50 YEARS WITH IMPACT

Journal of Applied Ecology

1913 2013 British Ecological Society CELEBRATING 100 YEARS

Journal of Applied Ecology 2014, **51,** 460–469 doi: 10.1111/1365-2664.12205

Impact of chronic exposure to a pyrethroid pesticide on bumblebees and interactions with a trypanosome parasite

Gemma L. Baron*, Nigel E. Raine and Mark J. F. Brown

School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK

Summary

1. Bees are exposed to pesticides when foraging in agricultural areas and growing evidence suggests that such compounds can be harmful to managed and wild populations. Given the economic and ecological importance of bees, and the evidence of widespread population declines, the full impacts of pesticides and their interactions with other stressors in the environment need to be investigated.

2. Here, we focus on the impacts of chronic exposure to the commonly used pyrethroid pesticide lambda (λ)-cyhalothrin on the bumblebee *Bombus terrestris* at both the individual and colony level. Furthermore, we investigated the interactions of pesticide exposure with a highly prevalent trypanosome parasite *Crithidia bombi*. Colonies were exposed to λ-cyhalothrin in the laboratory, and colony growth and reproductive output were monitored for up to 14 weeks. The potential interactions between the pesticide and *C. bombi* were investigated by quantifying the impact of pesticide treatment on susceptibility to, and success of experimental infections, as well as the survival of workers. Male survival after larval pesticide exposure was also monitored.

3. Pesticide-treated colonies produced workers with a significantly lower body mass. However, out of the twelve variables of colony development measured, this was the only metric that was significantly affected by pesticide treatment and there was no subsequent significant impact on the reproductive output of colonies.

4. Lambda-cyhalothrin had no significant impact on the susceptibility of workers to *C. bombi,* or intensity of parasitic infection.

5. Pesticide exposure did not cause differential survival in workers or males, even when workers were additionally challenged with *C. bombi*.

6. *Synthesis and applications*. Chronic exposure to λ-cyhalothrin has a significant impact on worker size, a key aspect of bumblebee colony function, particularly under conditions of limited food resources. This could indicate that under times of resource limitation, colonies exposed to this pesticide in the field may fail. However, the lack of other impacts found in this study indicate that further field trials are needed to elucidate this.

Key-words: agrochemicals, beneficial insects, bumble bees, colony success, ecosystem services, insecticide, neonicotinoid, parasite–pesticide interactions, pollinator, pollinator decline

Introduction

Wild bee populations are declining at a global scale (Williams 1982; Biesmeijer *et al.* 2006; Brown & Paxton 2009; Williams & Osborne 2009; Cameron *et al.* 2011). Given the economic and ecological importance of pollinating insects such as bees (Klein *et al.* 2007; Ollerton, Winfree & Tarrant 2011), an understanding of the underlying causes of these declines is vital (Potts *et al.* 2010a; Dicks *et al.* 2013; Vanbergen *et al.* 2013). Several factors have been implicated in declines, including habitat loss (Williams 1986; Osborne, Williams & Corbet 1991; Carvell *et al.* 2006), parasites and disease (Colla *et al.* 2006; Cameron *et al.* 2011; Meeus *et al.* 2011), and the introduction of non-native species (Thomson 2004; Stout & Morales 2009). There is also

*Correspondence author. E-mail: gemma.baron.2011@live.rhul.ac.uk

mounting evidence that bees are regularly exposed to pesticides (Chauzat *et al*. 2009; Mullin *et al*. 2010) and that some of these compounds are detrimental to bees, even at sublethal levels (Johnson *et al*. 2010; Cresswell 2011; Gill, Ramos-Rodriguez & Raine 2012; Henry *et al*. 2012; Whitehorn *et al*. 2012; Bryden *et al*. 2013).

Most research into the impacts of pesticides on bees has focused on honeybees *Apis mellifera* L., due to their extensive use in commercial pollination globally, and concerns over widespread honeybee losses in the USA (vanEngelsdorp *et al*. 2008) and Europe (Potts *et al*. 2010b). However, protecting the diverse wild bee community is equally important for commercial pollination and maintaining wild ecosystems (Westerkamp & Gottsberger 2000; Klein *et al*. 2007; Breeze *et al*. 2011; Garibaldi *et al*. 2013). Bumblebees are key pollinators of agricultural crops and wild plants (Corbet, Williams & Osborne 1991), but their annual life cycle, relatively small colony size and different foraging strategies to honeybees are traits which are likely to make them more vulnerable to pesticide exposure (Thompson 2001). Furthermore, recent evidence suggests that honeybees and bumblebees vary in their sensitivity to a neonicotinoid pesticide (Cresswell *et al*. 2012). Recent studies have demonstrated sublethal effects of pesticides on bumblebee fecundity (Laycock *et al*. 2012), queen production (Whitehorn *et al*. 2012) and foraging ability (Gill, Ramos-Rodriguez & Raine 2012).

