

Can Costs of Pesticide Exposure for Bumblebees Be Balanced by Benefits from a Mass-Flowering Crop?

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Supporting Information

ABSTRACT: Mass-flowering crops provide forage for bees but also contain pesticides. Such pesticide exposure can harm bees, but our understanding of how this cost is balanced by forage benefits is limited. To provide insights into benefits and costs, we placed bumblebee colonies in 18 landscapes with conventional red clover fields treated with the neonicotinoid thiacloprid (flowers + pesticide), untreated organic red clover fields (flowers), or landscapes lacking clover fields (controls). Colonies grew heavier near thiacloprid-treated clover compared to controls lacking clover, while colonies near untreated clover did not differ from colonies in neither of the other landscape types. Thiacloprid treatment effectively controlled pests and increased bumblebee crop visitation. However, colony production of queens and males did not differ among landscape types. In conclusion, thiacloprid application in clover appears to be of low risk for bumblebees. More generally, neonicotinoids may not be equally harmful when used in flowering crops and effective low-risk pest control in such crops could potentially benefit bumblebees and crop pollination.



INTRODUCTION

Bees (Hymenoptera: Anthophila) are affected by numerous drivers in agricultural landscapes.¹ A key limiting factor for bee population sizes is lack of flowers,² but mass-flowering crops (MFCs) can provide abundant resources that mitigate this.^{3,4} The pollen and nectar resources provided by MFCs are, however, often contaminated with pesticides^{5,6} which can act as another driver by negatively affecting bee survival and reproduction.^{7–10} Evaluations of how pesticide-treated MFCs affect bees under realistic field settings have most often assessed bee performance at treated fields compared to nontreated control fields of the same crop.^{11–13} A critique of this study design is that nontreated MFCs are an unrealistic alternative and that any bans on pesticide use are likely to increase the cultivation of less pest-sensitive nonflowering crops, such as cereals, which therefore could represent alternative controls.^{13,14} There is, however, a large uncertainty in how cropping patterns would change after a pesticide ban,^{15,7} which calls for research into multiple scenarios. Furthermore, including only the treated MFC and landscapes lacking both the MFC and the pesticide confounds the potential benefits of the forage resources and the potential costs of the pesticide exposure. A more complete design that can separate the benefits and costs would include landscapes with pesticide-treated and untreated MFCs as well as landscapes lacking the MFC.

Among pesticides potentially affecting bees negatively, particular attention has been given to neonicotinoid insecticides.^{7,9,16–18} Neonicotinoids are a widely used and effective class of insecticides, applied as foliar spray, seed

treatment, through irrigation, and via soil application against insect pests from a diversity of orders and in a range of crops.¹⁹ Neonicotinoids are frequently used in MFCs such as oilseeds,^{7,8} fruit, vegetables, and berries,^{19,20} as well as in seed production;²¹ crops that often benefit in yield from insect pollination.²² Outdoor use of the three neonicotinoids, clothianidin, imidacloprid, and thiamethoxam, was, however, recently banned in the European Union, due to risks for bees.²³ Two other neonicotinoids, acetamiprid and thiacloprid, remain in use both in the European Union and globally.²⁴ These are generally less bee-toxic^{25–27} and degrade more quickly in the environment²⁸ than the three banned compounds. The lower-risk properties may, however, be counteracted by more liberal criteria for usage, for example, as spray treatments in flowering crops.¹¹ Little is, however, known about how spray applications of acetamiprid or thiacloprid in MFCs affect bees (but see Ellis et al.²⁰ and Havstad et al.²⁹).

Bumblebees (Apidae: Bombus) are important pollinators that frequently visit MFCs.³⁰ They are eusocial, producing workers during much of the season and then switch to production of new queens and males at the end of the season.³¹ The ability of the colony to produce new queens depends on the availability of floral resources in the surrounding landscape.³² Pesticide use in MFCs can interfere

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with the ability of the colony to benefit from floral resources through lethal³³ or sublethal effects, e.g., impaired foraging ability^{34,35} on its worker force, which can set back colony growth and reproduction.^{7,18}

In this study, we quantified the costs and benefits of pesticide use in an MFC for bumblebees. Our focal MFC was red clover grown for seed, where bumblebees are important pollinators^{36,37} and thiacloprid is used to control seed-eating weevils.²¹ We compared the performance in terms of growth and reproduction of 54 *Bombus terrestris* L. colonies in six matched landscape triplets. Each triplet contained a landscape with a thiacloprid-treated, conventionally managed MFC (flowers + pesticide), a landscape with an untreated, organically managed MFC (flowers) and a landscape without the MFC (control). Landscapes were typical for southern Swedish intensively farmed areas, with agricultural land dominated by annual crops such as cereals. We verified the bees' use of the crop by quantifying the proportion of red clover pollen brought back to the colonies and by comparing bumblebee visitation to crop flowers in thiacloprid-treated and untreated plots in 19 red clover seed fields over 2 years in a separate field experiment. We also assessed bumblebee exposure to the pesticide by quantifying thiacloprid in bee-collected pollen following thiacloprid application.

MATERIALS AND METHODS

The study was conducted over 3 years. In 2011 and 2013, we used a split plot setup within fields, which was powerful for detecting effects of the thiacloprid application on bumblebee flower visitation, but this smaller scale setup was not suitable for evaluating effects on bumblebee colony performance. For this, we used a whole field-scale study setup across replicated landscapes in 2015.

Bumblebee Colony Performance. We placed three commercially reared *B. terrestris* colonies (Natupol N, Koppert Biological Systems) in the center of each of 18 landscapes ($r = 2$ km, containing the majority of expected foraging by the species^{38,39}), divided into three landscape types (organic clover, conventional clover, control without clover), in 2015 (Figure 1). Six of the landscapes were centered on organically managed red clover seed fields, where no pesticides were used. These were matched with another six landscapes centered on conventionally managed red clover seed fields treated with Biscaya (Bayer; 240 g L⁻¹ thiacloprid) before or during early crop bloom (Table S2). The matching was based on clover cultivar and type (diploid or tetraploid and early or late blooming), size of the clover field, geographical proximity, and land use (based on digital land use information from the Integrated Administration and Control System and Swedish national land cover data) within 2 km (see Supporting Methods). The final six landscapes lacked clover fields within 2 km and were matched with the clover landscapes based on geographical proximity and landscape land use (same as above), to form six triplets of landscapes (Figure 1). Further details about the study design are available in Supporting Methods.

