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A landscape scale study of the net effect of proximity to a neonicotinoid-treated crop on honey bee and bumble bee colonies

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Abstract

Since 2013 the European Commission has restricted the use of three neonicotinoid insecticides as seed dressings on bee-attractive crops. Such crops represent an important source of forage for bees, which is often scarce in agro-ecosystems. However, this benefit has often been overlooked in the design of previous field studies, leaving the *net impact* of neonicotinoid treated crops on bees relatively unknown. Here we determine the combined benefit (forage) and cost (insecticide) of oilseed rape grown from thiamethoxam-treated seeds on *Bombus terrestris* and *Apis mellifera* colonies. In April 2014, 36 colonies per species were located adjacent to three large oilseed rape fields (12 colonies per field). Another 36 were in three nearby locations in the same agro-ecosystem, but several kilometres distant from any oilseed rape fields. We found that *Bombus* colony growth and reproduction were unaffected by location (distant versus adjacent) following the two month flowering period. *Apis* colony and queen survival were unaffected. However, there was a small, but significant negative relationship between honey and pollen neonicotinoid contamination and *Apis* colony weight gain. We hypothesize that any sub-lethal effects of neonicotinoid seed-dressings on *Bombus* colonies are potentially offset by the additional foraging resources provided. A better understanding of the ecological and agronomic factors underlying neonicotinoid residues is needed to inform evidence-based policy.

Keywords: honey bees; bumble bees; insecticides; neonicotinoids; seed-treatments; thiamethoxam; oilseed rape

Introduction

Neonicotinoids have been in use since the early 1990's and are now the most widely used insecticide class globally[1]. These versatile neurotoxicants are most commonly used as seed dressings [1], providing systemic protection to young plants against herbivorous insects.

However, detectable residues can occur in the pollen and nectar of treated crops. Laboratory-led studies which directly fed 'field realistic doses' to individual honey bees[2] and to bumble bee colonies[3,4] identified sub-lethal effects in both species.

Such findings have led to concern that neonicotinoids might be a key factor in the declining population of many bee species[5] and resulted in the 2013 European Commission (EC) moratorium [6]. This precautionary measure reflects the vital role of bees in crop pollination[7] and highlighted the need for further data. Sub-lethal effects, across a range of doses and durations of exposure, have now been recorded by laboratory-led studies[8,9]. However, field studies have generally found no affect on either honey bee[10,11,12,13] or bumble bee[14,15,16] colonies.

The only field studies showing adverse effects on bumble bees colonies[10,17] used insecticide-free fields of oilseed rape (*Brassica napus*) as a control. Thus, it is not possible, from these data alone, to determine the overall *net impact* of the presence of the seed-treated crop on bee populations. This is because access to neonicotinoid treated mass-flowering crops, such as oilseed rape, can involve both benefits (abundant floral resources[18,19]) as well as costs (insecticide exposure[10,17]) for bees. Because forage resources are not abundant in modern agricultural landscapes[20], neonicotinoid-treated mass flowering crops may represent a better *net* environment than a field of an unattractive crop, such as wheat, which provides neither nectar nor pollen for bees. In this study we determine the net effect of these benefits and costs by monitoring both honey bee (*Apis mellifera*) and bumble bee (*Bombus terrestris audax*) colonies adjacent to, versus distant from, large agricultural fields of oilseed rape grown from neonicotinoid-treated seeds.

Materials and Methods

Study Location & Experimental Design

We assessed the impact of thiamethoxam (and its metabolite clothianidin) exposure on honey bee (*A. mellifera*) and bumble bee (*B. terrestris audax*) colonies foraging naturally on the seed-treated bee-attractive oilseed rape in a landscape setting. During February and March 2014 we selected six rural sites in a 6 x 20 km zone of predominately agricultural land in the South Downs, Sussex, UK to place our bee colonies. Three sites were adjacent (<5 m) to large oilseed rape fields (0.38, 0.55, 0.64 km^2) grown by commercial farmers from seeds planted in late summer 2013, before the implementation of the EC moratorium, that had been treated with thiamethoxam (Cruiser oilseed rape®, Syngenta Ltd., Basel, Switzerland) and a fungicide (Table S4, Supporting Information). The three 'distant' sites were 1.25 km, 3.05 km and 4.55 km from the nearest oilseed rape field boundary, see Fig. 1. The distribution of oilseed rape fields and the land-use types in the study area were plotted via an aerial survey on 12 May 2014 (Fig. 1), which showed that the proportion of oilseed rape in our study area (2.6%) was close to the UK average (3.0% in 2013-14;[21]). Since any possible treatment effect could be confounded with site differences, the study sites were all selected to be as similar as possible in terms of elevation, soil type, exposure and land use. Although honey bees can forage at distances of up to 12 km, average foraging distances are short, <1.1 km, during the oilseed rape spring blooming period (April-May; [22]), with oilseed rape fields located >2km from hives being little visited [23,24]. Likewise, the study bumble bee species, *B. terrestris*, normally forage within <1 km of their nests[24]. Therefore, our design was expected to result in zero or low foraging on oilseed rape by the 'distant' colonies.

