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THIAMETHOXAM: ASSESSING FLIGHT ACTIVITY OF HONEYBEES FORAGING ON TREATED OILSEED RAPE USING RADIO FREQUENCY IDENTIFICATION TECHNOLOGY

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Abstract: The present study was designed to assess homing behavior of bees foraging on winter oilseed rape grown from seed treated with thiamethoxam (as Cruiser OSR), with 1 field drilled with thiamethoxam-treated seed and 2 control fields drilled with fungicide-only–treated seed. Twelve honeybee colonies were used per treatment group, 4 each located at the field edge (on-field site), at approximately 500 m and 1000 m from the field. A total of nearly 300 newly emerged bees per colony were fitted (tagged) with Mic3 radio frequency identification (RFID) transponders and introduced into each of the 36 study hives. The RFID readers fitted to the entrances of the test colonies were used to monitor the activity of the tagged bees for the duration of the 5-wk flowering period of the crop. These activity data were analyzed to assess any impact on flight activity of bees foraging on the treated compared with untreated crops. Honeybees were seen to be actively foraging within all 3 treatment groups during the exposure period. The data for the more than 3000 RFID-tagged bees and more than 90 000 foraging flights monitored throughout the exposure phase for the study follow the same trends across the treatment and controls and at each of the 3 apiary distances, indicating that there were no effects from foraging on the treated crop. Under the experimental conditions, there was no effect of foraging on thiamethoxam-treated oilseed rape on honeybee flight activity or on their ability to return to the hive. *Environ Toxicol Chem* 2016;35:385–393. © 2015 SETAC

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INTRODUCTION

Studies in the laboratory have clearly demonstrated that insecticides have the potential to affect the ability of bees to return to the hive through effects on memory, knockdown, or flight capacity [1,2]. However, the challenge for studies conducted under laboratory conditions is to demonstrate their relevance to the field environment, particularly in terms of exposure profile, no choice, and often a single dose in the laboratory versus foraging on a treated crop [3]. To further develop our understanding of effects on honeybee behavior under realistic field conditions, there has been increasing interest in the use of sensors on free-flying forager bees to determine their ability to return to the hive [4–6]. To date, however, these studies have used artificial exposure either by feeding the bees directly [4] or by allowing them access to a feeder rather than foraging on treated crop [5,7]. There is also a shortage of reliable data on foraging activity and lifespan in free-flying bees in realistic crop scenarios on which to base risk-assessment scenarios [8].

The present study used radio frequency identification transponders (RFID tags) on free-flying honeybees (*Apis mellifera* L.) to determine their lifespan and foraging/homing activity on untreated winter oilseed rape and winter oilseed rape grown from seed treated with thiamethoxam (as Cruiser OSR). Because foraging activity may be affected by the distance to the forage source [4], study hives were located at the field edge (on-field site) and approximately 500 m (0.5 km site) and 1000 m (1.0 km site) from the fields of oilseed rape.

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MATERIALS AND METHODS

Field sites

The study was conducted on the east coast of the United Kingdom (Lincolnshire) in an area remote from both other flowering winter oilseed rape and from flowering crops to provide isolation of the bee colonies and to maximize foraging on the field sites. The location was identified as suitable for the present study because the principal crops grown in the area are intensive field vegetables, such as cabbages and cauliflowers, and there was negligible use of neonicotinoid pesticides. Three study fields (2 control and 1 treated) were used, each at least 2 ha and at least 5 km apart. There were no recorded applications of neonicotinoid compounds in any of the 3 fields for the 2 cropping seasons prior to drilling of the test crops.

All study fields were drilled with winter oilseed rape seed (variety Cabernet and all treated with the fungicides fludioxinil and metalaxyl-M) in October 2012. The 2 control fields were drilled with seed treated only with fungicide prior to the treated field being drilled with seed treated with fungicide and 420 g thiamethoxam/100 kg seed (0.020 mg thiamethoxam/seed) using the same drill. The drilling rate was 4.11 kg seed/ha on the control sites and 4.15 kg seed/ha on the treated site.

Honeybee colonies

In total, 36 normally developed, queen-right honeybee colonies (*A. mellifera* L.) were sourced from a local beekeeper for use in the present study. There were 12 colonies per study field (2 control fields and 1 treated field), 3 apiary sites per study field (on-field, at 0.5 km, and at 1 km from the field) and 4 hives per apiary. Colonies were housed in national hives using British Standard frames. Colonies were set up to be as similar to each another as possible; each hive contained a queen of similar age (successfully mated and proven laying queens from the previous season), at least 11 frames including brood across at least

3 brood frames, at least 5000 bees, and sufficient frames of stores to prevent starvation if foraging was limited by weather conditions during the early phase of flowering; remaining frames were empty. Colonies of a relatively small size were required for the study to minimize the possibility of congestion at the hive entrance as all bees had to enter and leave the hive through the small entrance tunnel formed by the RFID readers. Colonies were selected that had low incidence of minor brood disease (chalkbrood, sacbrood, and baldbrood) and in which both American foulbrood (causative agent *Paenibacillus larvae*) and European foulbrood (causative agent *Melissococcus plutonius*) were clinically absent. The colonies used had not been treated with a varroacide within 4 wk of the start of the trial.

Colonies were placed on all the study fields at the same time, when the crop on the least developed (treated) field was at approximately 20% flowering. All apiary sites were selected on the basis of meeting the standard requirements for apiary location (including being level, with good air and water drainage, with good shelter from the elements, and not directly north-facing). Apiaries were located at positions (allowing for geographical and landownership constraints) that would place them at the farthest point possible from the other treatment groups to minimize risk of drift between treatments (this risk had already been minimized by the distance between the fields). Where possible, the apiary sites were also placed to minimize the risk of a direct flight line between apiaries associated with the same field to reduce the risk for potential drift between apiary sites within the same treatment group.