The colonies were randomly assigned to landscapes and placed in the field 17–18 June 2015. Thiacloprid was applied by the farmers as part of their regular field management 8–30 days after colonies had been placed in the field, which corresponds to the early part of the colony development at the field sites (Tables S1 and S2). All colonies had a natal queen, and neither colony weight nor the number of workers differed

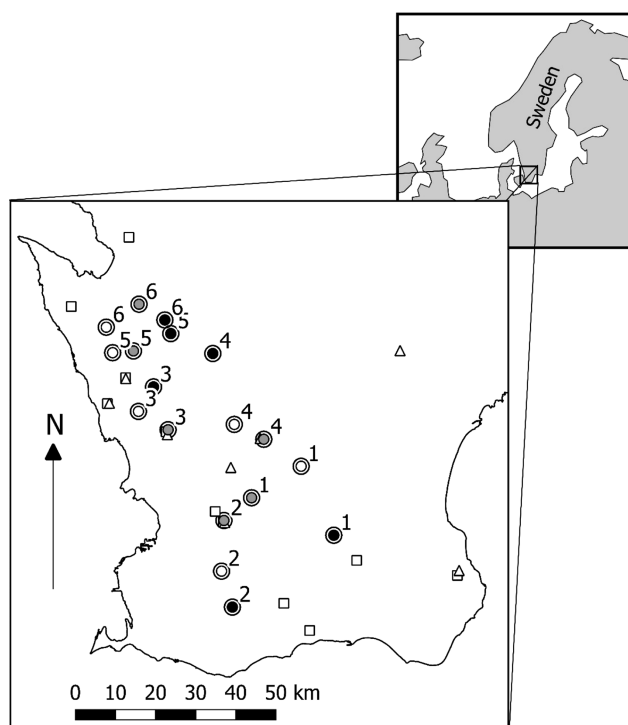


Figure 1. Location of the studied clover fields and landscapes in southern Sweden. White circles, control landscapes without clover; gray circles, thiacloprid-treated conventional clover; black circles, untreated organic clover for the 2015 study. Larger circles, 2 km radius landscapes. The numbers indicate triplets of matched landscapes 2015. Squares, clover fields 2011; triangles, clover fields 2013. Maps were created in QGIS 3.6.0 based on shape files from gadm.org and naturalearthdata.com.

between the three landscape types when the colonies were placed in the field (Table S3). Colonies were housed in a ventilated wooden box in the shade and did not receive any supplementary feeding after field placement. The exit hole was fitted with a queen excluder that allowed workers and males but not queens to enter and exit, based on the queen's larger body size, to allow for complete assessment of queen production. Colony growth was assessed by weighing the colonies (as bumblebee colony weight is highly correlated with worker force and brood production,^{3,40} but less invasive to measure) during three consecutive survey rounds: 3–13 July, 16–27 July, and 29 July–6 August. The colonies were collected and terminated by freezing after the third weighing round. All nine colonies within a landscape triplet were weighed and collected on the same day. To estimate reproductive output, colonies were dissected to count the number of new queens, males, large queen cocoons (width, >12 mm⁷), and small worker/male cocoons. Worker and male cocoons cannot be separated without opening the cocoons, which we did not have resources to do. Instead, to estimate pupal identity, we assumed that the colony had switched from producing workers to reproductives if there were new queens and/or males present in the colony and thus that the small cocoons contained males.⁴¹ Number of adults and cocoons were grouped by caste to represent the colony reproductive output of new queens and males.

Bumblebee Flower Use. Bumblebee use of red clover was determined by (1) the proportion of red clover pollen brought back to the colonies 2015 and (2) bumblebee visitation to crop

flowers in areas of red clover fields with and without thiacloprid application 2011 and 2013.

Pollen loads were collected from bumblebees returning to the colonies during the second survey round (16–27 July 2015), corresponding to the peak bloom period of the red clover fields. Bees were caught using a hand net and transferred to a holding tube, where pollen loads were removed into individual tubes before the bee was released. Pollen loads from 10 bees were collected in each landscape, with the exception of four landscapes where only 9, 9, 1, and 0 bees with pollen could be sampled within the up to 1 h sampling period. Samples were kept in a cooler throughout the sampling day and brought back to the lab to be frozen (−20 °C). Of the 159 samples, nine contained pollen of two colors and two contained pollen of three colors, indicating pollen from multiple plant species, and the proportion of the sample belonging to each color was visually estimated. A small amount of pollen from each sample and color was melted together with a cube of fuchsin-colored gel on a microscope slide and left to solidify. A minimum of 100 pollen grains per slide were determined to either red clover or other plant species under a microscope (25–60× magnification), using a pollen reference library at the Department of Biology, Lund University, collected in the study region.

Bumblebee flower visitation was surveyed in thiacloprid-treated and untreated plots in 19 conventionally managed red clover fields in a separate field experiment; 11 fields in 2011 and another 8 fields in 2013 (Figure 1). Each field had two adjacent 50 m long and 16–24 m wide plots (corresponding to the width of the farmer's spray equipment) along a field edge. One plot was excluded from insecticide treatment, whereas the other was treated with Biscaya (Bayer; 240 g L^{−1} thiacloprid, Table S2) in bud or early flowering stage of the crop. In 12 fields, insecticide-treated plots also received one pyrethroid application (Table S2). To quantify how insecticide treatment affected the main insect pests, which are *Protapion* spp. weevils,²¹ we quantified pest densities in treated and untreated plots (see Supporting Methods).