The vast majority of recent available data on the sublethal impacts of pesticides on bumblebees focuses on neonicotinoids, whilst other pesticide classes remain relatively understudied. This stands in contrast to the fact that the usage of pesticides such as pyrethroids is widespread and increasing, for example pyrethroid usage in the UK has nearly doubled since the early 1990s (FERA 2012), and given the recent EU moratorium on neonicotinoid usage for crops attractive to bees, use of alternative pesticides is likely to increase further. Here, we investigate the impacts on *Bombus terrestris* L. colonies of exposure to a widely used pyrethroid insecticide, lambda-cyhalothrin (λ-cyhalothrin). This pesticide is sprayed during the flowering period on a range of crops, such as oilseed rape *Brassica napus*, which provide an important bumblebee foraging resource (Westphal, Steffan-Dewenter & Tscharntke 2003; Knight *et al*. 2009). Lambda-cyhalothrin is applied to large areas of agricultural crops in the UK throughout the spring and summer (e.g. 43% of oilseed rape was treated with this pesticide in 2012; Garthwaite *et al*. 2012a). Bumblebee colonies in agricultural landscapes are therefore likely to be exposed to low levels of this compound over extended periods of time (chronic exposure) whilst foraging on flowering crops. Gill, Ramos-Rodriguez & Raine (2012) found that *B. terrestris* colonies exposed to λ-cyhalothrin had higher levels of worker mortality during the early stages of colony development. Our study expands on this by exploring the long-term impact of chronic exposure to λ-cyhalothrin on *B. terrestris* colony growth and the production of queens and males.

In order to understand the full impacts of pesticides on bumblebees in the wild we also need to consider other stressors, such as parasites, which are likely to influence colony success. Interactions between pesticides and parasites could result in a greater impact than the sum of each stressor acting individually (a synergistic interaction), which has been demonstrated in both vertebrates (Kiesecker 2002) and invertebrates (Coors *et al*. 2008). Such interactions have received some attention in honeybees (Alaux *et al*. 2010; Vidau *et al*. 2011; Aufauvre *et al*. 2012; Pettis *et al*. 2012), and more recently, bumblebees (Fauser-Misslin *et al*. 2014). Whilst the above studies explore the impacts of chronic pesticide exposure in adult bees, little is known about how larval exposure to a pesticide impacts on adult survival, or how this interacts with parasite infection. Here, we address these important questions in the bumblebee *B. terrestris*. Bumblebees are hosts to a wide range of parasites (Schmid-Hempel 1998), the most prevalent of which in Europe is *Crithidia bombi* Lipa and Triggiani (Shykoff & Schmid-Hempel 1991). This gut parasite infects a range of bumblebee species (Ruiz-González *et al*. 2012) and is transmitted via contaminated faeces within the natal colony and on flower surfaces when foraging (Durrer & Schmid-Hempel 1994). *Crithidia bombi* occurrence in wild bumblebee populations varies spatiotemporally, and across species and caste, but prevalence levels of up to 47·5% have been reported in spring *B. terrestris* queens and up to 80% in workers (Shykoff & Schmid-Hempel 1991). This parasite has been shown to increase mortality in nutritionally stressed *B. terrestris* workers (Brown, Loosli & Schmid-Hempel 2000) and reduce queen fitness after a stressful hibernation period (Brown, Schmid-Hempel & Schmid-Hempel 2003; Yourth, Brown & Schmid-Hempel 2008). The likelihood of bumblebees encountering stress from a combination of parasite and pesticide exposure in the field is therefore high, and the interactions between these stressors need to be determined.

In this study, we addressed the following questions: 1. How does chronic exposure to λ-cyhalothrin affect *B. terrestris* colony growth and reproductive output? 2. Are workers exposed to λ-cyhalothrin as larvae more susceptible to infection by *C. bombi*? 3. Do larval exposure to λ-cyhalothrin, *C. bombi* or a combination of both have an impact on the survival of workers? 4. Is male survival affected by larval exposure to λ-cyhalothrin?