Monitoring bee activity within the crop

Once per day (except on a few days when there was persistent rain), the foraging activity of bees within the flowering plots was recorded. This was done by walking an approximate 100 m × 1 m transect within a randomly chosen flowering section of the crop over a 10-min period. The numbers, species, and behavior of bees (including non-*Apis* bees) were recorded.

Tagging bees

The RFID transponders (Mic3-Tags, 1.0 mm × 1.6 mm × 0.5 mm; Microsensys), each with a unique identification, were attached to the dorsal thorax of honeybees using adhesive (shellac). Adult bees were emerged from sealed brood combs collected from National Bee Unit (donor) colonies in the field (North Yorkshire) and returned to the laboratory for hatching (frames were maintained at approximately 34 °C and 65% relative humidity). In total, 273 bees were tagged in each of the 36 colonies by placing Mic3 RFID transponders (tags) on 3 consecutive cohorts of newly emerged bees (<24 h old) approximately 1 wk apart (100 bees/colony were tagged for the first 2 cohorts and 73/colony for the final cohort). Within 48 h of tagging each cohort of bees was released into the study hives in the field using queen introduction cages.

Observations

Transponders were read by paired antennae ("reading" units) mounted on the entrance of the hive so that the bees were forced to walk through the tunnel formed by the paired antennae when entering or exiting the hive, and collected data, with time stamp, were stored on a data logger as .xml files. Each reader scanned every 100 ms. The direction of travel could be identified by establishing which reader was triggered first (i.e., inner reader followed by outer was an outward journey, and outer reader followed by inner was an inward journey). Analysis of the data allowed the movements in and out of the hive and foraging activity of tagged bees to be monitored. A bee was regarded as having left the hive when it had passed through both the inner and outer readers and had not been rescanned by the outer reader for more than 5 s; the time taken was the last scan time to register from the outer reader. A bee was regarded as returning to a hive as soon as it was registered through both the outer and the inner readers; the time taken was the first scan time to register from the inner reader. The activity of the tagged bees was monitored for the duration of the exposure phase—approximately 5 wk of flowering (16 May–20 June 2013).

A Tinytag® data logger was placed on each site (next to colonies at the plot edge) to record air temperature and humidity during the course of the exposure phase. Additional weather data were obtained from meteorological office sources from a local weather station to provide supporting evidence, including wind speed and rainfall information.

Palynological analysis

Pollen samples were collected from bees returning to the hives at each apiary site at 2 time points (approximately 1 wk apart) during peak flowering of the oilseed rape. Samples consisted of pollen collected as corbicular loads from returning foragers combined from the 4 hives located on each apiary site. Samples were stored frozen and returned to the laboratory for palynological analysis.

Each of the samples was homogenized by mixing the pollen loads with an Eppendorf pestle and then suspending in deionized water. An aliquot of the resulting suspension was then transferred onto a glass microscope slide and dried before staining with standard safranin solution. Microscopic examination was carried out at 400× magnification with a calibrated eyepiece graticule. An analysis of 100 grains per slide was carried out by examining the first 100 intact pollen grains encountered on the slide (any clearly damaged grains were excluded from the identification process). Where possible, each grain was identified to at least the family level [9].

Bee disease monitoring

Because bee diseases may affect the foraging behavior of bees, at least 100 adult bees were collected from each hive on 2 occasions, once before the colonies were moved to the test apiaries and once after removal to the out-apiary post-exposure. Total nucleic acid, both RNA and DNA, were extracted from the adult bee samples using an automated magnetic particle extraction system. The first step in the process was to grind each of the 60 bee samples in buffer to release nucleic acid. Samples were then incubated at 65 °C to further help lysis of cells. Once incubated, samples were centrifuged to produce a clear lysate. This clear lysate was then mixed with silica-coated magnetic particles at the start of the extraction system. The nucleic acid was attracted to the silica, which then became bound to the particles. The automated process, using a magnetic rod, then mixed and passed the beads through a series of washes. These washing steps were to clean and purify the nucleic acid by precipitating out unwanted inhibitors. The particles were then heated in an aqueous buffer to break the bond between the silica and the nucleic acid to produce a clean extract of nucleic acid. The quality of this extract was assessed by the use of an "internal housekeeping gene" (EF1). This gene is present in all bees, and by looking for this gene at this level (criteria cycle threshold

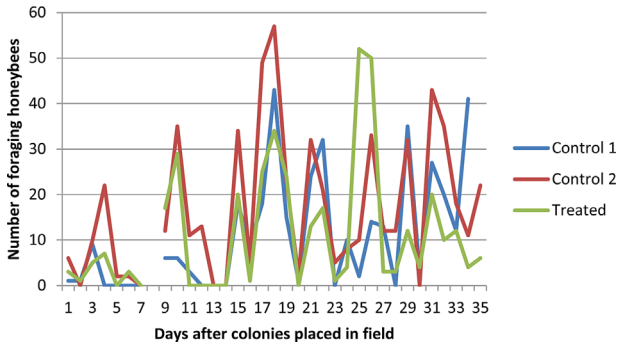

Figure 1. Numbers of honeybees observed within the crop during a daily 10-min transect count. Where data are missing, this is because of adverse weather conditions (i.e. persistent rain) preventing meaningful assessments.

value <27) within the extract, it is possible to determine the quality of the extract. If a high level of this gene is detected in the extract, it demonstrates that the extraction has been successful and that any pathogen DNA/RNA present in the original sample will have been extracted equally well. Following this confirmation of quality, the extracts were tested for the presence or absence of known bee pathogens. Eleven pathogens were screened for by performing a specific one step real-time reverse-transcriptase polymerase chain reaction (TaqMan®). The pathogens tested for were *Nosema apis*, *Nosema ceranae*, deformed wing virus, black queen cell virus, chronic bee paralysis virus, European foulbrood, Kashmir bee virus, sacbrood virus, slow paralysis virus, Israeli acute paralysis virus, and acute bee paralysis virus. Each sample was tested in duplicate and analyzed using sequence detection software. The software provides a numerical value in the form of a cycle threshold value. These values were used to qualitatively score the samples. A cycle threshold value of <36 was defined as positive, 36–40 as low positive, and >40 as negative.

