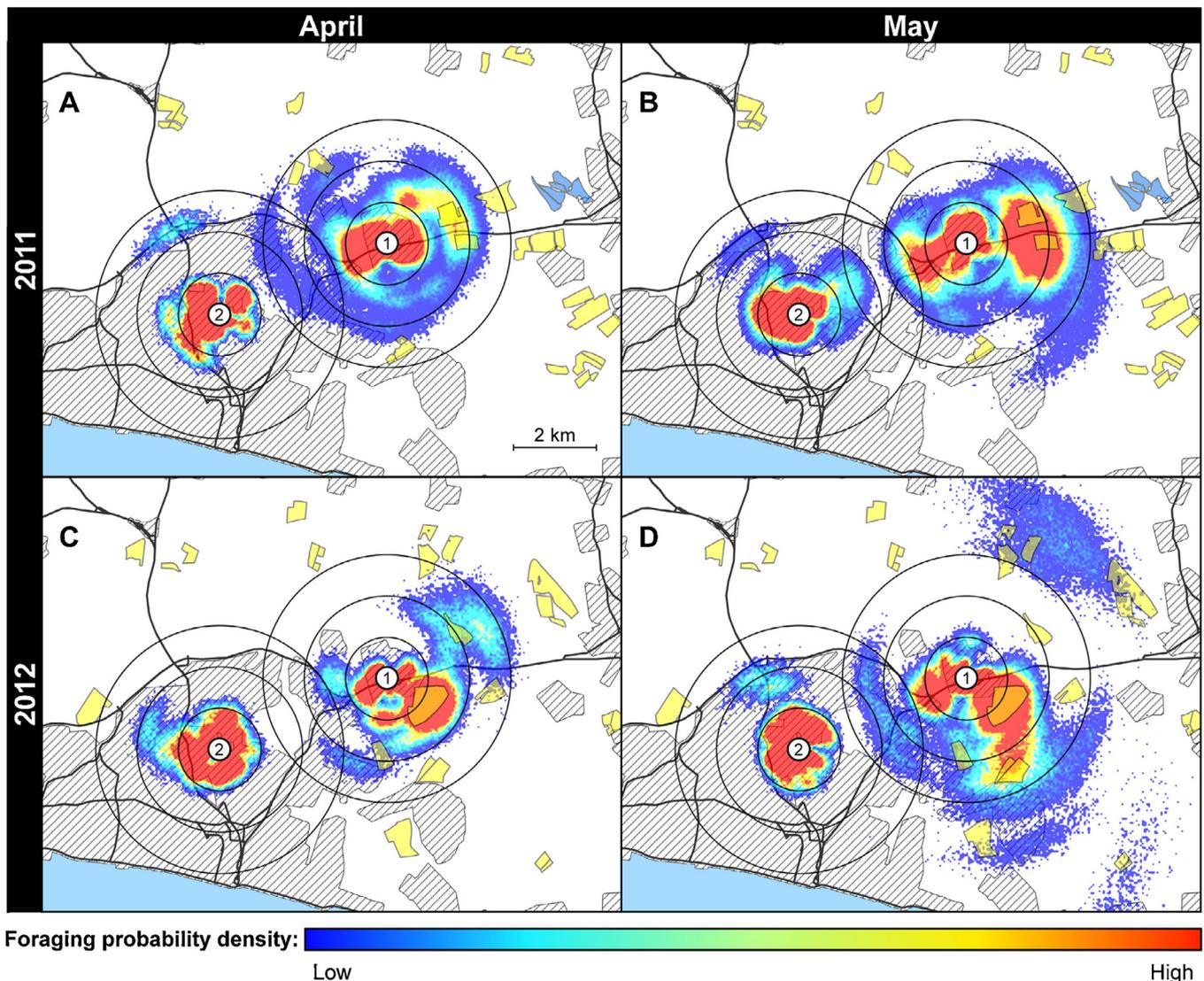
morphotypes were not identified, with the exception of dandelion (*Taraxacum officinale*), which was identified from a combination of morphology (Marciniuk and Rudzińska-Langwald, 2008) and pellet colour (Kirk, 2006).