During early oilseed rape bloom (10% of flowers on the main raceme open), on 2-4 April 2014, we set out 72 honey bee colonies and 72 commercially reared *B. terrestris audax* (tomato Audax type) colonies, 12 per site. The bloom stages of the three study fields were temporally synchronized. Near the end of the oilseed rape bloom (10% of flower buds remaining), 20-22 May, all 72 honey bee colonies were moved from their spring study sites to

six 'common' apiaries. Each common apiary housed two hives from each spring study sites. Common apiaries were located within a 8 x 12 km area of predominately agricultural land in the South Downs. Their locations were selected so that each had a similar quality of surrounding habitat within honey bee forage range. All colonies were moved at night. For one year, at approximately monthly intervals, we measured honey bee colony performance: (i) hive weight change, (ii) frames of brood, (iii) colony survival and, (iv) queen survival/replacement. The bumble bee colonies were collected at the end of the oilseed rape bloom, half after six weeks (16 May) and half after eight weeks (30 May). Colony performance was later determined by quantifying: i) adult bee populations, ii) number of cocoons, iii) nest weight change and, iv) final nest volume.

Spring study location (within treatments) was not found to significantly affect any of our measures of honey bee (Table S1) or bumble bee (Table S2) colony performance (Supporting Information) Land-use types (urban, scrub/trees, grassland and arable) within 2km of the study location did not differ significantly between treatments (Table S3; Supporting Information). Thus, indicating that forage availability was similar across the six study locations. During the first week of the experiment there was a conspicuous absence of alternative foraging resources. However, several Rosaceae species and *Taraxacum officinale* began to bloom during the second half of April. Mean wind speeds and temperatures recorded (HoldPeak HP-866B) at the study locations did not differ significantly between treatments (ANOVA; $p > 0.05$).

Honey Bee Colony Management

Colonies were managed according to standard UK beekeeping methods and housed in hives consisting of a single 'commercial' brood chamber (11 frames of 43.8 x 25.4 cm, volume 56.4 litres). Each hive was given a queen excluder and additional boxes ('supers') of wax combs for honey storage as required. We removed and extracted the honey from one to two full supers

(equivalent to c. 15-30 kg) per colony during June and July. Colonies had access to both honey stores and empty frames throughout the experimental period. Colonies were equalized on 31 March or 1 April 2014 during unfavourable foraging conditions to ensure that the vast majority of foragers were within the hive and worker population could be assessed. Each had a marked laying queen, 4 frames of brood, 6 frames of adult worker bees, 2-3 frames of honey, 0.5-1 frames of pollen and two frames of empty wax foundation comb. Based on visual inspection all colonies were apparently disease free.

Any colonies with failed queens were made queen-right with mated queens at the earliest opportunity. As a preventative measure each colony was given two Apistan strips (Vita Europe; Basingstoke, UK) in August 2014 and twice treated with oxalic acid, 2.25g via sublimation, in December 2014 and January 2015 [26] to control varroa mites (*Varroa destructor*). During the swarming period (May-June) additional inspections were made every nine days to destroy queen cells and prevent swarming. Additionally, we employed a modified version of the Brother Adam swarm prevention technique [27] between 15 May and 2 June 2014. This involved removing the queens from all 72 colonies for 10-14 days. During this period queens were housed in mating nuclei with several hundred workers and all queen cells were destroyed in the original colonies.

Bumble Bee Colony Management

Colonies were supplied by Syngenta Bioline (Clacton-on-Sea, England), were approximately 10 weeks old and contained a queen and approximately 60 workers on delivery (4 April). They were housed in the normal commercial hive boxes, consisting of an 8.16 litre nest cavity inside a plastic membrane protected by a cardboard box, with a layer of cotton wool for insulation. As the hives were placed outdoors, each was given a polythene 'roof' to protect the cardboard box from rain and tied to a wooden stand staked one metre high above ground to prevent water ingress and badger predation.

The hive entrances were then opened and the colonies allowed to forage naturally. The suppliers advised that these colonies would reach their reproductive stage approximately six weeks after delivery. At this point bumble bee colonies are at their maximum size and weight. Therefore, to assess colony performance and reproductive success, half of the hives (six from each site) were collected after six weeks (16 May) and half after eight weeks (30 May), near the end of the oilseed rape bloom (c. 10% of flower buds remaining). Thus, the adjacent colonies had 6 or 8 weeks' opportunity to forage on oilseed rape. Two days before colony collection, the hive entrances were switched (from open to an inward-pointing cone option provided by the hive manufacturer) to allow bees to enter the hive, but not to exit. This ensured that on collection our hives contained all the bees living in the colony, including foragers. Hives were then stored at -20 °C until they were sorted to take samples and quantify nest contents.