Residue analysis of crop-collected pollen and nectar samples

Pollen and nectar samples were collected from the crop plants in each field by hand and analyzed for residues of thiamethoxam and the metabolite CGA322704 (commonly referred to as clothiandin) with limits of quantitation of 0.5 μg/kg nectar and 1 μg/kg pollen according to the method reported by Pilling et al. [10].

Analysis of outputs from data loggers

The data for each hive were interrogated to extract the unique tag identification number, date and time that a tagged bee had either entered or exited the hive, and the duration of the trip. This was summarized to determine the activity of the tagged bees for each hive for the exposure period, including the mean active period for the bees, the mean duration of individual flights, the mean number of flights per bee, and the mean total flight time per bee over the exposure period. Bees that had drifted between hives on the apiary site (a natural phenomenon) were detected by referring to the unique identification numbers of bees introduced to each hive. All drifted bees—that is, bees detected at hives to which they were not introduced—were excluded from the flight/foraging analysis. All bees detected at the hive they were placed in after tagging were defined as "home" bees. In the foraging analysis, home bees were considered to be active when they had started to forage and had shown at least 1 single complete in/out trip.

Despite the extensive amount of data generated, the fundamentals of the experimental design are such that, when it comes to making formal statistical comparisons between treatments, the only true replication is at the field level. Therefore, in terms of independent experimental units, the design contains 1 treatment and 2 controls, and statistical analysis is not appropriate.

Table 2. Total numbers of bees detected as drifted from other colonies at the same site or (in parentheses) from colonies placed at other sites

Cohort	On-field	0.5 km	1 km
Control 1			
1	17 (16)	5 (12)	9 (19)
2	9 (0)	7 (0)	11 (0)
3	11 (0)	4 (0)	16 (0)
Control 2			
1	5 (43)	4 (18)	3 (43)
2	13 (0)	11 (0)	12 (1)[b]
3	16 (1)[a]	8 (0)	5 (0)
Treated			
1	5 (15)	3 (50)	13 (23)
2	15 (0)	6 (0)	34 (0)
3	20 (0)	10 (0)	12 (0)

[a]Forager from 1 km of control 2 site.
[b]Forager from 0.5 km of control 2 site.

RESULTS

Bee activity within the crop

Throughout the course of the exposure phase *A. mellifera* were seen to be active within the crop at all 3 field sites (Figure 1). The honeybees seen were actively foraging for

Table 1. Identity of pollen collected from foragers returning to the colonies at each site and sampled twice at peak oilseed rape flowering Treated

	% Pollen type identified in forager pollen sample[a]								
	Control 1			Control 2			Treated		
Pollen family	On-field	0.5 km	1 km	On-field	0.5 km	1 km	On-field	0.5 km	1 km
Aceraceae	—/—	—/—	27/27	—/—	—/—	—/2	—/—	—/—	—/—
Cruciferae(Brassica napus; OSR)	99/76	91/38	2/2	100/95	85/8	41/19	88/69	82/90	87/34
Aquifoliaceae (Ilex)	—/—	—/—	—/—	—/—	—/—	—/2	—/—	18/7	—/—
Hippocastanaceae (Aesculus carneas)	—/—	9/33	33/29	—/—	—/—	16/—	—/—	—/—	—/24
Rosaceae	—/13	—/17	32/17	—/5	13/83	42/72	10/23	—/3	4/—
Umbelliferae	–/–	—/—	—/—	—/—	—/—	—/—	—/—	—/—	3/22
Unidentified	1/11	—/12	6/25	—/—	2/9	1/7	2/8	—/—	6/20

[a]Data are presented as sample 1/sample 2 (taken at peak flowering approximately 1 wk apart).

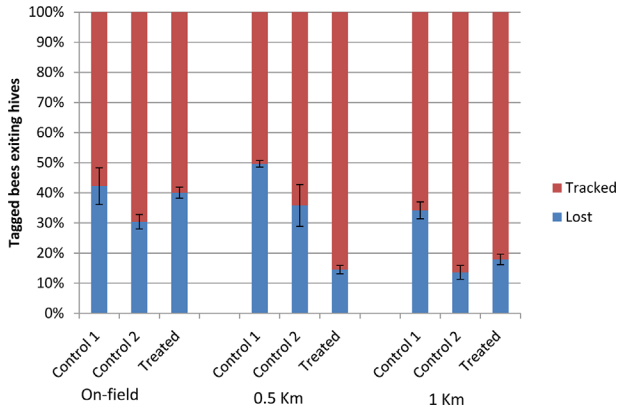

Figure 2. Proportion of tagged bees that were lost from their home colony (with standard error; for n, see Table 3).

nectar or pollen on the crop, and no abnormal behavior of the bees was noted. The peaks of activity in Figure 1 are associated with periods of warmer, drier weather.

Palynology

Oilseed rape was the most prevalent pollen identified in corbicular samples collected from the returning foragers at most apiary sites (Table 1). The exceptions were the 1 km site at control 1, where the most prevalent pollen was Hippocastanaceae (*Aesculus carneas*), with only 2% of pollen identified as oilseed rape, and both the 0.5 km and 1.0 km sites at control 2 at the second time of sampling, where the predominant pollen identified was Rosaceae. However, it was confirmed that the honeybees collected at the hive entrance, particularly those on the treated plot, had been actively foraging on the experimental plots and that a large proportion of the pollen was collected from these plots.