### 3. Results and discussion

Fig. 1 maps the foraging locations as probability distributions based on dance decoding. There was effectively no overlap in the foraging between the two apiaries. In the urban apiary, most foraging (93%) was within 1 km, and almost all (99%) within 2 km. In the rural apiary, on average 60% of foraging was within 1 km, with more foraging occurring at 1–2 km and concentrated around oilseed rape fields. The distortions of the dance density distribution by the oilseed rape fields (Fig. 1) show that this crop is more attractive, on average, than the surrounding landscape, which is concurrent with the results of Riedinger et al. (2015) on wild bees. However, the total proportion of foraging on oilseed rape fields was limited. In the urban location, the mean proportions were 0% for April in both years, and 0% and 0.02% for May in 2011 and 2012,

respectively. In the rural location, the mean proportions during the two years were 2.2% (2011) and 21.8% (2012) for April and 8.0% (2011) and 26.1% (2012) for May (full data including confidence intervals are given in Table 1). But, the noise associated with the spatial information encoded in waggle dances was, in many cases, considerably greater than the size of the oilseed rape fields. This could lead to underestimation of foraging in the oilseed rape fields, if most foraging is inside the fields, or to overestimation, if most foraging is outside but close to the fields. However, this should not change the general results that oilseed rape was not the dominant source of forage for the rural hives and a negligible source for the urban hives.

As part of crop rotation and pest management, farmers generally do not plant oilseed rape in the same field in consecutive years. This can be seen in the field locations in Figs. 1 and 2, which all changed from 2011 to 2012. Honey bees are known to make economic foraging decisions, with greater foraging distance increasing costs and having an effect on decisions to forage and recruit nestmates to foraging locations via the waggle dance (Cresswell et al., 2000; Seeley, 1994; Seeley et al., 2000). Thus, the locations advertised by waggle dances



**Fig. 1.** Probability density distributions of honey bee foraging from (1) the rural apiary at Laboratory of Apiculture & Social Insects and (2) the urban apiary at Dorothy Stringer School in relation to oilseed rape (yellow) and linseed (darker blue, 2011 only) fields in April and May 2011 (A and B) and 2012 (C and D). Landscape comprised a mixture of rural (white) and urban (hatched) areas bordered by the English Channel to the south (lighter blue). Circles around apiary locations are 1, 2 and 3 km buffers. Colour spectra show the range of foraging probabilities, as determined by simulated waggle dance locations, binned into 25 m × 25 m quadrats, from blue (1) to red (620–6985, depending on dataset). The definition of urban areas follows the Ordnance Survey maps for GIS (vector 'Meridian 2') provided by Digimap service (EDINA, <http://digimap.edina.ac.uk>).