Our colonies each had an internal syrup feeder (volume: 1.5 litres) provided by the supplier. On average 577 g sucrose solution was consumed in the period after colonies were set up by the supplier, but only 117 g of consumption was during the experimental period, indicating that there was abundant nectar available at this time. The presence of syrup feeders may have reduced the amount of nectar collected by our study colonies and thus the neonicotinoid residues consumed. However, previous research has shown that the total amount of honey in a bumble bee nest does not influence forager behaviour[28]. Moreover, bumble bee colonies are known to collect c. 50 g of nectar per day[29]. Therefore, the amount of syrup consumed during the experimental period is equivalent to only 2-3 days of foraging and only c. 5% of a colony's energy use over the study period. Consequently, the volume of syrup consumed per colony did not significantly affect any of our measures of performance or reproductive success (Table S2, Supplementary Material).

Measuring Honey Bee Colony Performance

158 At approximately monthly intervals from 2 April 2014 to 20 April 2015 we quantified four
159 measures of honey bee colony performance: (i) hive weight (from which we determined
160 weight change, after allowing for the weight of any additional hive equipment added or
161 removed from each hive), (ii) frames of brood (iii) colony survival and (iv) queen
162 survival/replacement.

163 The majority (~74%[30]) of hive weight change is due to variations in the amount of stored
164 honey. To determine colony weight, hives (including the brood chamber and additional
165 supers) were suspended and weighed using a digital hanging scale (PCE Instruments, model:
166 PCE-HS 150N, Accuracy: ± 0.20 kg) immediately prior to being moved into spring study
167 sites and thereafter at approximately monthly intervals. Weighing was undertaken during poor
168 weather conditions so that the majority of foragers were within the colony. At monthly
169 intervals we also inspected all hives and estimated the number of sealed brood frames per
170 colony (to the nearest quarter of a frame).

171 *Measuring Bumble Bee Colony Performance*

172 Bumble bee colony performance was determined by quantifying several measures of
173 productivity per colony: i) number and caste of adult bees present: workers, males or queens;
174 ii) number of sealed and uncapped cocoons, sorted as worker or male (small cocoons) versus
175 queen (large cocoons); iii) nest weight change; and iv) final nest volume.

176 The number of adult worker, male and queen bees in each colony was determined by first
177 separating the females from the males by examining the hind legs for corbiculae (pollen
178 baskets), which are found in both queens and workers but not males. The females were then
179 differentiated by size, with the queens being the conspicuously larger of the two castes[28].

180 The number of sealed (i.e. containing a developing bee) and uncapped cocoons per colony
181 was determined by grouping together worker and male cocoons and separating them from the

larger queen cocoons. This was achieved by measuring the width of 100 cocoons per six colonies (one from each study locations) to determine a cut-off size (i.e. the lowest value between the two peaks) for queens and workers/males[12]. Separating male cocoons from those of workers was not possible due to their overlapping sizes.

To measure colony weight change the plastic membrane with the nest inside it and the internal feeder of each hive were weighed separately on a portable balance (model 1066, Salter Ltd., Tonbridge, sensitivity 1g) on the day of colony delivery (4 April 2014) and again on the day the colony was collected from the field (16 or 30 May). Nest volume was determined by filling the empty space in each of the 8.16 litre plastic hive membranes with small polystyrene packing chips. The chips were then weighed to the nearest mg using a analytical balance (TE-64, Sartorius), which allowed the calculation of chip volume, and from this the volume of the nest.

Pollen Analysis

We determined the proportion of honey bee foraging on oilseed rape pollen by analysing two random samples of pollen pellets, per colony, collected during the early (12 April 2014) and mid bloom periods (23 April 2014). 100 pollen pellets (equal to c. 1g) from each colony from each date were initially sorted by colour and then weighed. Pellets that were within the oilseed rape pollen colour spectrum (bright yellow to light green[31]) were subsequently examined at x 600 magnification using a compound transmission light microscope (model N-117N, Brunel Microscopes Ltd., Chippenham). Tens of pollen grains per sample were confirmed as oilseed rape by size, shape and surface texture in comparison to voucher specimens collected directly from oilseed rape flower anthers in April 2014. We also identified a small number (5-10 per sample) of pollen loads from alternative forage resources during the oilseed rape bloom.

To determine the proportion of bumble bee pollen foraging on oilseed rape we examined 100 pollen shells (exines) in three faecal samples per colony (n = 300 exines per colony). This is possible because the hard exine layer of a pollen grain passes through the digestive system of bumble bees without bursting[32].Three samples from three sub-locations were taken from the faecal pile within the nest of each colony. Individual samples were placed on a microscope slide and distilled water was slowly added via a dropper. The sample was then squeezed and the faeces removed. The remaining liquid contained thousands of pollen exines. Samples were then mounted and examined microscopically (see above for details). We counted the proportion of oilseed rape exines present in a transect of 100 visible exines. Transects began approximately in the centre of the microscope slide and the direction taken alternated between the four directions provided by the controls on the microscope slide stage (i.e. up, down, left, right).