Residue analysis of crop-collected samples

No residues of either thiamethoxam or the metabolite CGA322704 were detected in any of the samples of plants, flowers, pollen, or nectar collected from the control fields above the level of quantification (limits of quantitation for thiamethoxam were 1.0 μg/kg in whole plants, flowers, and pollen and 0.5 μg/kg in nectar; limit of quantitation for CGA322704 in all sample matrices was 1.0 μg/kg).

Residues of 1.0 μg thiamethoxam/kg and 3.0 μg CGA322704 (metabolite)/kg were detected in pollen from the treated crop. Nectar from the treated crop contained 1.8 μg thiamethoxam/kg and no detectable metabolite.

Analysis of outputs from data loggers

Problems with the reliability of the readers/loggers resulted in complete datasets being available for 2 to 3 colonies at each site for the entire 5-wk exposure period. Only data from colonies with complete data records were included in the analysis.

Drifting of bees between hives was observed in the first cohort introduced (cohort 1), and the data showed that this took place at the holding apiary where all colonies were located together prior to being moved to the study fields (i.e., they were present in the hive they had drifted to when first detected by the readers). Drifting after relocation to the test field sites occurred occasionally between hives at the same apiary site and on only 2 occasions occurred between apiary

Table 3. Summary flight data for tagged bees (all cohorts)

		Foraging life span (d)	Total no. of flying days per bee	Mean trip duration (if trips >1) (min)	Flights per bee per flying day	Mean total flight duration per bee per flying day (min)	Mean total flying time per bee, all cohorts (hours)
On-field							
Control 1	*n*	252					
	Mean	7.9	5.0	43.5	2.7	85.3	9.05
	SE	0.4	0.3	7.5	0.2	12.1	1.02
Control 2	*n*	375					
	Mean	11.5	8.1	62.7	3.4	183.3	21.87
	SE	0.44	0.4	11.18	0.14	39.70	1.79
Treated	*n*	214					
	Mean	9.0	5.5	41.5	2.4	83.6	7.94
	SE	0.6	0.4	11.2	0.1	16.3	1.01
0.5 km							
Control 1	*n*	212					
	Mean	9.5	6.2	71.8	2.5	127.4	12.18
	SE	0.6	0.4	22.1	0.1	27.4	1.41
Control 2	*n*	269					
	Mean	16.1	10.3	46.3	2.9	102.4	18.85
	SE	0.56	0.4	6.54	0.12	9.33	1.33
Treated	*n*	377					
	Mean	14.1	9.8	39.5	3.9	116.0	22.55
	SE	0.4	0.3	3.9	0.1	8.5	1.82
1 km							
Control 1	*n*	337					
	Mean	14.6	8.6	49.2	2.7	106.4	12.92
	SE	0.46	0.3	17.99	0.10	33.40	1.53
Control 2	*n*	433					
	Mean	14.0	9.3	46.6	3.5	130.1	24.51
	SE	0.40	0.3	4.82	0.15	8.63	1.56
Treated	*n*	536					
	Mean	10.9	6.6	30.5	3.7	82.8	10.06
	SE	0.3	0.2	7.2	0.1	11.2	0.69

sites for the study field; this was within the same treatment. All drifted bees (i.e., those detected at hives to which they were not introduced) were excluded from the analysis.

Identification of tagged bees

Forager bees that had drifted between colonies were identified within the data (Table 2). Most of those identified as drifting between colonies placed on different sites (99%, $n = 239$) reflected drifting on the home apiary site before the colonies were moved to the field sites (cohort 1), with only 2 (1%) instances of bees drifting between apiary sites within later cohorts, both within the same treatment (control 2). For bees identified at their home colony, >70% of all tagged bees introduced to the hives were detected by the readers at least once.

The proportions of bees detected that left the colony in which they were placed at the start of the study and that did not return are shown in Figure 2. Up to 50% of bees from each colony were recorded as exiting but not returning to the colony within the study period (e.g., if they drifted to another colony within the same apiary site), and these were defined as "lost" (Figure 2). There were no apparent differences between the treated and control sites and no obvious differences between the treatments at each distance.

After removing the drifted and lost bees from the dataset, data were analyzed for 3005 foragers originating from their home colony and completing at least 1 foraging trip, providing data for more than 90 000 foraging flights. These flights are summarized by treatment and distance in Table 3. Each bee made a mean of 2.5 flights/d to 3.9 flights/d, with a mean total foraging time per day of 82 min to 183 min (1.3 h to 3 h), with no obvious differences between the treated and control sites.

Time to first foraging

The first time the tagged bees left the hive after they were placed on the field sites is shown in Figure 3. This shows no clear differences between the treatments in times of peaks of forager first flights. The peaks in activity after 2 d to 3 d primarily reflect activity of the first cohort; peaks after 4 d of activity reflect the second cohort; and peaks after 8 d to 10 d reflect the third cohort of bees, which were introduced immediately after the colonies had been moved to the field.

Lifespan of tagged bees

The mean lifespans (days between introduction into the colony and last record) of the bees in the control 1, control 2, and treated fields are shown in Table 4; and the survival curves and foraging survival curves for the tagged bees are shown in Figure 4. The lifespan for the third cohort was artificially shortened by the fact that they were placed in the colonies on day 0 (when the colonies were placed in the field) and the majority made their first flights around day 10. With an exposure period finishing on day 35, the absolute maximum lifespan detectable in this experiment was thus 35 d, whereas a number of bees in the first and second cohorts were identified with maximum lifespans of 46 d to 48 d and 37 d to 39 d, respectively.