Bumblebees were surveyed two to four times in each field during crop bloom July 7–August 3 2011 and June 24–July 24 2013, with surveys at least 4 days after thiacloprid application. Weather was warm, dry, and calm (temperature at least 16 °C, wind not higher than 4 Beaufort) during surveys. The observer walked slowly along a 50 m transect in the center of each plot and aimed to net all bumblebees (except queens) visiting flowers in a 1 m wide area. Queens and individuals that were not caught were counted and field-identified to species. Collected specimens were identified to species in the laboratory following Edwards and Jenner,⁴² Loken,⁴³ and Prys-Jones and Corbet.⁴⁴ *B. terrestris*, *Bombus lucorum* L., *Bombus magnus* Vogt and *Bombus cryptarum* Fabricius were summarized as *B. terrestris* agg. because they are difficult to separate morphologically.⁴⁵ We determined crop flower density each time bumblebees were surveyed by counting the number of red clover inflorescences with five or more open flowers in four 0.25 m² quadrats per transect.

Bumblebee Pesticide Exposure. Bumblebee pesticide exposure was determined by quantifying thiacloprid residues in (1) pollen brought back to the colonies during red clover bloom 2015 and (2) pollen collected by bumblebee foragers in red clover fields 1, 2, 4, and 16 days following Biscaya application 2013.

Pollen brought back to the colonies during red clover bloom (16–27 July 2015, 6–19 days following thiacloprid application, Table S2) was collected as described previously. Pollen from each field was pooled and analyzed for thiacloprid at the Laboratory for Organic Environmental Chemistry and Ecotoxicology at the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences in Uppsala, Sweden. Samples were weighed (mean (range): 0.19 g (0.0076–0.42 g)), homogenized, extracted, cleaned, and dried before dissolved in acetonitrile and analyzed using liquid chromatography with tandem mass spectrometry. Apart from thiacloprid, samples were simultaneously assessed for residues of four other neonicotinoids (Table 1). Limits of detection (LODs) and limits of quantification (LOQs) based on spiking experiments are listed in Table 1.

Table 1. Mean and Range of Neonicotinoid Concentrations (ng g^{−1}) in Pollen Brought Back by Bumblebee Foragers to Colonies in Three Landscape Types and Proportion of That Pollen Originating from Red Clover

	landscape type			LOD ^a	LOQ ^b
	control without clover	thiacloprid-treated clover	untreated organic clover		
thiacloprid	0.05 (<LOD–0.15)	21 (0.42–56)	0.07 (<LOD–0.24)	0.02	0.1
acetamiprid	<LOD	0.01 (<LOD–0.07)	<LOD	0.05	0.2
clothianidin	<LOD	<LOD	<LOD	0.5	2
imidacloprid	<LOD	<LOD	<LOD	0.5	2
thiamethoxam	<LOD	<LOD	<LOD	0.1	0.5
red clover ^c	0.037 (0.0064–0.19)	0.57 (0.28–0.82)	0.57 (0.27–0.82)		

^aLimit of detection. Values below the LOD were assumed to be zero when calculating means. ^bLimit of quantification. ^cEstimates are means (±95% confidence limits) based on model-estimated least-square means.

In 2013, pollen loads were collected from five bumblebee workers foraging in the red clover that were captured using a hand net in each of 8 fields at 1, 2, 4, and 16 days after Biscaya application (Table S2). Samples are missing from one field for days 1–4 due to lack of communication with the grower and from day 4 at another field because sampling in another field was prioritized. Pollen from each field and day was pooled and frozen. These samples were analyzed in the same way as the pollen samples from the pollen brought back to the colonies, but only for thiacloprid. Sample weights were (mean (range)): 0.13 g (0.022–0.23 g). LOD was 0.070 ng g^{−1} and LOQ was 0.21 ng g^{−1}. To estimate the amount of red clover pollen in these samples, pollen loads were classified to color categories. Small amounts of pollen were removed from the first five samples of each color category and thereafter every 10th sample of each color category. Samples were prepared on slides as previously described. A total of 100 pollen grains per sample were counted and determined to either red clover or other plant species. We estimated the average proportion red clover pollen 1, 2, 4, and 16 days after spray application based on the relative amounts of pollen color categories and the proportion red clover pollen in the color category subsamples.

Table 2. Bumblebee Colony Performance in Relation to Landscape Proportion Agricultural Land, Landscape Type, and Survey Round

	colony weight change (g)		queen production		male production	
	F_{df}	P	F_{df}	P	F_{df}	P
proportion agricultural land	0.41 _{1,15}	0.53	0.24 _{1,12}	0.63	0.84 _{1,14}	0.38
landscape type ^a	1.22 _{2,14}	0.33	0.27 _{2,13}	0.77	0.80 _{2,14}	0.47
round	26.78 _{2,63}	<0.0010				
type × round	3.08 _{4,70}	0.021				

^aControl without clover, conventional clover treated with thiacloprid, organic untreated clover.

Statistical Analyses. We used general (LMM, Proc MIXED), generalized linear mixed models (GLMM, Proc GLIMMIX) or Kruskal–Wallis test (Proc NPARIWAY) in SAS 9.4 for Windows (SAS, Cary, NC) to analyze data. Degrees of freedom were estimated with the Kenward Roger method.⁴⁶

To determine if bumblebee colony growth was related to landscape type, we specified an LMM with colony weight change from field placement as the dependent variable and landscape type, survey round, and their interaction as explanatory variables. Proportion agricultural land was also included as an explanatory variable since it was lower for organic clover landscapes (see [Supporting Methods](#)). Colony weight changes were treated as repeated measures of individual colonies over the three survey rounds, with covariance structure set to AR(1), since this gave the lowest Akaike information criterion (corrected for small sample size, AICc). Landscape and triplet identities were included as random variables accounting for the study design. Heterogeneous variance among landscape types was modeled using landscape type as the grouping factor in the repeated statement (yielding lower AICc). Least-square mean estimates among landscape types over rounds were compared using the slice option because of a significant interaction between round and landscape type. Contrasts were used to explore the differences in weight change among landscape types for the round that indicated the largest difference. We also tested for differences in colony weight and worker number at field placement among landscape types using an LMM assuming normally distributed residuals and a GLMM with Poisson distribution and a log link, respectively, with landscape and triplet identities included as random variables.