Materials and methods

Thirty early-stage *B. terrestris* colonies (containing a queen, brood and a mean of 8 (± 0·55 S.E.) workers) were obtained from Syngenta Bioline (Weert, the Netherlands). Colonies were kept in a dark room (red light was used for colony manipulation) at 25 °C. To ensure that colonies were healthy and developing normally, they were monitored for 18 days prior to allocation to a treatment group. All colonies were screened for the common parasites, *Crithidia bombi*, *Nosema bombi* and *Apicystis bombi*, by

microscopic examination of faecal samples from 19/24 queens (79%), and by dissection of 10% of workers present at the time of sampling (mean = 2 ± 0·2 S.E., range = 0–3). No infections were found in any colonies at this stage. A laboratory set-up was used to ensure that colonies remained parasite-free throughout the experiment.

The number of workers per colony was counted, and each colony matched to another of equivalent size. One colony in each pair was then randomly allocated to the 'pesticide' treatment group and the other to the 'control' group. Six of the 30 queens (control = 4, pesticide = 2) died within the first 4 weeks of treatment, due to damage caused to these colonies during transit. These colonies were excluded from the rest of the experiment.

COLONY GROWTH AND REPRODUCTIVE OUTPUT

Colonies were exposed to λ-cyhalothrin (Technical grade λ-cyhalothrin PESTANAL, Sigma-Aldrich) via the pollen feed provided, which was sprayed at a concentration of 37·5 ppm (the recommended application rate for oilseed rape: Syngenta Crop Protection UK, 2011), following the methods of Gill, Ramos-Rodriguez & Raine (2012). A stock solution of λ-cyhalothrin in acetone was prepared, and a sample of this was diluted each week with distilled water to obtain the required concentration. The same concentration of acetone was used for the control treatment. Pollen treatment took place at the same time every 7 days (the minimum interval between applications to a single crop: Syngenta Crop Protection UK, 2011). Defrosted frozen pollen pellets (Koppert Ltd, Haverhill, UK) were weighed into 10 g portions to create a single layer in a Petri dish (diameter 8·6 cm). Pollen was sprayed with the λ-cyhalothrin or control solution from a distance of 20 cm using a fine mist sprayer to ensure even coverage. Each Petri dish was then closed and kept in dry dark conditions for 15 hours (overnight) at 22 °C to ensure that the solution was absorbed into the pollen. All pesticide-treated pollen was combined and mixed, before being weighed into clean Petri dishes. The same process was repeated with the control-treated pollen. Samples of pollen treated in this way were analysed for λ-cyhalothrin residues using GC-MS (Food and Environment Research Agency, Sand Hutton, York). Further details can be found in Appendix S1 (Supporting information). The average residue in pollen samples treated with the pesticide was 0·247 mg kg^{-1} (± 0·021 S.E.), which is approximately a 100-fold reduction, similar to that found by Choudhary and Sharma (2008).

A standardized amount of treated pollen was provided to each colony once per week, based on an estimate of colony size (allowing 0·5 g per bee each week). The weekly treatment represents the minimum time interval between treatments of individual crops (Syngenta Crop Protection UK, 2011). Treated pollen was provided to the colony in a Petri dish for 3 days and then replaced with *ad libitum* untreated pollen for the remaining 4 days, this simulated the field scenario where bees will forage for pollen on pesticide-treated crops and untreated plants. This temporal protocol was chosen to account for daily fluctuations in pollen intake (observed in a pilot experiment, G.L. Baron, unpublished data). Colonies were also provided with *ad libitum* 50% Ambrosia (EH Thorne Ltd), an inverted sugar syrup solution. The mass of treated and untreated pollen removed from the feeding dishes by each colony was weighed to the nearest 0·1 g, on a weekly basis. In order to check that workers would forage on treated pollen and feed this to larvae, we undertook a pilot study using microcolonies, observing the behaviour of individual workers when provided with treated and untreated pollen (see Appendix S2 and Table S1 in Supporting Information).

Workers and males that died in the colony were discarded, whilst live males were kept for a survival experiment, or were frozen. All gynes (unmated queens) were removed from the colonies and frozen. The dates of the first male and gyne eclosion, foundress queen death and the onset of worker egg laying (competition point) were all recorded, as they represent the main phases of colony development (Duchateau & Velthuis 1988; Lopez-Vaamonde *et al*. 2009).