Chemical Analysis

To determine neonicotinoid concentrations of the honey stored by our honey bee colonies during the oilseed rape bloom period we collected honey samples from all 72 colonies near the end of the oilseed rape bloom (15 May 2014). Freshly sealed honey was collected from multiple previous empty frames and locations within each colony to provide a representative sample from the oilseed rape bloom period. To assess whether neonicotinoids were still present during the following winter we collected additional honey samples from sealed cells within all surviving colonies on 10 April 2015.

Honey bee pollen samples were collected for two 24h periods from all colonies during early (12 April 2014) and full bloom (23 April 2014) stages using pollen traps (Fairweather pollen trap; E.H. Thorne, Market Ransen, UK). To monitor the neonicotinoids present in the wider environment we also collected pollen samples from all colonies after the oilseed rape bloom

had finished (12 June 2014). All honey and pollen samples were stored at -20 °C prior to analysis.

During August 2014 and April 2015 we prepared 30 pooled honey bee samples, five per spring study location, for chemical analysis. Each sample consisted of a homogenised composite of 5g of honey or 2g pollen from each colony for each of the six spring locations. The 12 honey samples were: (i) six from material collected 15 May 2014 and (ii) six from 10 April 2015. The 18 pollen samples were: (i) six from pollen from pollen traps collected 12 April 2014, (ii) six from 23 April 2014 and (iii) six from 12 June 2014.

To determine the neonicotinoid residues present in the honey and pollen stored by our bumble bee colonies, one composite sample of honey and another of pollen was prepared for chemical analysis for each of the 6 sites. Initially, all the honey and pollen pots within each colony were collected. All colonies had at least 2 g of stored honey and all but six had 2 g of stored pollen (two from site D2, and one each from sites A1, A3, D1 and D3). All the available material from each colony was then homogenised into twelve composite samples (mean weight 42.3 g), one honey and one pollen per site.

Samples were analysed for neonicotinoid concentrations (thiamethoxam and its metabolite clothianidin) by SAL (Scientific Analysis Laboratory Ltd., Cambridge), an accredited (UK Accreditation Service) contract analytical laboratory that routinely analyses plant and food materials for the farming and food industries. SAL's extraction method is based on the QuEChERS extraction technique which uses water and acidified acetonitrile as an extraction solvent[33]. Magnesium sulphate and ammonium acetate (as a buffer) were added to induce solvent partitioning. Quantitation was assessed against a series of known calibration standards dissolved in a methanol:water solution. Deuterated clothianidin (Clothianidin-d3) was used as an internal standard pre-extraction, to correct for losses during extraction and to compensate for matrix effects (suppression or enhancement) during analysis. The limit of quantification

(LOQ) and detection (LOD) were 0.1 µg per kg for both thiamethoxam and clothianidin and for both pollen and honey.

Statistical Analysis

Statistical analyses were conducted using 'R' software (version 3.1.1[34]). We used Linear Mixed-Effect Model analysis (LMER, 'R' package lme4, version 1.1-7[35]) when the data met the assumptions of a normal distribution (i.e. z-scores for skew and kurtosis were between -1.96 and 1.96). When the data were positively skewed we used a Generalized Linear Mixed-Effect Model analysis (GLMER; 'R' command 'glmer') with a Poisson error structure. Spring 'location' was held as a random effect in LMER analysis of honey bee colony performance data. Hive 'location' and the quantity of 'syrup' consumed was held as a random effects in (G)LMER analysis of bumble bee colony performance data. (G)LMER analysis were simplified using backwards elimination of non-significant variables and model comparison using ANOVA. The maximum likelihood method was used to estimate p-values. As is the convention in most biological research we have used a significance a level of $p<0.05$.

To test the significance of our random effects (Table S1 and S2) we compared lme ('R' package version nlme, version 3.1-117[36]) models (including the random effect), with gls models (no random effect) with the same fixed effects structure[37]. These two models were then compared using a likelihood ratio test via ANOVA.

We also used Regression analysis (LM, 'R' function: lm) and one-way ANOVA ('R' function: aov). Homogeneity in variance between groups was tested using the Levene Test. Proportion data were logit transformed prior to analysis. All values are presented as mean ± 1 standard error. All R^2 values present are 'adjusted'.

To determine the overall level of neonicotinoid contamination per honey bee spring study location we calculated two weighted averages of the neonicotinoid residues present in the two

pollen (p) samples collected during the oilseed rape bloom (12 and 23 April 2014) and honey (h) samples (collected 15 May 2014). The first (Equation 1) was simply the average of the two: (p+h)/2. The second (Equation 2) allowed for the fact that honey bee colonies gather c. 6 times more nectar than pollen[38]: (p+6h)/7. In our statistical analysis we used both equations and they yielded approximately the same results (Fig. 2). Choice of equation made little difference to our statistical analyses due to the high correlation between the neonicotinoid contamination found in the pollen and honey samples per spring study location ($R^2 = 0.85$, df = 5, F = 20.38, $p = 0.011$). Samples with no detectable neonicotinoid contamination (<0.1 µg/kg) were given a zero value in analysis. Fig. 1was generated using ArcMap (ArcGIS Desktop 10.2, ESRI, USA).