**Table 1**  
Estimated % of foraging on oilseed rape by rural (LASI) and urban (DS) hives.

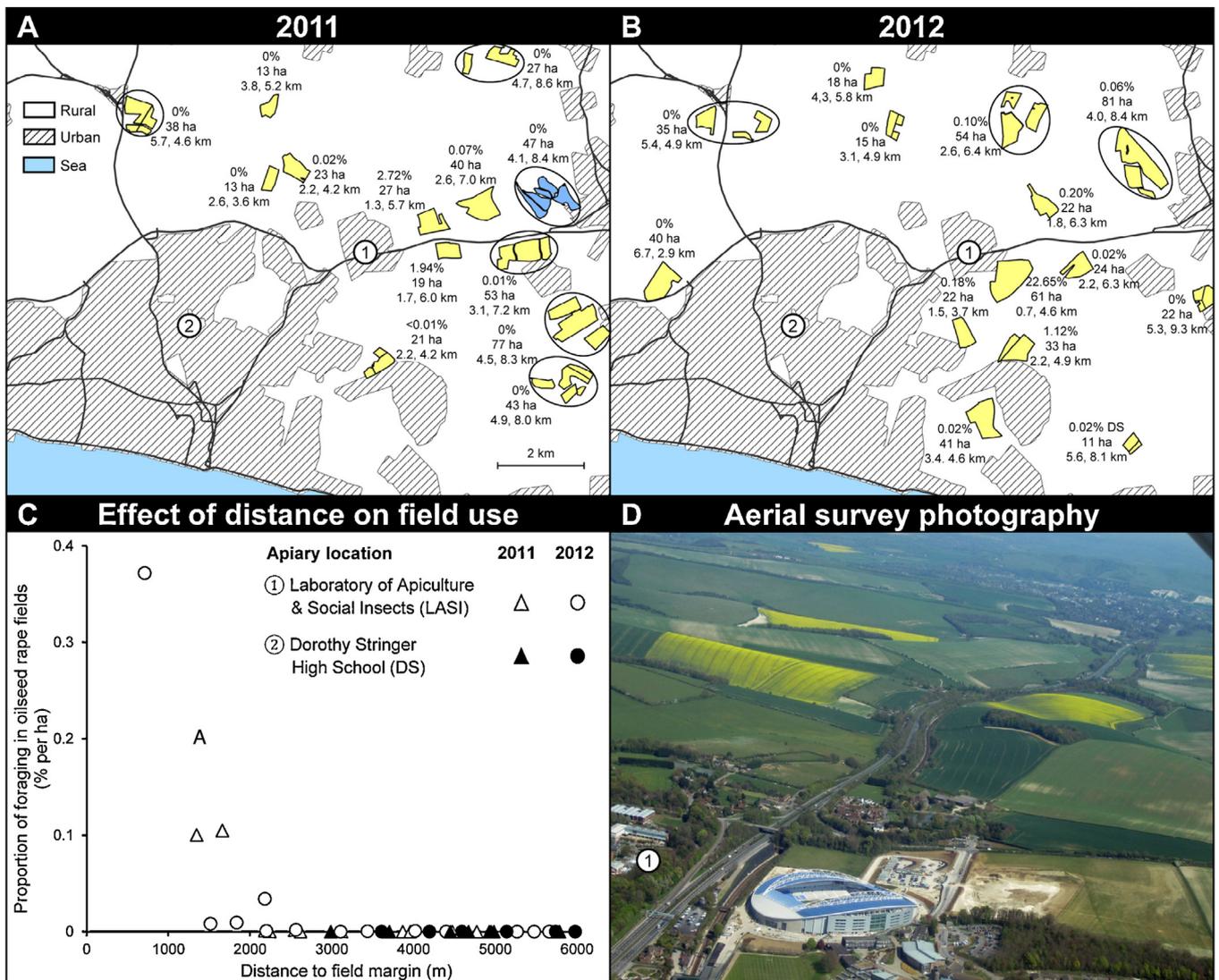
Laboratory of Apiculture & Social Insects (LASI)		% <sup>a</sup> of foraging on oilseed rape	95% CI <sup>b</sup> lower limit	95% CI <sup>b</sup> upper limit	Number of dances
2011	April	2.23%	1.17%	4.08%	460
	May	7.97%	5.57%	11.26%	362
2012	April	21.84%	15.94%	29.15%	150
	May	26.14%	20.63%	32.50%	209
Dorothy Stringer School (DS)					
2011	April	0.00%	–	–	61
	May	0.00%	–	–	234
2012	April	0.00%	–	–	94
	May	0.02%	0.00%	5.79%	76

<sup>a</sup> Percentage among simulated locations.

<sup>b</sup> Agresti–Coull (Brown et al., 2001) 95% confidence intervals for binomial proportion, where *p* (number of simulated locations within fields) and *n* (total number of simulated locations) are scaled back to the number of dances.