Pesticide treatment continued for 14 weeks. The peak time of λ-cyhalothrin application to crops in the UK is from April to July (in 2010, more than 100 000 ha of crops were treated with λ-cyhalothrin in each of these months; Garthwaite *et al*. 2010). As such, a 14-week period represents a worst-case scenario and mimics a situation where bumblebee colonies are collecting pollen over an extended period, from a range of treated crops which are treated at different times, with each crop potentially being treated multiple times.

Each colony was removed from the experiment and frozen 4 weeks after the queen's death, ensuring that all queen-laid offspring had eclosed. At this point a final count of workers, males and gynes within the colony was made. All living bees removed from the colonies were frozen at -20 °C. Frozen workers and males from each colony (when available) were randomly subsampled, and twenty of each caste were dried at 60 °C for 5 days, from which the average dry mass of workers and males was calculated for each colony (see Appendix S3 for an explanation of this procedure). All gynes produced were dried in the same way and weighed. The total dry mass of workers and sexual offspring (males and gynes) produced by each colony could then be estimated, by multiplying the total number of bees produced by their average dry mass.

WORKER INFECTION AND SURVIVAL

This stage of the experiment began 4 weeks after the start of pollen treatment to ensure that any workers removed from the colonies were exposed to the treated pollen throughout their larval development (average worker development time is 22 days: Duchateau & Velthuis 1988). Callow workers were only removed from colonies on days when untreated pollen was provided. Workers removed from each colony were allocated sequentially to a parasite or control treatment group, resulting in a fully crossed design (Table S2, Supporting information). Throughout the rest of the experiment, these workers were kept in plastic boxes (13 × 11 × 6·8 cm) containing a small amount of recycled paper cat litter (Waitrose) to remove excess moisture, and *ad libitum* untreated food (pollen and 50% Ambrosia solution) in a dark room at 22 °C. After 3 days each worker was removed from its box, starved for 3 hours and transferred into a vial containing a 20 μL droplet (inoculum) of 50% Ambrosia solution containing either 10 000 *C. bombi* cells or a control solution (acquisition and purification of *C. bombi* and the control solution are described below). Only bees which consumed all of the inoculum were included in the experiment. A dose of 10 000 cells lies within the range of *C. bombi* cells shed by infected workers which has been reported in previous studies (5000 cells μL^{-1} (Ruiz-González & Brown 2006) to 25 000 cells μL^{-1} (Logan, Ruiz-González & Brown 2005)). Therefore, workers in an infected colony will be

exposed to this level of the parasite if they ingest food contaminated with faeces.

Seven days after inoculation, faeces were collected from each bee, diluted with 0·9% insect Ringer solution (Thermo Fisher, Basingstoke, UK) to a concentration of 10%, thoroughly mixed, and the number of *C. bombi* cells per microlitre of faeces was counted using a Neubauer chamber.

Workers were monitored every day until death. Dead workers were placed into a −20 °C freezer within 24 hours. The hindgut of each worker was dissected out and checked microscopically for the presence of *C. bombi*.

MALE SURVIVAL

Males, which had been exposed to λ-cyhalothrin throughout their development, were removed from colonies in the same way as described above for workers. Males were kept in groups of up to ten in communal wooden boxes (24 × 14 × 10·5 cm), provided with *ad libitum* pollen and sugar water, and monitored every day until death.

CRITHIDIA BOMBI PURIFICATION PROTOCOL

Wild *B. terrestris* queens, naturally infected with only *C. bombi* (queens were also screened for *Nosema bombi* and *Apicystis bombi),* were collected from Windsor Great Park, Surrey, UK (latitude: 51·417432, longitude: −0·60481256). Local adaptations of a parasite to its host can cause variability in infectiveness to different host populations (Imhoof & Schmid-Hempel 1998; Yourth & Schmid-Hempel 2006). To select strains that would infect the commercial colonies used in our experiment, we infected workers from a commercial colony with a multitude of wild *C. bombi* strains and used only strains infective to these stock bees for subsequent experimental infections. Faeces from uninfected queens from the same wild population were fed to stock bees from the same colony to provide a control. Stock bees were kept in groups of up to 20 individuals in wooden boxes (24 × 14 × 10·5 cm) and fed *ad libitum* pollen and 50% Ambrosia solution. On the day of inoculation of experimental workers, faeces were collected from at least ten stock bees, then combined and diluted with 0·9% insect Ringer solution to make a 1 ml solution. *Crithidia bombi* were purified using a modified triangulation protocol developed by Cole (1970). The *C. bombi* cells in the resulting solution were counted using a Neubauer chamber, and the volume of solution that contained 10,000 cells bee^{-1} was diluted with 50% Ambrosia solution. The same protocol was followed for the control solution, using faeces from uninfected stock bees.