To determine if production of new queens and males were affected by landscape type, we specified two GLMMs with the number of queens and males, respectively, per colony as the dependent variables, landscape type and proportion agricultural land as explanatory variables, and landscape and triplet identities as random variables. We assumed negative binomial error distribution and used a log link because the Poisson assumption indicated overdispersion.

To explore the influence of the clover field size, in addition to the other explanatory variables, we ran additional models for colony weight change and production of reproductives only including colonies in landscapes with clover (see [Supporting Methods](#)).

To determine if red clover pollen collection by bumblebee colonies was affected by landscape type, we specified a GLMM with the average proportion red clover pollen per landscape as the dependent variable, landscape type as the explanatory variable, and triplet identity as a random variable, with a binomial error distribution and logit link. Contrasts were used

to explore the differences in proportion clover pollen between landscape types.

To determine if bumblebee flower visitation was affected by thiacloprid application, we specified a GLMM with thiacloprid treatment, year, and clover inflorescence density as explanatory variables and field identity as a random variable. The numbers of bumblebees per transect were summed across the survey rounds prior to analyses. We assumed Poisson error distribution and log link and the ln-transformed number of survey rounds was included as an offset. Predictions for least-square mean differences between treatments on the modeled ln scale were multiplied by 100 and interpreted as symmetric percentage differences.⁴⁷ To determine if crop bloom was affected by thiacloprid application, we similarly specified a model with treatment and year as explanatory variables and field identity as a random variable. The number of flowering inflorescences per square were averaged across the survey rounds prior to analyses and we assumed normal error distribution. We also explore if the pyrethroid applications affected the results (see [Supporting Methods](#)).

To determine if thiacloprid residues in pollen brought back to colonies differed among landscape types, we used the Kruskal–Wallis test with concentration of thiacloprid as the dependent variable and landscape type as the explanatory variable. We followed up on overall significant results with pairwise multiple comparisons using the Dwass, Steel, Critchlow–Fligner method.

RESULTS

Colony weight change over survey rounds was explained by an interaction between landscape type and round ([Table 2](#)). Comparisons among rounds showed that the change in colony weights was largest between landscape types during the second survey round ($F_{2,17} = 3.08$, $P = 0.071$) and did not differ during the first ($F_{2,17} = 0.31$, $P = 0.74$) or third ($F_{2,17} = 1.00$, $P = 0.39$) rounds ([Figure 2](#)). Colonies in landscapes with thiacloprid-treated clover had gained more weight at the second round than colonies in landscapes lacking clover ($F_{1,18} = 5.79$, $P = 0.027$), while colonies in landscapes with untreated organic clover did not differ from colonies in landscapes lacking clover ($F_{1,15} = 2.30$, $P = 0.15$) or colonies in landscapes with thiacloprid-treated clover ($F_{1,20} = 0.38$, $P = 0.54$) ([Figure 2](#)).

The number of new queens or males at termination did not differ among landscape types ([Table 2](#) and [Figure 3](#)).

Bumblebee colonies differed in their collection of red clover pollen among landscape types ($F_{1,9} = 11.34$, $P = 0.0036$, [Table 1](#)). Comparisons between landscape types showed that colonies in landscapes with either thiacloprid-treated ($F_{1,9} = 22.21$, $P = 0.0011$) or untreated organic clover ($F_{1,9} = 20.56$, $P = 0.0014$) collected a larger proportion of red clover pollen

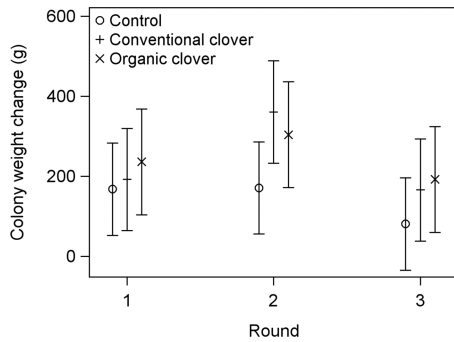


Figure 2. Mean ($\pm 95\%$ confidence limits, CL) weight change since field placement in relation to survey round and landscape type ($n = 6$ landscapes per type). Means and CL are based on model-estimated least-square means.

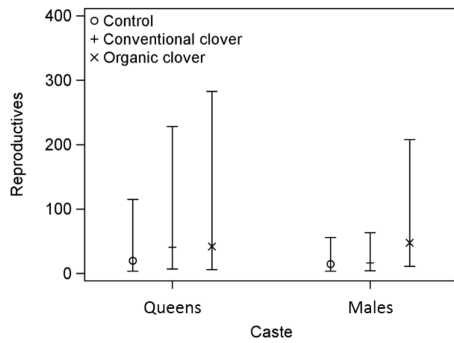


Figure 3. Mean ($\pm 95\%$ confidence limits, CL) production of new queens and males per colony in relation to landscape type ($n = 6$ landscapes per type). Means and CL are based on model-estimated least-square means.

than those in landscapes lacking clover and did not differ from each other ($F_{1,9} < 0.01$, $P = 0.95$, Table 1).

Bumblebee flower visitation, mainly by *B. terrestris* agg. (Table S4), was on average 13% (1.3–25% (95% confidence limits)) higher in thiacloprid-treated plots compared to adjacent untreated plots (Table 3). Crop bloom density was not affected by thiacloprid treatment (Table 3). Thiacloprid treatment reduced pest abundance by 93% (Figure S1).

Thiacloprid residues in pollen brought back to the colonies differed by landscape type ($H_2 = 11.34$, $P = 0.0035$). Comparisons between the landscape types showed that colonies in landscapes with thiacloprid-treated clover were exposed to higher thiacloprid levels than colonies in landscapes with untreated organic clover ($Z = 2.89$, $P = 0.011$) or in landscapes without clover ($Z = 2.76$, $P = 0.016$), while

thiacloprid exposure did not differ between colonies in landscapes with organic clover and landscapes lacking clover ($Z = 0.38$, $P = 0.92$, Table 1).