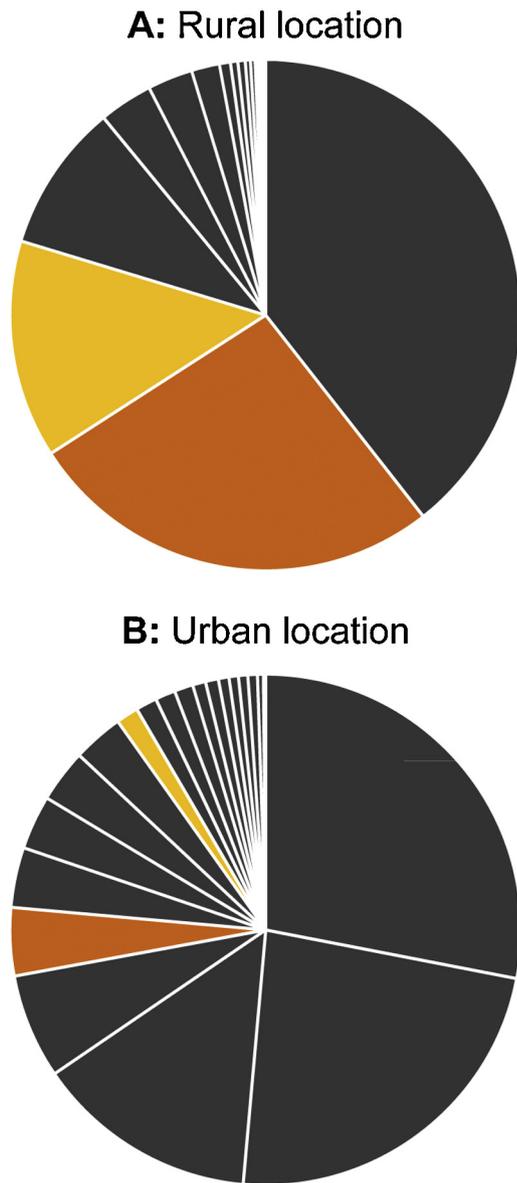
do not represent all foraging locations currently being used by a colony, but only the most profitable locations to which the recruits are being directed (reviewed in Dyer, 2002). The greater amount of foraging on oilseed rape in 2012 from the rural apiary seems to be a

consequence of crop rotation, which resulted in a large field of oilseed rape (61 ha) only 0.7 km from the rural hives at its closest point. This one field accounted for almost a quarter (22.7%) of all spring foraging and almost all (93%) spring foraging on oilseed rape.



**Fig. 2.** Percentage of total spring (April and May) foraging on particular oilseed rape (yellow) and linseed (darker blue, 2011 only) fields, in addition to field area and distances from the Laboratory of Apiculture & Social Insects (LASI) and Dorothy Stringer High School (DS) apiaries, respectively, in 2011 (A) and 2012 (B). Circled groups of neighbouring fields are treated as one unit. All foraging was from colonies at the LASI apiary, with the exception of one field in 2012 (marked DS) in which the 0.02% foraging level was from the DS apiary. (C) Proportion of total foraging per hectare of field or field group as a function of apiary distance to the closest field margin. (D) Aerial photo taken on 19/04/2011 showing four fields of oilseed rape on both sides of the A27 highway east of the rural apiary location (marked 1 on photo). The definition of urban areas follows the Ordnance Survey maps for GIS (vector ‘Meridian 2’) provided by Digimap service (EDINA, <http://digimap.edina.ac.uk>).

■ Oilseed rape ■ Dandelion



**Fig. 3.** Diversity of pollen morphotypes based on pollen pellets collected by bees from three hives each at the rural (A) and urban (B) apiaries. Only pollen of oilseed rape (*Brassica napus*) and dandelion (*Taraxacum officinale*) was identified. The remaining pollen morphotypes are shown in grey and were not identified.

In 2011 the two closest fields were adjacent at distances of 1.3 and 1.7 km, and had a combined area of 46 ha. They received only 4.7% of all spring foraging, but 98% of total spring foraging on oilseed rape. Fig. 2C shows that the amount of foraging per unit area of oilseed rape declined greatly with distance. There was almost zero foraging at distances greater than 2 km, which demonstrates that oilseed rape cannot be profitably exploited at greater distances at this time of year in relation to other available forage. Similarly, the fields of linseed at 4.1 km from the rural apiary did not receive any visits.