Results and Discussion

Our key finding was that there was no significant effect on the survival of either bumble bee or honey bee colonies, despite the honey bee colonies being monitored for a full year after exposure to neonicotinoid treated oilseed rape. As such, our results, and those of all other field studies[11,12,13,15,17] strongly suggest that honey bee colonies are not severely harmed by chronic, low-level neonicotinoid exposure. However, this overall result masks a number of more subtle findings.

Firstly, we noted that in the adjacent study locations less than half of the pollen collected (*Apis*: 49 %; *Bombus*: 41 %) was oilseed rape (Table 1), despite immediate proximity to large fields of this crop during bloom. Colonies in the three distant locations collected significantly less oilseed pollen than adjacent colonies for both species (*Apis*: 9%; ANOVA, $F_{1,5} = 14.1$, $p = 0.020$; *Bombus:* 2%; $F_{1,5} = 97.5$, $p<0.001$). The greater use of oilseed rape pollen by honey bee versus bumble bee colonies is expected given their greater foraging range. Pollen analysis also indicated that the predominant alternative forage across all six study sites were *Prunus spinosa* (~15%) and *Salix* spp. (~15%). Other less common (<5% across study sites) species

304 included: *Allium ursinum* (site D3), *Crocus* spp. (A1), *Endymion non-scriptus* (A1, D1, D2)
305 *Taraxacum officinale* (ubiquitous), *Malus domestica* (A2), *Pyrus communis* (A1), and *Vicia*
306 *faba* (A3, D2).

307 Secondly, the mean neonicotinoid residues in the honey and pollen in our colonies during
308 oilseed rape bloom were at the low end of the range reported previously[11, 13, 14,15]. The average
309 residues (thiamethoxam + clothianidin) of the stored honey and pollen sampled from adjacent
310 colonies were low (*Apis*: 0.76 ppb; *Bombus:* 0.26 ppb) and was below the detection levels
311 (<0.1 ppb) in the majority of samples collected from distant locations (*Apis*: 0.21 ppb;
312 *Bombus*: <0.1 ppb). Overall, average residues were marginally greater at adjacent versus
313 distant locations for both species (ANOVA; *Apis*: $F_{1,5} = 8.1$, $p = 0.048$; *Bombus:* $F_{1,5} = 9.2$, p
314 = 0.039). This significance level was likely reduced due to the relatively high levels of oilseed
315 rape foraging at site D1. Furthermore, it should be noted that our composite analysis (i.e.
316 consisting of samples from 12 colonies) may partially mask inter-site and between-site
317 variation.

318 It is noteworthy that these concentrations are more than 15 times lower than those reported by
319 Rundlöf et al.[12] (*Apis*: 12.4 ppb, *Bombus*: 5.4 ppb), which found negative impacts on bumble
320 bee colonies, but not honey bee colonies. There are a number of reasons for this difference.
321 Firstly, Rundlöf et al.[12] analysed nectar and pollen sampled directly from foraging bees in
322 their study fields. By contrast, we analysed stored pollen and honey, which was mostly
323 collected from flower species other than oilseed rape (i.e. <50% oilseed rape identified in
324 pollen samples), which is equivalent to a 2-3 fold dilution. However, neither of these factors
325 would appear to be sufficient to account for the great difference in residue levels between the
326 two studies. We hypothesize that this is because of the contrasting ways that the oilseed rape
327 was grown. The Swedish fields of oilseed rape were planted in spring and bloomed in summer
328 ("spring-sown"), while we studied crops planted in late summer/autumn and blooming in

329 spring ("winter-sown"). Because spring-sown rape has a shorter growth period, mature plants
330 generally have a smaller mass during flowering than spring-blooming rape[39]. Previous
331 research shows that smaller seed-treated plants have higher neonicotinoid residues[40].
332 Furthermore, due to their water solubility, neonicotinoids are readily leached from seed-
333 dressings, leaving a variable amount, ranging from 2-20%, of the active ingredient to be
334 absorbed by the plant's root system[41]. Winter is the time of *'maximum transport of*
335 *agrochemical pollutants to watercourses*' (November to April in Britain) [42]. As a result, it is
336 likely that a relatively high proportion of neonicotinoid dressing are leached from the crop
337 root zone. Again this may result in lower residues being found in the pollen and nectar of
338 winter-sown oilseed rape. Because the majority of European Union oilseed rape is winter-
339 sown (94%[43]), this hypothesis warrants further research.

340 Thirdly, we found no significant differences in the weight gain or nest volume between
341 adjacent and distant bumble colonies, see Fig 2. The only differences were that adjacent
342 colonies had greater numbers of adult male (61%; LMER, χ^2 (1) = 3.9, p = 0.049) and worker
343 (111%;, LMER, χ^2 (1) = 9.7, p = 0.002) bees. However, the overall reproduction of the two
344 groups of colonies was not significantly different (i.e. number of queen and male/worker
345 cocoons were similar). This also contrasts with the results of two recent field studies[12,17],
346 which found a reduction in the number of queen cocoons. We hypothesize that this disparity
347 reflects both the differences in agronomic practices (discussed previously) and experimental
348 design.