Residues of thiacloprid in pollen collected by foragers in red clover fields were on average 1640 ng g^{-1} pollen 1 day after thiacloprid applications and then declined sharply to 28.1 ng g^{-1} 16 days after the applications (Figure 4). The proportion of red clover pollen was on average 0.75 (0.72–0.78) over the 4 sample days (Figure 4).

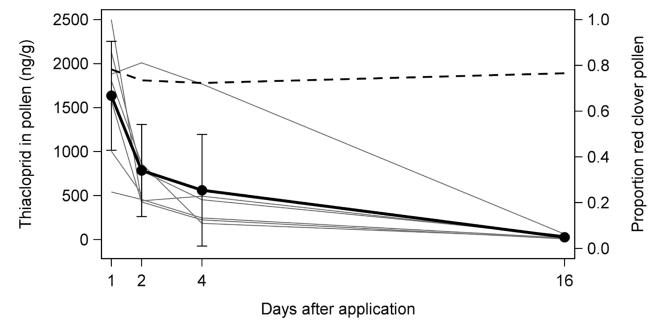


Figure 4. Thiacloprid concentration (solid lines) and proportion red clover pollen (dashed line) in bumblebee collected pollen 1, 2, 4, and 16 days after spray application with thiacloprid in eight red clover seed fields 2013. Means (solid circles, black lines) and 95% confidence limits (error bars) are based on raw data. The gray lines represent the thiacloprid concentrations for the individual fields.

DISCUSSION

We show that an MFC provides forage resources that can benefit bees, as well as that it is a source of pesticide exposure. However, contrary to what we expected of pesticide exposure, bumblebee colonies grew heavier in landscapes with red clover fields treated with the neonicotinoid thiacloprid compared to in otherwise similar landscapes that lacked red clover seed fields, and they did not differ in weight change from colonies in landscapes with untreated organic clover. This occurred despite that colonies in landscapes with thiacloprid-treated clover were exposed to higher thiacloprid levels than those in either landscapes lacking clover or landscapes with organic clover. We provide support that thiacloprid application in red clover can effectively control seed predating weevil pests and that such treated clover is more frequently visited by bumblebees than untreated clover in the same field. Bumblebee queen and male production was highly variable and did not differ among landscape types. Use of pesticides in general, and neonicotinoids in particular, is widely assumed to be costly for bees due to their now well-documented negative

Table 3. Bumblebee Crop Flower Visitation and Crop Bloom Density in Red Clover Fields in Relation to Pesticide Treatment and Year

		bumblebee visitation			crop bloom density		
	categories	F_{df}	P	estimate ^a	F_{df}	P	estimate ^a
treatment	untreated	5.12 _{1,34}	0.030	6.2 (4.1–9.6)	0.78 _{1,18}	0.78	52 (46–58)
	thiacloprid-treated			7.1 (4.6–11)			53 (47–58)
year	2011	8.94 _{1,14}	0.0095	12 (7.0–21)	<0.01 _{1,17}	>0.99	52 (45–59)
	2013			3.7 (1.9–7.1)			52 (44–60)
crop bloom		6.01 _{1,34}	0.020	0.012 (0.0050)			

^aEstimates are means ($\pm 95\%$ confidence limits) based on model-estimated least-square means and the model-estimated slope (with standard error within parenthesis) for the continuous variable inflorescence density.

effects on bee survival and reproduction. Here, we do not find evidence of such costs.

This study is a first attempt to separate the benefits and costs for bumblebees of a pesticide-treated MFC by including treated MFC landscapes with the flower resource and the pesticide (nutritional benefit and cost of pesticide exposure), untreated MFC landscapes with the flower resource but not the pesticide (only the nutritional benefit), and control landscapes without the flower resource (neither the nutritional benefit nor the cost of pesticide exposure). That midseason weight gain of colonies was largest in landscapes with pesticide-treated clover indicates that in our case, the pesticide treatment does not inflict costs. Because colony weight gain in landscapes with the untreated clover was inseparable from the weight gains in the other landscape types, we cannot, however, conclude on the relative role of the clover versus the thiacloprid treatment for explaining the higher colony weight gains. Irrespective of the explanation, we can, however, conclude that in a scenario where a ban on thiacloprid also would mean that farmers replaced mass-flowering clover with non-MFCs,^{13,14} this is likely to lead to reduced bumblebee colony weight gains in such landscapes.

Our finding that bumblebee visitation was 13% higher in thiacloprid-treated clover compared to in adjacent untreated clover supports that the pesticide treatment of the MFC may benefit bumblebee flower attraction or resource availability. We have previously shown a similar pattern of reduced bumblebee density in untreated organic compared to insecticide-treated conventional white clover (*Trifolium repens* L.) seed fields.⁴⁸ One possible explanation for increased flower visitation in pesticide-treated clover is that bumblebees may prefer to forage from neonicotinoid-treated flowers.^{49,50} We think, however, that the most likely explanation is that flower quality in untreated plots decreased, although flower quantity remained similar. This is supported by the over 90% decreased pest loads of seed-eating weevil larvae in the clover inflorescences with thiacloprid treatment but no effect on inflorescence density. Pest infestation can decrease flower attractiveness for pollinators and their visitation to crop flowers.^{51,52} *Protapion* spp. seed weevils lay their eggs in red clover buds, and the larva eats ovules and developing seeds during crop flowering and seed maturation,⁵³ but to what extent this may damage flowers or affect pollen and nectar resources available to pollinators has not been examined. Because colonies in landscapes with thiacloprid-treated and untreated organic clover collected similar proportions red clover pollen, we hypothesize, however, that the reduced pest infestation due to pesticide use primarily has a positive effect on crop nectar production. This has been shown in oilseed rape crops, where pest damage to nectaries results in reduced nectar production.^{52,54}