Flight duration and time between flights

The duration of flights made by individual bees and the time between flights on the same day were analyzed and the distributions compared between the treatments (Figure 5). More than 97% of the flights on all sites were less than 120 min (Table 5), and the small number of more

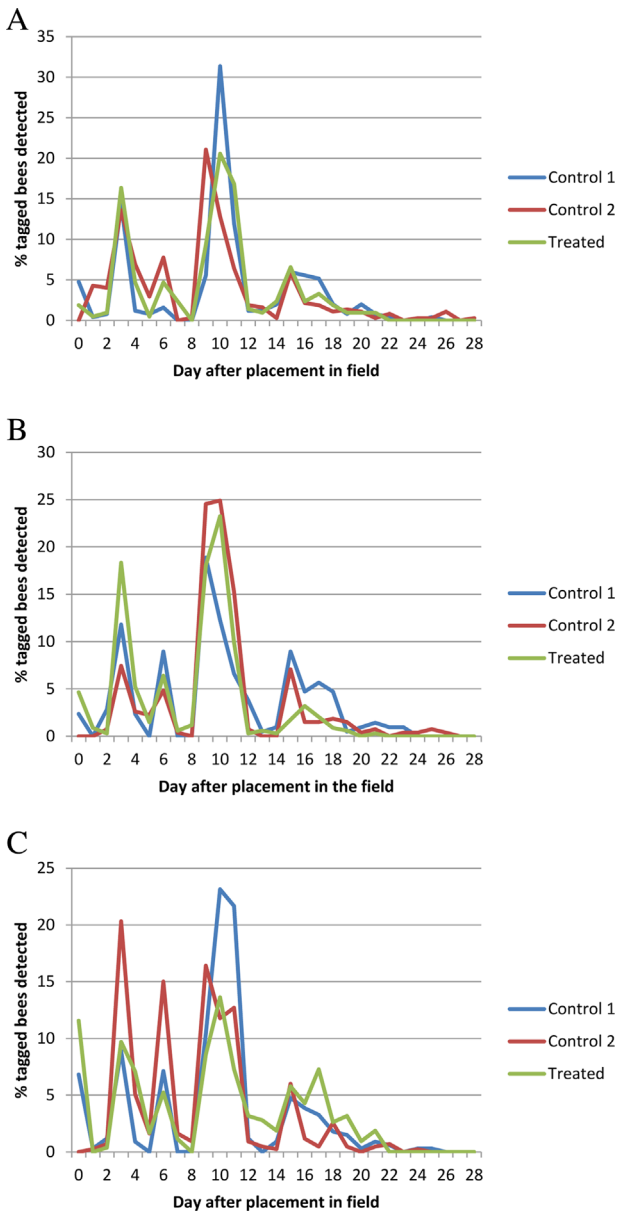

Figure 3. Time of first foraging of bees introduced when the colonies were in place on the field and therefore with maximum exposure: (A) on-field colonies; (B) colonies 0.5 km from the field; (C) colonies 1 km from the field (for numbers of bees detected, see Table 3).

extensive flight times probably reflected the occasional drifting between hives on the same site. There were no apparent differences between the sites in the duration of flights (see mean flight times in Table 3). Flights of less than 1 min probably represent early orientation flights. Similarly, there were no apparent differences between the treatments in the duration of the time spent between flights on the same day (Figure 5).

Bee disease monitoring

At the start of the study, only 2 colonies (1 colony in each apiary at the control 1 on-field and treated 1 km sites) tested negative for both *N. apis* and *N. ceranae* and only 1 colony was negative for black queen cell virus (in control 1 0.5 km apiary site). There were only occasional instances of other viruses detected, primarily deformed wing virus and sacbrood virus,

Table 4. Life span of tagged bees introduced into each colony[a]

Treatment	On-field	0.5 km	1 km
All cohorts			
Control 1	20.8 ± 0.5	23.4 ± 0.6	26.8 ± 0.5
Control 2	23.7 ± 0.5	31.5 ± 0.7	25.3 ± 0.5
Treated	21.5 ±0.7	26.5 ± 0.5	24.4 ± 0.3
Cohort 1			
Control 1	25.7 ± 1.1 (56)	29.3 ± 1.2 (58)	29.6 ± 1.1 (72)
Control 2	26.6 ± 0.9 (107)	37.2 ± 0.9 (109)	31.7 ± 0.8 (113)
Treated	28.0 ±1.3 (56)	30.9 ± 0.7 (120)	29.1 ± 0.7 (165)
Cohort 2			
Control 1	20.6 ± 0.7 (102)	21.0 ± 0.9 (93)	25.8 ± 0.9 (120)
Control 2	23.6 ± 0.7 (141)	30.2 ± 0.9 (113)	22.8 ± 0.7 (155)
Treated	21.8 ± 1.1 (77)	24.9 ± 0.7 (143)	23.4 ± 0.5 (221)
Cohort 3			
Control 1	18.2 ± 0.7 (94)	21.6 ± 1.1 (61)	26.3 ± 0.7 (145)
Control 2	21.2 ± 0.8 (127)	21.7 ± 1.4 (47)	23.3 ± 0.7 (165)
Treated	16.8 ± 0.8 (81)	23.8 ± 0.8 (114)	20.8 ± 0.6 (150)

[a]Values in parenthesis are numbers of bees (*n*) per cohort; for *n* at each site/location, see Table 3.

mostly at low levels. At the end of the exposure period, only 1 colony from the treated site (from the 1 km apiary site) and 2 from the control 1 site (1 colony from the on-field apiary site and 1 colony from the 0.5 km apiary site) were positive for *N. apis*. Levels of black queen cell virus remained consistently high across all treatment groups, and incidence of all other viruses remained negligible.