349 Our experimental design investigated the combined effect of the benefits (additional floral
350 resources) and costs (insecticide exposure) of proximity to oilseed rape on bee colony
351 performance, versus being located at a distance (neither cost nor benefit). This contrasts with
352 the alternative design used by most previous field studies[10, 11,13,15,16,17], which compared the
353 cost and benefit of seed-treated oilseed rape (nearby treated crop fields) versus the benefit

354 alone (nearby insecticide-free crop fields). Therefore, the contrasting results of this study (and
355 similar studies[13,15]) and those which used insecticide-free oilseed rape as a control, suggest
356 that the sub-lethal effects of neonicotinoid seed-dressings may be offset or even balanced by
357 the additional foraging resources provided by the treated crop. However, further work is
358 required to test this hypothesis.

359 Fourthly, over one year honey bee colonies adjacent to oilseed rape during the two-month
360 bloom gained 24% less weight than the distant colonies. During the first month of bloom
361 (April 2014) colonies adjacent to oilseed rape gained significantly more weight than did the
362 distant colonies (LMER, χ^2 (1) = 13.68, p <0.001). This indicates that easy access to this mass
363 flowering crop provided a short-term increase in honey production, detected as colony weight
364 gain. However, this benefit was not seen during the second month of bloom and was actually
365 reversed (LMER, χ^2 (1) = 4.72, p = 0.030). Furthermore, this pattern was repeated in the
366 following month (June), during which all colonies were under equal foraging conditions in six
367 common apiaries (LMER, χ^2 (1) = 9.63, p = 0.002). However, it is noteworthy that during
368 these months there was considerable variation of the performance of colonies within each
369 treatment (i.e. Adjacent and Distant), Figure S1, Supplementary Materials. From July-
370 November 2014 no significant differences were recorded in terms of colony weight change
371 between treatments.

372 Furthermore, mean neonicotinoid concentrations in honey and pollen collected during spring
373 2014 were negatively correlated with cumulative hive weight gain per spring study location
374 (Fig. 3a, LM, R^2 = 0. 72, df = 5, F= 13.67, p = 0.021). This indicates, in line with laboratory-
375 led data, that neonicotinoid exposure may indeed impair honey bee orientation and/or
376 foraging ability[2,8]. Interestingly, this data also suggests that there is no negative effect of
377 neonicotinoid exposure on honey bee colonies below a threshold of 0.5 ppb (Fig. 3a and 3b).
378 Importantly, however, weight gain across the year was not related to the proportion of pollen

collected from oilseed rape (LM, $R^2 = 0.09$, df = 5, F= 1.52, $p = 0.285$), see Fig. 3b. These contrasting results indicate that ready access to this mass flowering crop is not in itself detrimental to colony performance. This would have been a very unlikely result given that proximity to oilseed rape is known to augment the abundance of several bee species[18,19]. One reason for this seeming inconsistency in the results is that oilseed rape pollen collection was not perfectly correlated with the neonicotinoid levels in our honey ($R^2 = 0.68$) and pollen samples ($R^2 = 0.60$). This may be a because mean neonicotinoid residues in the oilseed rape plants in one of our study fields (that next to spring study location A2) was 80% lower than those recorded at the other two fields[39]. However, due to the design of our experiment it is difficult to separate effects of neonicotinoid exposure from the possible effects of exposure to oilseed rape [44]. An alternative explanation for this disparity is that honey bee colonies may have been exploiting alternative forage sources for nectar versus pollen.

Fifth, we found that during the ensuing winter honey bee colonies that had previous been adjacent to oilseed rape the previous spring had slightly fewer frames of brood (LMER, December 2014: χ^2 (1) =, p <0.001, February: df = 1, $\chi^2 = 3.85$, $p = 0.049$ and March 2015: χ^2 (1) = 4.03, $p = 0.045$) than those previously distant (Fig. 2b). Analysis of winter honey stores (samples taken on 10 April 2015) detected neonicotinoid residues (0.1 ppb) in colonies from two of the three previously adjacent spring study locations (A2 and A3). These two locations had the highest contamination levels in spring 2014 (Table 1). One obvious interpretation of these data is that reduced winter brood rearing occurred because these colonies were consuming contaminated oilseed honey stored during the previous spring. However, our samples size (i.e. number of colonies) was low at this stage, which may have compromised our results.

This 'next winter' effect, however, was short lived and the lower brood rearing of colonies from the previously adjacent study sites during February and March 2015 appear to be a

consequence of reduced number of brood frames at the turn of the year. The evidence for this is that colony growth (the proportional increase in the number of frames of brood per colony) was similar for both groups of colonies between January-February (LMER, χ^2 (1) = 0.09, p = 0.755) and February-March (LMER, χ^2 (1) = 3.06, p = 0.080). In addition, the previously distant colonies lost more weight than previously adjacent colonies during February 2015 (χ^2 (1) = 6.37, p =0.012). This was presumably a consequence of the greater brood rearing in the colonies from the distant spring study sites, which will have used up more of a colony's winter food supplies.