Bumblebee reproduction did not differ among landscape types despite greater colony growth in landscapes with thiacloprid-treated clover compared to control landscapes lacking clover. Successful reproduction of bumblebee colonies has previously been linked to abundance of forage resources.^{3,4,32} However, the timing of the MFC bloom in relation to the colony cycle of annual social insects is suggested to have differential effects on production of workers and reproductives.⁵⁵ Bumblebees would reproductively benefit relatively more from mid- to late-season resources like red clover compared to early season resources, but the effects on reproduction may be too weak to be notable in empirical

studies.⁵⁵ In addition, bumblebee reproductive parameters are known to be variable,^{41,56} and the confidence intervals for, in particular, queen production were very wide for all landscape types in our study. Our previous work suggests that conventionally managed red clover do support production of males and new queens at a landscape scale,⁴ although this study was conducted prior to the introduction of thiacloprid in clover seed production in Sweden.²¹ A laboratory study using *B. terrestris* microcolonies that were chronically exposed to thiacloprid indicated that 1200 ng g⁻¹ nectar was the lowest concentration expected to have a negative effect on reproduction, measured as production of males.²⁶ While the use of microcolonies may not accurately reflect bumblebee reproduction in queen-right colonies in the field, bumblebees in our study were generally exposed to thiacloprid concentrations well below 1200 ng g⁻¹, indicating a limited risk.

The previous two field studies focusing on effects of thiacloprid use on bumblebee colonies found negative and mixed impacts on colony performance.^{20,29} These contrasting results may be related to different exposure levels stemming from higher recommended spray rates: raspberries, U.K.: 120 g ha⁻¹ thiacloprid,²⁰ red clover, Norway: 96 g ha⁻¹,²⁹ versus 72 g ha⁻¹ in red clover in Sweden. For example, Ellis et al.²⁰ found up to 771 ng g⁻¹ thiacloprid residues in colony food stores. In our study, such levels were only exceeded in pollen loads of bumblebee foragers in the red clover fields the first few days after thiacloprid application. Another possibility is that the timing of the pesticide application in relation to the bumblebee colony development stage influences the outcome for colony performance.

Although further studies with increased replication and all observations done in the same fields and year are needed to evaluate impacts on reproduction and on species other than *B. terrestris*, we conclude that use of thiacloprid for pest control in red clover seed production appears to be of low risk to bumblebees. This is in stark contrast to our finding that clothianidin seed treatment in spring-sown oilseed rape grown in the same region resulted in reduced bumblebee colony growth and reproduction.⁷ Neonicotinoids can thus not be considered as a homogeneous group in assessments of risks to bees.²⁷ The fact that improved pest control has both a direct positive effect on yield by limiting pest damage and an indirect positive effect by promoting flower visitation by crop pollinators might explain why promoting pest control and crop pollination in tandem synergistically enhance crop yield.^{57,58} However, if such forage benefits are not translated into reproductive output, pollinator population sizes will not be enhanced. To optimize pollinator conservation, crop pollination, pest control, and yields, further research is needed on integrated pest management strategies for flowering crops using methods which are of minimal risk for pollinators.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02789.

Study design; colony growth and reproduction in relation to clover field size; effect of thiacloprid treatment on pest abundance; additional analyses of bumblebee crop visitation in relation to pesticide treatment; pest densities in relation to thiacloprid treatment; bumblebee crop visitation in relation to

insecticide treatment; details on insecticide treatments, clover field size and landscape land use; bumblebee species in the clover fields; and bumblebee colony development in relation to clover field size (PDF)