DISCUSSION

This is an extensive dataset for free-flying tagged bees foraging on untreated and pesticide-treated crops and provides data for more than 3000 bees and 90 000 flights over a period of 5 wk. Together, these data suggest that the forager bees were behaving normally in the presence of the tags. The tagged bees started foraging within 4 d to 10 d after they were placed in the colonies, with foraging activity followed throughout the 5 wk of flowering of the oilseed rape crop. The time of first foraging was identified as peaking 8 d to 10 d after introduction of the cohort, and this is in accordance with other studies [11]. The total lifespan of the tagged bees and number of days spent foraging are also within the expected range (Table 6). The forager daily mortality rate (based on flight lifespan) of 5.6% to 13.0% (all based on the first 2 cohorts) was lower than the 15.3% assumed by Khoury et al. [12]. The total time spent foraging can also be assessed against the expected maximum foraging distance of 800 km of an individual [11]. The maximum mean foraging time was 24.5 h at the 1 km site, and assuming a maximum flight speed of 7.5 m/s, this equates to 661 km if all time outside the hive was spent flying.

The dataset also highlights the need to take into account key behaviors and effects of other factors on foragers. For example, drifting between colonies within an apiary site is a natural occurrence and may be greater during orientation flights; in the present study, an average of 9% of identified individuals at each site originated from other colonies (with no clear differences between treatments or cohorts). This has the consequence that

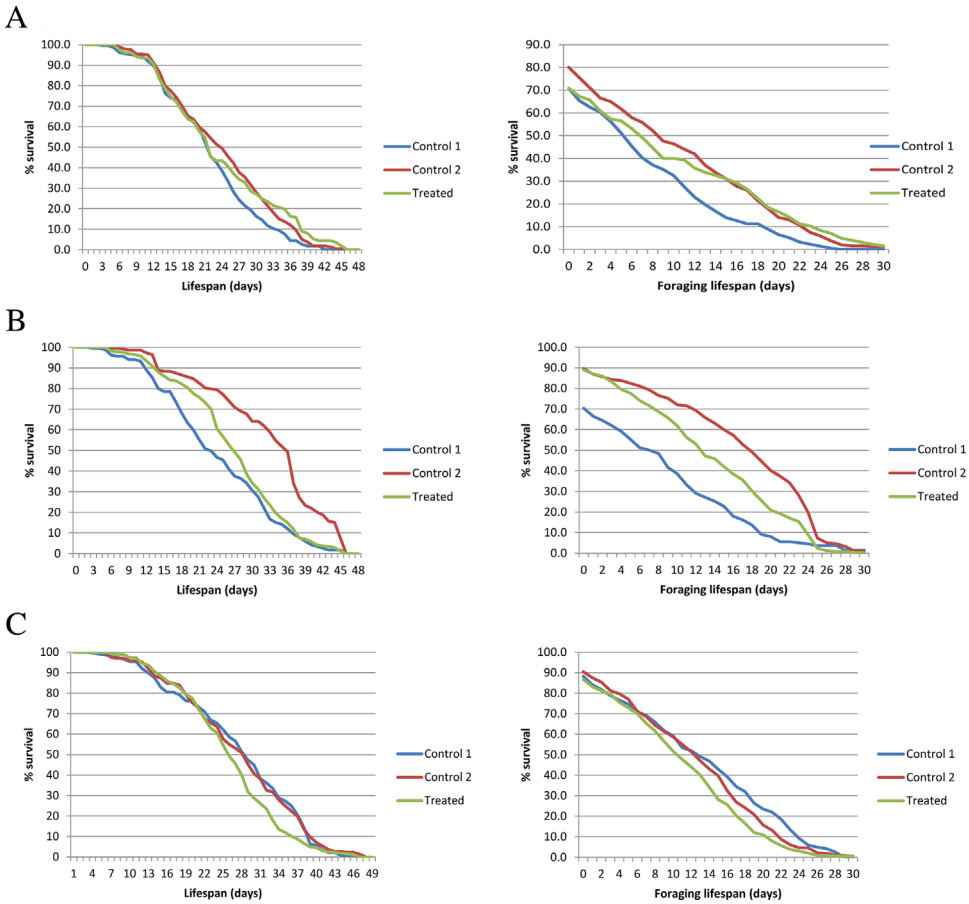

Figure 4. Survival curves for total lifespan and foraging life of bees introduced to the colonies in cohorts 1 and 2 based on date of introduction into the colonies and the time when the tag was last recorded: (A) on-field colonies (control 1 n = 158, control 2 n = 248, treated n = 133); (B) colonies 0.5 km from the field (control 1 n = 151, control 2 n = 222, treated n = 267); (C) colonies 1 km from the field (control 1 n = 192, control 2 n = 268, treated n = 386).