This winter effect, however, was small and could be avoided if beekeepers managed their hives to ensure that honey from neonicotinoid-treated crops was not used for winter stores. This is one practical recommendation arising from the present study. However, the presence of neonicotinoid residues in winter stores would be unavoidable in non-managed or wild honey bee colonies.

One general recommendation from our study is the need for greater understanding of the wide variation in neonicotinoid residue levels recorded in the pollen and nectar of seed-treated crops and nearby wildflowers[45]. This would enable more sophisticated and nuanced policies relating to the use of neonicotinoids, focusing on restricting their use in connection with agronomic practices that result in high residues in nectar and pollen. Our results suggest that one such source of variation in contamination levels is the timing of oilseed rape sowing (i.e. spring versus late summer planting). In addition, the temporary lifting of the EU moratorium in certain parts of the United Kingdom[46] highlights the fact that pest insect problems may also vary regionally[47]. Consideration of these factors is crucial to minimise harm to bees and other non-target organisms while still allowing farmers to produce oilseed rape without undue crop damage by insect herbivores or greater reliance on alternative insecticides. Indeed, in the year following the EC moratorium the volume of insecticide (mostly pyrethroids) sprayed onto

English oilseed rape fields increased 2.5-fold[48]. This was despite a concomitant decrease in the total area of this crop. Unfortunately, alternative insecticides applied to oilseed rape are often sprayed during flowering, April-May (37%[49]), and little is known of the potential impact on bees or, indeed, other pollinator groups.

The principal rationale behind the EU moratorium on the use of neonicotinoid seed dressings was the concern that these agents might be exacerbating bee population declines. However, inconsistencies in field data to date make it difficult to determine whether neonicotinoids are indeed a key factor in ongoing bee declines, and highlight the difficulties faced by policy makers. Our study indicates no effect on honey bee and bumble bee colony reproduction and survival. The data, therefore, do not support the hypothesis that population declines of honey bees and bumble bees are due to neonicotinoid seed-treatment of winter-sown oilseed rape.

Ultimately, there are many causes responsible for the declining populations of bees and pollinating insect species and many of them are likely linked to modern agricultural practices[20,50]. Notably, the general decline in the United Kingdom farmland biodiversity[49], including that of bees and wasps[50], predates the widespread use of neonicotinoids (in use since 1991). As such, the current debate regarding neonicotinoids should not divert the attention of policy makers, researchers, interest groups and the media from the wider picture.

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455 **Associated Content**

456 Supporting Information. Tables and figures as noted in the text.

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586 **Table 1** Area of oilseed rape in flower within 0.5 and 2 km of each of the Adjacent (A1-A3) and
587 Distant (D1-D3) spring study locations. Mean percentage of bumble bee (*Bombus terrestris audax*)
588 faecal oilseed rape pollen exines identified (n = 300 per colony) and honey bee (*Apis mellifera*)
589 collected oilseed rape pollen pellets. Neonicotinoid contamination (thiamethoxam + clothianidin) of *B.*
590 *terrestris* and *A. mellifera* stored pollen and honey per study site.

		Oilseed rape pollen collection, mean ± s.e.m (%)		**Pollen residues, thiamethoxam + clothianidin, (µg/kg)**		**Honey residues, thiamethoxam + clothianidin, (µg/kg)**		
Spring Study Location	Oilseed rape (km^2) within 0.5 & 2km	*Bombus terrestris*	*Apis mellifera*	*Bombus terrestris*	*Apis mellifera,* early & mid bloom	*Bombus terrestris*	*Apis mellifera*, bloom period	*Apis mellifera*, ensuing winter
A1	0.30 0.60	40.5 ± 4.8	40.5 ± 2.4	0.49	1.05 0.63	0.23	1.51	0.1
A2	0.28 0.55	41.3 ± 3.6	53.6 ± 3.4	0.18	0.47 0.29	<0.1	0.79	<0.1
A3	0.33 0.91	41.9 ± 4.9	51.7 ± 2.7	0.42	0.64 0.29	0.25	1.18	0.1
D1	0.0 0.59	2.97 ± 1.0	21.6 ± 2.4	<0.1	0.39 0.15	<0.1	0.70	<0.1
D2	0.0 0.0	1.0 ± 0.3	8.2 ± 1.4	<0.1	<0.1 <0.1	<0.1	0.46	<0.1
D3	0.0 0.0	0.9 ± 0.2	0.2 ± 0.0	<0.1	0.16 <0.1	<0.1	<0.1	<0.1