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M.R. conceived the experiments, collected and analyzed data, and wrote the manuscript. O.L. analyzed data and co-wrote the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E. L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **2015**, *347*, No. 1255957.
- (2) Carvell, C.; Bourke, A. F. G.; Dreier, S.; Freeman, S. N.; Hulmes, S.; Jordan, W. C.; Redhead, J. W.; Sumner, S.; Wang, J.; Heard, M. S. Bumblebee family lineage survival is enhanced in high-quality landscapes. *Nature* **2017**, *543*, 547–549.
- (3) Westphal, C.; Steffan-Dewenter, I.; Tschamntke, T. Mass flowering oilseed rape improves early colony growth but not sexual reproduction of bumblebees. *J. Appl. Ecol.* **2009**, *46*, 187–193.
- (4) Rundlöf, M.; Persson, A. S.; Smith, H. G.; Bommarco, R. Late-season mass-flowering red clover increases bumble bee queen and male densities. *Biol. Conserv.* **2014**, *172*, 138–145.
- (5) Botías, C.; David, A.; Horwood, J.; Abdul-Sada, A.; Nicholls, E.; Hill, E.; Goulson, D. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. *Environ. Sci. Technol.* **2015**, *49*, 12731–12740.
- (6) Pettis, J. S.; Lichtenberg, E. M.; Andree, M.; Stitzinger, J.; Rose, R.; vanEngelsdorp, D. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLoS One* **2013**, *8*, No. e70182.
- (7) Rundlöf, M.; Andersson, G. K. S.; Bommarco, R.; Fries, I.; Hederström, V.; Herbertsson, L.; Jonsson, O.; Klatt, B. K.; Pedersen, T. R.; Yourstone, J.; Smith, H. G. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* **2015**, *521*, 77–80.
- (8) Woodcock, B. A.; Isaac, N. J. B.; Bullock, J. M.; Roy, D. B.; Garthwaite, D. G.; Crowe, A.; Pywell, R. F. Impacts of neonicotinoid use on long-term population changes in wild bees in England. *Nat. Commun.* **2016**, *7*, No. 12459.
- (9) Henry, M.; Cerrutti, N.; Aupinel, P.; Decourtye, A.; Gayrard, M.; Odoux, J. F.; Pissard, A.; Rüger, C.; Bretagnolle, V. Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. *Proc. R. Soc. B* **2015**, *282*, No. 20152110.
- (10) Woodcock, B. A.; Bullock, J. M.; Shore, R. F.; Heard, M. S.; Pereira, M. G.; Redhead, J.; Ridding, L.; Dean, H.; Sleep, D.; Henrys, P.; Peyton, J.; Hulmes, S.; Hulmes, L.; Sárospataki, M.; Saure, C.; Edwards, M.; Genersch, E.; Knäbe, S.; Pywell, R. F. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science* **2017**, *356*, 1393–1395.
- (11) Godfray, H. C. J.; Blacquière, T.; Field, L. M.; Hails, R. S.; Petrokofsky, G.; Potts, S. G.; Raine, N. E.; Vanbergen, A. J.; McLean, A. R. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proc. R. Soc. B* **2014**, *281*, No. 20140558.
- (12) Lundin, O.; Rundlöf, M.; Smith, H. G.; Fries, I.; Bommarco, R. Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. *PLoS One* **2015**, *10*, No. e0136928.
- (13) Ratnieks, F. L. W.; Balfour, N. J.; Carreck, N. L. Have suitable experimental designs been used to determine the effects of neonicotinoid insecticides on bee colony performance in the field? *J. Apic. Res.* **2018**, *57*, 586–592.
- (14) Balfour, N. J.; Al Toufailia, H.; Scandian, L.; Blanchard, H. E.; Jesse, M. P.; Carreck, N. L.; Ratnieks, F. L. W. Landscape scale study of the net effect of proximity to a neonicotinoid-treated crop on bee colony health. *Environ. Sci. Technol.* **2017**, *51*, 10825–10833.
- (15) Sahlin, U.; Rundlöf, M. Differences in the strengths of evidence matters in risk-risk trade-offs. *J. Risk Res.* **2017**, *20*, 988–994.
- (16) Siviter, H.; Koricheva, J.; Brown, M. J. F.; Leadbeater, E. Quantifying the impact of pesticides on learning and memory in bees. *J. Appl. Ecol.* **2018**, *55*, 2812–2821.
- (17) Gill, R. J.; Ramos-Rodriguez, O.; Raine, N. E. Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* **2012**, *491*, 105–108.
- (18) Whitehorn, P. R.; O'Connor, S.; Wackers, F. L.; Goulson, D. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* **2012**, *336*, 351–352.
- (19) Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A. Overview of the status and global strategy for neonicotinoids. *J. Agric. Food Chem.* **2011**, *59*, 2897–2908.
- (20) Ellis, C.; Park, K. J.; Whitehorn, P.; David, A.; Goulson, D. The neonicotinoid insecticide thiacloprid impacts upon bumblebee colony development under field conditions. *Environ. Sci. Technol.* **2017**, *51*, 1727–1732.
- (21) Lundin, O.; Rundlöf, M.; Smith, H. G.; Bommarco, R. Towards integrated pest management in red clover seed production. *J. Econ. Entomol.* **2012**, *105*, 1620–1628.
- (22) Klein, A. M.; Vaissiere, B. E.; Cane, J. H.; Steffan-Dewenter, I.; Cunningham, S. A.; Kremen, C.; Tschamntke, T. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B* **2007**, *274*, 303–313.
- (23) European Commission. Neonicotinoids. https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en (accessed 5 December, 2018).
- (24) Simon-Delso, N.; Amaral-Rogers, V.; Belzunces, L. P.; Bonmatin, J. M.; Chagnon, M.; Downs, C.; Furlan, L.; Gibbons, D. W.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D. P.; Krupke, C. H.; Liess, M.; Long, E.; McField, M.; Mineau, P.; Mitchell, E. A. D.; Morrissey, C. A.; Noome, D. A.; Pisa, L.; Settele, J.; Stark, J. D.; Tapparo, A.; Van Dyck, H.; van Praagh, J.; van der Sluijs, J. P.; Whitehorn, P. R.; Wiemers, M. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ. Sci. Pollut. Res.* **2015**, *22*, 5–34.
- (25) Iwasa, T.; Motoyama, N.; Ambrose, J. T.; Roe, R. M. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Prot.* **2004**, *23*, 371–378.