### 3.1. Amount of foraging on oilseed rape indicated by pollen analysis

The proportion of oilseed rape pollen pellets was 13.8% (total  $n = 3436$ ) and 3.8% (total  $n = 1344$ ) from the rural and urban hives, respectively. Hives in each location were similar in the proportions

of oilseed rape pollen collected, being 13.4%, 13.3% and 16.8% among the three rural hives and 2.2%, 5.1% and 6.1% among the three urban hives. Similarly, Odoux et al. (2012) found that the oilseed rape pollen comprised no more than 29% of the total pollen collected by honey bees in an agricultural landscape where oilseed rape covered 5% of the land within 2.5 km of the apiary. However, both our data and those of Odoux et al. (2012) are likely to be overestimates due to possible false positives, as pollen of other wild Brassicaceae species can be difficult or impossible to distinguish from oilseed rape (Odoux et al., 2012). This may also explain why we detected oilseed rape pollen in the urban samples even though foraging in oilseed rape fields was not indicated by dance decoding.

Overall the pollen data support the waggle dance data and strengthen the conclusion that oilseed rape did not dominate foraging. The remaining pollen was categorized into 31 morphotypes, 20 from the rural samples and 22 from the urban. The leading four morphotypes accounted for 89.1% and 72.1% of all pollen in the rural and urban locations, respectively (Fig. 3). A common wildflower, the dandelion, was the second most important pollen type in the rural location (26.5%) and fifth (6.6%) in the urban location. Oilseed rape was the third most important in the rural and tenth in the urban location. The remaining types were not identified, but appeared to be mainly Rosaceae. This matches casual observations that in spring there are many hawthorn (*Crataegus monogyna*) and blackthorn (*Prunus spinosa*) bushes in bloom, as well as wild and cultivated fruits, such as cherries and plums (*Prunus* spp. and their cultivars), all Rosaceae.

### 3.2. Potential risks of exposure to neonicotinoid insecticides

Overall, the data show that spring-flowering oilseed rape is a variable forage resource for honey bees, and was not the dominant fraction of both the pollen samples and the foraging locations shown by dance decoding. Honey bees are capable of foraging at distances of over 10 km (Beekman and Ratnieks, 2000; Ratnieks, 2007). As a result, the colonies at both the rural and urban apiaries had many oilseed rape fields within range. In this study, however, most foraging was within 2 km of the apiaries, whether to fields of oilseed rape or not. But these results should be taken with caution as only two apiary locations were studied. However, each location was studied over two years.

One of the key requirements in the design of experiments investigating colony-level effects of insecticides on social bees is the use of environmentally-realistic doses (Cresswell et al., 2012). However, the results of this study indicate that many earlier laboratory and semi-field studies were based on unrealistic exposure scenarios. For example, Bryden et al. (2013) fed bumble bee colonies with neonicotinoid-treated sucrose solution under laboratory conditions with no access to the outside world, thus assuming the colonies forage exclusively on a treated crop. Whitehorn et al. (2012) used a similar feeding regime, with the exception that colonies were moved to the field after the treatment phase. Gill et al. (2012), allowed the bees to forage outside, but provided a treated solution to the colonies with “a similar amount of active ingredient as if they had been foraging exclusively on a crop with 5 ppb imidacloprid in the nectar”. Henry et al. (2012) constructed models predicting honey bee colony failure based on individual-level harm on homing ability and the proportion of foragers in a colony exposed to a treated crop, which was assumed to be 50–90%.

In reality, the levels of colony exposure to a treated crop are variable, but may often be lower than assumed above, even in landscapes where that crop is abundant, as there are often many alternative flowers available. Using pollen analysis, Pettis et al. (2013) showed that honey bee foraging on flowering agricultural crops, where the hives are placed into or beside the crop for pollination, ranges from almost 100% (almond) to nearly 0%

(e.g. watermelon, pumpkin). Similarly, Cutler and Scott-Dupree (2014) found that bumble bee (*Bombus impatiens*) colonies placed directly next to corn fields during pollen shed collected very little, on average 0.6%, of their pollen from corn, whether the corn was treated with clothianidin or not. With regard to oilseed rape, most of the UK crop is planted in late summer or autumn to bloom next spring, when honey bee foraging distances are at their seasonal minimum, indicating high floral abundance in the environment (Couvillon et al., 2014; Beekman and Ratnieks, 2000). Indeed, in this study a common wildflower, the dandelion, was approximately twice as important a pollen source as oilseed rape in the rural location (26.5% vs 13.8%), where most of the foraging on oilseed rape occurred.