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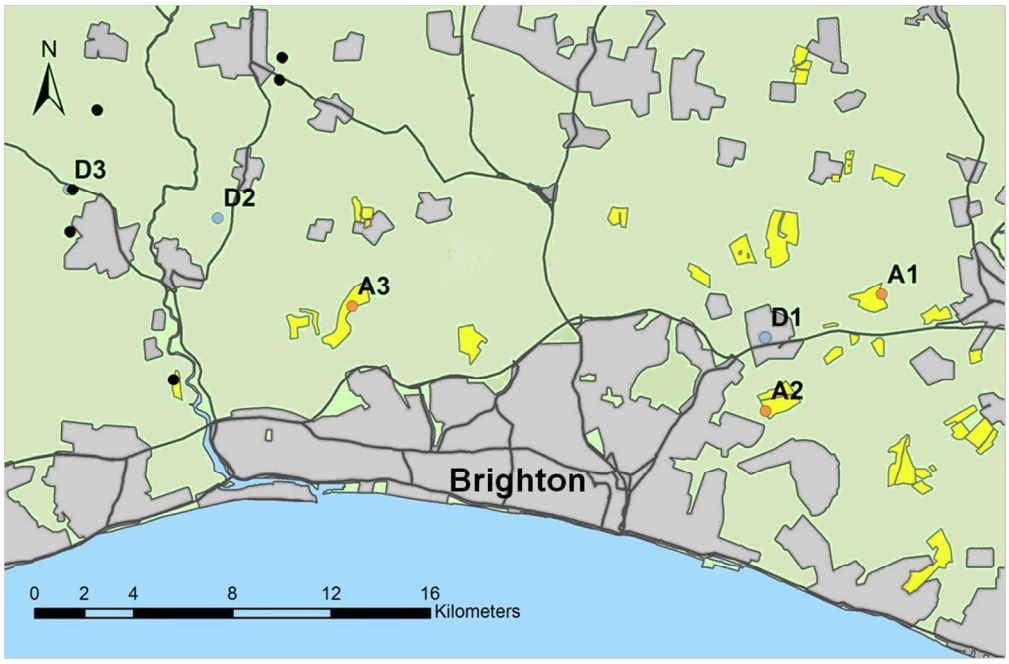

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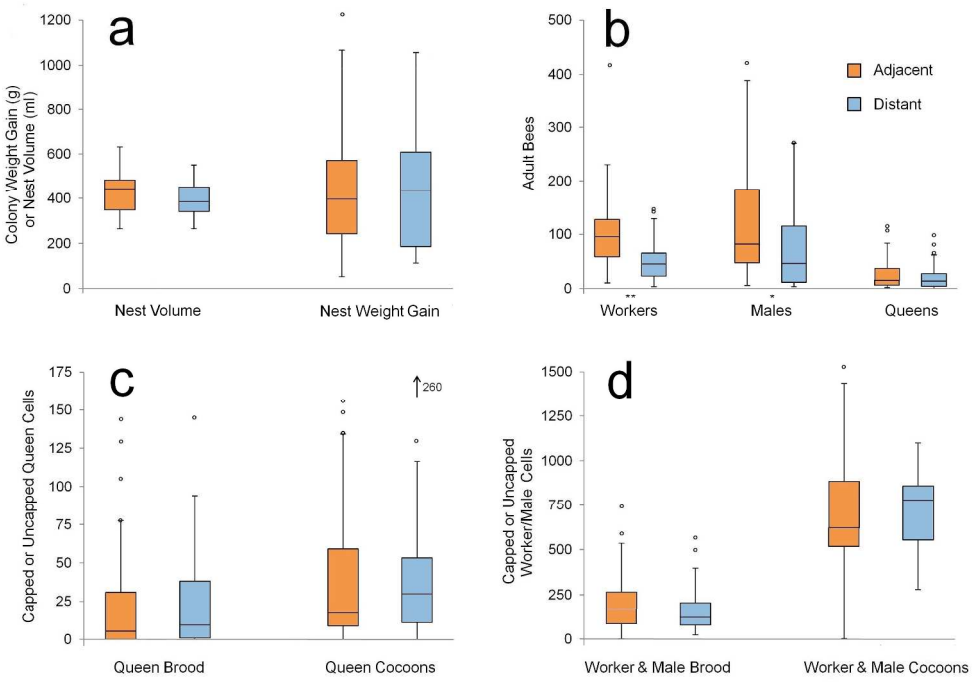

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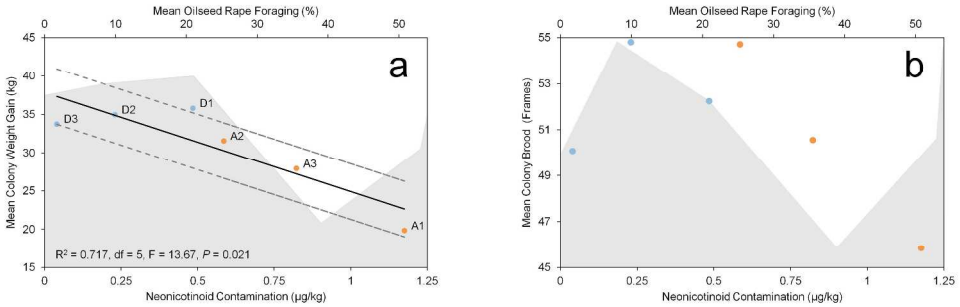

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