- (26) Mommaerts, V.; Reynders, S.; Boulet, J.; Besard, L.; Sterk, G.; Smagghe, G. Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* **2010**, *19*, 207–215.
- (27) Manjon, C.; Troczka, B. J.; Zaworra, M.; Beadle, K.; Randall, E.; Hertlein, G.; Singh, K. S.; Zimmer, C. T.; Homem, R. A.; Lueke, B.; Reid, R.; Kor, L.; Kohler, M.; Benting, J.; Williamson, M. S.; Davies, T. G. E.; Field, L. M.; Bass, C.; Nauen, R. Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. *Curr. Biol.* **2018**, *28*, 1137–1143.
- (28) Goulson, D. An overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* **2013**, *50*, 977–987.
- (29) Havstad, L. T.; Øverland, J. I.; Valand, S.; Aamlid, T. S. Repellency of insecticides and the effect of thiacloprid on bumble bee colony development in red clover (*Trifolium pratense* L.) seed crops. *Acta Agric. Scand., Sect. B—Soil Plant Sci.* **2019**, *69*, 439–451.
- (30) Kleijn, D.; Winfree, R.; Bartomeus, I.; Carvalheiro, L. G.; Henry, M.; Isaacs, R.; Klein, A. M.; Kremen, C.; M'Gonigle, L. K.; Rader, R.; Ricketts, T. H.; Williams, N. M.; Adamson, N. L.; Ascher, J. S.; Baldi, A.; Batary, P.; Benjamin, F.; Biesmeijer, J. C.; Blitzer, E. J.; Bommarco, R.; Brand, M. R.; Bretagnolle, V.; Button, L.; Cariveau, D. P.; Chifflet, R.; Colville, J. F.; Danforth, B. N.; Elle, E.; Garratt, M. P. D.; Herzog, F.; Holzschuh, A.; Howlett, B. G.; Jauker, F.; Jha, S.; Knop, E.; Krewenka, K. M.; Le Feon, V.; Mandelik, Y.; May, E. A.; Park, M. G.; Pisanty, G.; Reemer, M.; Riedinger, V.; Rollin, O.; Rundlof, M.; Sardinias, H. S.; Scheper, J.; Sciligo, A. R.; Smith, H. G.; Steffan-Dewenter, I.; Thorp, R.; Tscharnkte, T.; Verhulst, J.; Viana, B. F.; Vaissiere, B. E.; Veldtman, R.; Westphal, C.; Potts, S. G. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nat. Commun.* **2015**, *6*, No. 7414.
- (31) Goulson, D. *Bumblebees: Behaviour, Ecology and Conservation*, 2nd ed.; Oxford University Press: Oxford, 2009.
- (32) Crone, E. E.; Williams, N. M. Bumble bee colony dynamics: quantifying the importance of land use and floral resources for colony growth and queen production. *Ecol. Lett.* **2016**, *19*, 460–468.
- (33) Thompson, H. M. Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* **2001**, *32*, 305–321.
- (34) Feltham, H.; Park, K.; Goulson, D. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* **2014**, *23*, 317–323.
- (35) Gill, R. J.; Raine, N. E. Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Funct. Ecol.* **2014**, *28*, 1459–1471.
- (36) Free, J. B. *Insect Pollination of Crops*; Academic Press: London, 1993.
- (37) Rundlöf, M.; Lundin, O.; Bommarco, R. Annual flower strips support pollinators and potentially enhance red clover seed yield. *Ecol. Evol.* **2018**, *8*, 7974–7985.
- (38) Greenleaf, S. S.; Williams, N. M.; Winfree, R.; Kremen, C. Bee foraging ranges and their relationship to body size. *Oecologia* **2007**, *153*, 589–596.
- (39) Osborne, J. L.; Martin, A. P.; Carreck, N. L.; Swain, J. L.; Knight, M. E.; Goulson, D.; Hale, R. J.; Sanderson, R. A. Bumblebee flight distances in relation to the forage landscape. *J. Anim. Ecol.* **2008**, *77*, 406–415.
- (40) Lefebvre, D.; Pierre, J. Hive weight as an indicator of bumblebee colony growth. *J. Apic. Res.* **2006**, *45*, 217–218.
- (41) Duchateau, M. J.; Velthuis, H. H. W. Development and reproductive strategies in *Bombus terrestris* colonies. *Behaviour* **1988**, *107*, 186–207.
- (42) Edwards, E.; Jenner, M. *Field Guide to the Bumblebees of Great Britain and Ireland*; Ocelli Limited: Eastbourne, 2005.
- (43) Loken, A. Studies on Scandinavian bumble bees (Hymenoptera, Apidae). *Norw. J. Entomol.* **1973**, *20*, 1–218.
- (44) Prys-Jones, O. E.; Corbet, S. A. *Bumblebees*; Cambridge University Press: Cambridge, 1986.
- (45) Murray, T. E.; Fitzpatrick, U.; Brown, M. J. F.; Paxton, R. J. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. *Conserv. Genet.* **2008**, *9*, 653–666.
- (46) Littell, R. C.; Milliken, G. A.; Stroup, W. W.; Wolfinger, R. D.; Schabenberger, O. *SAS for Mixed Models*, 2nd ed.; SAS Institute Inc.: Cary, N.C., 2006.
- (47) Cole, T. J. Sympercents: symmetric percentage differences on the 100 loge scale simplify the presentation of log transformed data. *Stat. Med.* **2000**, *19*, 3109–3125.
- (48) Lundin, O.; Svensson, G. P.; Larsson, M. C.; Birgersson, G.; Hederström, V.; Lankinen, Å.; Anderbrant, O.; Rundlöf, M. The role of pollinators, pests and different yield components for organic and conventional white clover seed yields. *Field Crops Res.* **2017**, *210*, 1–8.
- (49) Kessler, S. C.; Tiedeken, E. J.; Simcock, K. L.; Derveau, S.; Mitchell, J.; Softley, S.; Radcliffe, A.; Stout, J. C.; Wright, G. A. Bees prefer foods containing neonicotinoid pesticides. *Nature* **2015**, *521*, 74–76.
- (50) Arce, A. N.; Ramos Rodrigues, A.; Yu, J.; Colgan, T. J.; Wurm, Y.; Gill, R. J. Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged exposure. *Proc. R. Soc. B* **2018**, *285*, No. 20180655.
- (51) Muola, A.; Weber, D.; Malm, L. E.; Egan, P. A.; Glinwood, R.; Parachnowitsch, A. L.; Stenberg, J. A. Direct and pollinator-mediated effects of herbivory on strawberry and the potential for improved resistance. *Front. Plant Sci.* **2017**, *8*, No. 823.
- (52) Lindström, S. A. M.; Klatt, B. K.; Smith, H. G.; Bommarco, R. Crop management affects pollinator attractiveness and visitation in oilseed rape. *Basic Appl. Ecol.* **2018**, *26*, 82–88.
- (53) Notini, G. Undersökningar rörande på rödklöver levande spetsvivlar (*Apion* Herbst) 1. deras förekomst, levnadssätt och utvecklingshistoria. *Medd. Statens Växtskyddsanstalt* **1935**, *9*, 1–63.
- (54) Kirk, W. D. J.; Ali, M.; Breadmore, K. N. The effects of pollen beetles on the foraging behaviour of honey bees. *J. Apic. Res.* **1995**, *34*, 15–22.
- (55) Hovestadt, T.; Mitesser, O.; Poethke, A.; Holzschuh, A. Explaining the variability in the response of annual eusocial insects to mass-flowering events. *J. Anim. Ecol.* **2018**, *88*, 178–188.
- (56) Müller, C. B.; Schmid-Hempel, P. Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. *Ecol. Entomol.* **1992**, *17*, 343–353.
- (57) Sutter, L.; Albrecht, M. Synergistic interactions of ecosystem services: florivorous pest control boosts crop yield increase through insect pollination. *Proc. R. Soc. B* **2016**, *283*, No. 20152529.
- (58) Lundin, O.; Smith, H. G.; Rundlöf, M.; Bommarco, R. When ecosystem services interact: crop pollination benefits depend on the level of pest control. *Proc. R. Soc. B* **2013**, *280*, No. 20122243.