Summer-flowering oilseed rape may present a different picture. Summer is a more challenging foraging season than spring for honey bees in the study area, with mean foraging distances several times greater (Couvillon et al., 2014). If the same fields of oilseed rape had been in bloom in July, they would probably have received more foragers from the study hives. Hayter and Cresswell (2006) showed that summer-flowering oilseed rape can have c. 300× greater bee density than spring-flowering. This would have increased its potential benefit as a food source, but also increased the level of exposure to insecticides. However, overall exposure to summer-flowering oilseed rape is probably much less than to spring-flowering in the UK, as the acreage of summer-flowering varieties is only 2% of the total oilseed rape acreage (Garthwaite et al., 2012).

Under field conditions, it is also possible that pesticide residues in nectar and pollen may themselves help to reduce foraging on treated crops by acting as repellents. Some insecticides are well known for their repellent effects on pollinators [e.g. pyrethroids (Thompson, 2001 and references therein)]. But in the case of neonicotinoids the situation is less clear. An early study found that honey bees take longer to return to a syrup feeder treated with the neonicotinoid imidacloprid (Bortolotti et al., 2003). However, the concentrations used were 100 ppb or more, which are ten or more times greater than the maximum detected in nectar (Table 6 in EFSA, 2012). More recently, Eiri and Nieh (2012) showed that imidacloprid at more realistic doses, as low as 0.21 ng per honey bee, raises the response threshold to sucrose via the proboscis extension reflex, and lowers the tendency to make waggle dances. If bees foraging on a treated crop made fewer dances, this could reduce the recruitment of nestmates to a treated area. Similarly, Easton and Goulson (2013) found that pan traps containing imidacloprid at concentrations of 0.01 and 1 ppb had a repellent effect on flying beetles and flies. Furthermore, Thompson et al. (2015) showed that bumble bees (*Bombus terrestris*) exhibit a reduction in consumption of sucrose solution treated with imidacloprid at concentrations as low as 1 ppb and clothianidin as low as 10 ppb; no consumption reduction was detected with thiamethoxam at concentrations 1–10 ppb, but 100% mortality occurred at 100 ppb. It is possible that the repellent effects of neonicotinoids could play a role under field conditions, although further research is needed to determine whether this is the case or not. It is also possible that foraging on oilseed rape would have been greater if the fields were not treated with neonicotinoids, although it is impossible to determine this using our data due to the lack of controls (i.e. colonies within range of fields that are known to be untreated).

The decision on whether to permit the use of neonicotinoid seed treatments on bee attractive crops should be based on a careful weighing of their benefits in reducing insect damage and all possible negative effects to bees and the wider environment (Goulson, 2013). However, little is known about their effects in the environment and on species other than the honey bee, *A. mellifera*, and one bumble bee, *B. terrestris* (Goulson, 2013; Godfray et al., 2014). Indeed, the

current EU moratorium restricts neonicotinoid use only on bee-attractive crops (European Commission, 2013), highlighting that possible effects on bees are at the forefront of the issue. The effects on social bee colonies can most reliably be determined in field experiments, which generally show no or low risk to honey bee and bumble bee colonies exposed to neonicotinoid-treated crops (Cutler and Scott-Dupree, 2007; 2014; Cutler et al., 2014; Pilling et al., 2014; Pohorecka et al., 2012). In contrast, the relevance of laboratory or semi-field studies to field conditions should be interpreted with caution, especially where a single dose has been used, given that several factors affect field exposure and that there is variability and uncertainty in these factors (Carreck and Ratnieks, 2014). Our findings should help base future studies on more realistic levels of one of these factors, and highlight the fact that under field conditions bees will normally have a choice of forage sources.

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