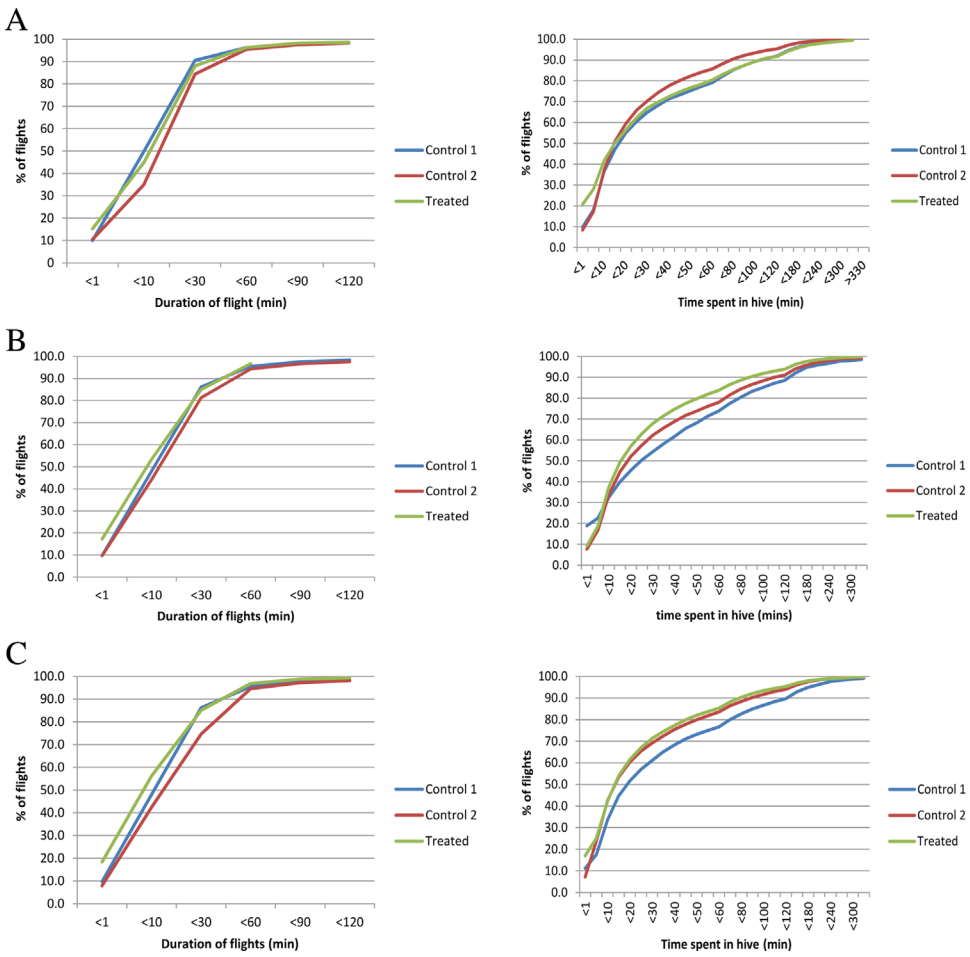

Figure 5. Distribution of flight times and times between flights for bees introduced to the colonies for all flights recorded: (A) on-field colonies (control 1 n = 5005, control 2 n = 14 748, treated n = 4128); (B) colonies 0.5 km from the field (control 1 n = 2383, control 2 n = 10 078, treated n = 16 559); (C) colonies 1 km from the field (control 1 n = 9749, control 2 n = 17 170, treated n = 9779).

studies examining forager return with multiple hives on a site should take into account the scale of drifting between colonies when assessing loss rates for foragers. The reasons for the lost bees in the present study are unclear but may include the presence of *N. ceranae* and/or black queen cell virus in the colonies. Wolf et al. [6] used harmonic radar to track honeybees from a 120-m release site to their home colony and reported that homing failure increased from 6.3% to 8.6% in controls to 34.2% in *N. ceranae*–infected bees. However, "lost" bees in the present study may have lost their tags or drifted to other colonies within the same apiary, in the same way as drifted bees were identified within monitored colonies. The data were not interrogated to identify whether lost bees could be located because they may have drifted to colonies where data loggers failed, resulting in incorrect interpretations.

Honeybees (and other bees) were seen to be actively foraging on the crop within all 3 treatment groups during the exposure period. Considering that residues of both thiamethoxam and the metabolite CGA322704 were found within the nectar and pollen samples collected from the treated site, it can be assumed that any bees foraging on the treated crop would be exposed to these residues. It is highly likely that some or all of the honeybees seen foraging within the crops were from the study colonies. This is supported by the fact that across all apiary sites and treatments (with the exception of the control site 2, 1.0 km apiary) high levels of oilseed rape pollen were identified from corbicular loads of returning foragers on at least 1 occasion. Because of the location and distances involved, it is unlikely that the bees used within the present study foraged on any of the other fields or other oilseed rape fields, which were located at least 5 km distant. Therefore, these data also allow comparison with reports of the potential adverse effects of neonicotinoids on memory, orientation, and foraging.

Table 5. Percentage of flight times >120 min

Treatment	On-field	0.5 km	1 km
Control 1	2.4	2.4	1.3
Control 2	2.3	2.9	2.6
Treated	1.7	1.8	1.6

Henry et al. [4] reported the homing success of tagged bees released after dosing in the laboratory in 4 varying experimental scenarios. The probability of return within 4 h of 74 to 118 untreated established foragers released 1 km from their home colony was 81% in a familiar landscape and 57.6% in an unfamiliar landscape. In the present study, 66% to 86% of 501 to 512 bees foraging on the 2 control fields 1 km from their apiary site made at least 1 successful (returned to their own hive) foraging trip. Because the bees in the present study were on their first foraging trip, they could be considered as equivalent to those released in an unfamiliar landscape; Henry et al. [4] used established foragers. For those bees that made at least 1 successful round-trip (the average was 28.9 ± 1.7 to 40.7 ± 3.0

Table 6. Comparison of data generated in the present study with published data

Forage source	No. of bees introduced	No. of bees with data	Mean forager life span (days)	Number of flight days	Daily mortality rate	Reference
Oilseed rape, 5000 workers	6279	3005	20.8 ± 0.5 to 31.5 ± 0.7	7.7–17.7 (cohorts 1 and 2)	5.6–13	Present study
"Natural" setting	120	33		7.7 ± 0.75	13	[20]
Abundant forage	240	27		9.7 ± 0.9	10.3	[21]
Access to flight cage (1-h feed)	480	60	21.0	11.3	8.8	[22]
Access to flight cage (2-h feed)	960	175–183	30.7–32.9	4.7–5.3	18.8–21.2	[22]
Access to flight cage (24-h feed)	480	113	20.4	7.3	13.7	[22]
Natural forage	960	288–335	25.6–26.3	3.3–4.9	20.4–30.3	[22]
Natural forage: Low pollen hoarding strain	250–350	113–246	26.5–26.7	3.6	27.8	[22]
Natural forage: High pollen hoarding strain	250–530	165–168	23.2–234	3.3–6.1	16.4–20.4	[22]
Natural forage	250	250		6.6 ± 0.3 to 6.8 ± 0.2	14.7–15.1	[23]
Natural forage: Large hive (9000 workers)	796	609–671	22.3 ± 7.6 to 22.8 ± 9.4	6.5 ± 5.3 to 7.5 ± 6.6	13.3–15.3	[24]
Natural forage: Small hive (4500 workers)	796	680–709	26.4 ± 9.7 to 26.6 ± 8.9	6.7 ± 6.0 to 8.8 ± 6.9	11.4–14.9	[24]

foraging trips over a lifetime) and thus may be considered as established foragers foraging in a familiar landscape, >97% of bees returned within 2 h. This is a higher return ratio than suggested by Henry et al. [4] and may add support to the concern that capture/confinement and handling/dosing of forager bees in the laboratory influence their survival [13].

Henry et al. [4] reported that dosing of 74 to 118 established foragers in the laboratory with 1.34 ng thiamethoxam/bee (representing an estimated total daily exposure) significantly reduced their homing success from 81% in a familiar landscape and 57.6% in an unfamiliar landscape to 68.1% and 33.9%, respectively. In the present study, 82% of 653 bees foraging on the treated field 1 km from their apiary site made at least 1 successful (returned to their own hive) foraging trip and >98% of established foragers returned within 2 h, a return far greater than that suggested by Henry et al. [4] and equivalent to the bees foraging on the control fields.

There have also been a number of laboratory studies on the effects of thiamethoxam on memory, with conflicting results from no effects reported on learning or olfactory performance [14] to a significant decrease of olfactory memory at 0.1 ng thiamethoxam/bee and significant impairment of learning performance with no effect on memory at 1 ng thiamethoxam/bee [15]. At higher doses (3 ng/bee), significant effects were reported in a test of associative learning in a complex maze [16]. Although Henry et al. [4] did not report the distribution of flight times in their study, it may be expected that if bees are impacted, resulting in lost bees, then any bees returning to the hive also will take a greater time because of deviations during their foraging and return flight resulting from effects on memory [17,18]. However, in the present study, the distribution of flight times in all treatments was similar. Bees made between 2.5 ± 0.1 flights/d and 3.9 ± 0.1 flights/d, and the mean trip duration (excluding trips of <1 min, which were likely to be orientation flights [11]) for individual flights of bees located 1 km from the oilseed rape crop was 30.5 ± 7.2 min for bees on the treated site and 47.8 ± 5.0 min to 49.2 ± 18.0 min for bees on the control sites. This variation may be a result of the higher level of foraging on the oilseed rape on the treated site; 34% to 87% oilseed rape pollen was present in pollen loads returned to colonies on the treated site compared with 2% and 19% to 41% on the control 1 and control 2 sites, respectively. Similar data were generated at the 0.5 km apiary sites, where the foraging on the control oilseed rape was greater with flight times of 39.5 ± 3.9 min for bees on the treated site and 46.3 ± 6.5 min to 71.8 ± 22.2 min for bees on the control 2 and control 1 sites, respectively, again possibly because of the lower percentage of foraging on oilseed rape in the latter stage of the study. The flight times were also similar for bees from colonies on-field, with flight times of approximately 40 min to 60 min on the treated and control fields. Together, these data suggest that the bees foraging on the treated oilseed rape were behaving in a comparable manner to those foraging on the 2 control fields.

Schneider et al. [5] used RFID to measure the return of bees from an artificial feeder located 7 m from the hive and observed no effects at field realistic concentrations of imidacloprid or clothiandin but at higher concentrations identified prolonged periods in the hive before foraging again. These data suggest that the response of those bees able to return to the hive was to remain within the colony to recover. However, this effect was not observed in the present study. The duration of time spent in the hive between flights made on the same day is similar across the treatments, with means ranging from 30.2 ± 0.4 min to 38.8 ± 0.1 min in colonies on treated sites and 30.7 ± 0.4 min to 50.7 ± 1.8 min in colonies on control sites; the numbers of flights per day were also similar.

These data can also be used to assess the possible exposure of the foraging bees to the thiamethoxam in the nectar of the treated crop. Based on a requirement of 12 mg sugar/h to fly [8], the foraging behavior of the bees suggests that per flight they would expend 6 mg sugar to fuel flight (assuming they spend the entire time flying) and collect/carry 40 mg nectar per load [11]. In this scenario, based on 1.8 μg thiamethoxam/kg nectar, the bees collected 0.072 ng thiamethoxam per nectar load and consumed 0.036 ng thiamethoxam (based on 30% sugar in nectar, assuming all flight is fueled by the collected nectar and the absorption of thiamethoxam into the gut is linear with that of the nectar). With a mean of 4 foraging trips/d, this equates to 72 mg nectar/d to fuel flight (just fewer than 2 foraging trips) if all energy was sourced from collected nectar and results in exposure to a total of 0.14 ng thiamethoxam/d. In fact, the metabolism of thiamethoxam within the bee and the time course of exposure over the course of the day are likely to result in the body burden being far lower. Thus, the modeled exposure scenario used by Henry et al. [4] was at least an order of magnitude greater than that encountered by the bees foraging on treated oilseed rape and probably even greater when the exposure profile/metabolism is taken into account.

In conclusion, there has been significant interest in the effects of neonicotinoids on honeybee foragers' ability to return to the hive through effects on learning and memory. A number of laboratory studies have shown the potential for effects, but the exposure levels used have been questioned [19]. The present study showed that RFID tags can be used to track free-flying bees foraging on treated crops and therefore are the most realistic reflection of real-world scenarios. Under the conditions of the present experiment there was no effect of exposure to thiamethoxam-treated oilseed rape on forager lifespan or ability to return to the hive even when crops were located up to 1 km from the apiary site.

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Data availability—Data can be accessed by contacting the corresponding author (Helen.thompson@syngenta.com).

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