ANALYSIS

Multivariate and univariate ANOVAS were used to analyse the impacts of pesticide treatment on colony development and productivity data (Appendix S4, Supporting information).

In order to examine any differences in pollen consumption between pesticide and control treatment groups, and any differences within each colony in the consumption of treated and untreated pollen, a mixed-design ANOVA was performed (Appendix S4, Supporting information).

A G-test was used to test for differences among treatment groups in the prevalence of *C. bombi* both 7 days post-exposure and at death. A nested ANOVA was used to analyse the infection intensity of *C. bombi* (based on cell counts in faeces samples 7 days after parasite exposure) with the natal colony of each bee nested within the pesticide treatment.

A generalized linear mixed model (GLMM) was used to test for differences among treatment groups in worker survival. The model used a gamma (log-link) distribution and included survival time (days) as the response variable, pesticide and parasite treatment as fixed factors, and colony as a random factor. Male survival was analysed in the same way, with only pesticide treatment as a fixed factor.

All data analyses were performed using IBM SPSS, versions 19 and 20.

Results

Pesticide treatment had a significant overall effect in both MANOVAS (MANOVA 1, $F_{7,\ 11} = 3{\cdot}406$, $P = 0{\cdot}034$; MANOVA 2, $F_{6,\ 16} = 3{\cdot}331$, $P = 0{\cdot}025$). In the first MANOVA (Table 1), this was driven by a significantly lower mean worker dry mass in pesticide treated colonies compared to control colonies (ANOVA, $F_{1,\ 17} = 9{\cdot}846$, $P = 0{\cdot}006$: Fig. 1). In the second MANOVA no uniform trend in the effects of pesticide treatment on the dependent variables was apparent (Table 2), so a discriminant analysis was used to explore the underlying drivers of the difference between treatment groups. One significant discriminant function (Wilk's lambda = 0·435, $\chi^2_6 = 15{\cdot}798$, $P = 0{\cdot}015$) was identified: the number of males produced, the total dry mass of sexual offspring produced and the difference between these were the major factors driving this discriminant function. This is likely to be due to differences in male and gyne production between pesticide and control colonies; on average, pesticide-treated colonies produced a greater number of males with a higher mean dry mass (Table 2), but fewer gynes with a lower mean dry mass (Table 3) compared to controls. However, these differences were not individually significant within the MANOVA. Similarly, neither the overall dry mass of sexual offspring produced (Tables 1 and 2), nor the timing of key colony developmental events, such as the competition point (ANOVA, $F_{1,16} = 0{\cdot}616$, $P = 0{\cdot}444$) and the number of days until the first male emerged (ANOVA, $F_{1,20} = 2{\cdot}563$, $P = 0{\cdot}125$), were affected by pesticide treatment (Table S3, Supporting information). In both MANOVAS, the number of workers at the start of the experiment had a significant overall effect (MANOVA 1, $F_{7,11} = 3{\cdot}601$, $P = 0{\cdot}029$; MANOVA 2, $F_{6,16} = 3{\cdot}178$, $P = 0{\cdot}030$), with individually significant effects on the number of workers produced, number of males produced, the total dry mass of sexual offspring and the number of worker mortalities (Tables 1 and 2).

The power of our data to detect differences between treatment groups may differ across variables (Fig. S2, Supporting information). Whilst effect sizes for the mean dry mass of workers, mean dry mass of males and number of days until male production have tight confidence intervals, suggesting that these results are reliable, effect

Table 1. Colony development data from 20 *B. terrestris* colonies treated with either the pesticide λ-cyhalothrin or a control solution, used in statistical analysis including worker mass as a variable. Data shown are colony means (± S.E.) and n indicates the number of colonies per treatment group. Test statistics are from individual ANOVAs for the variable in each row. The overall MANOVA was significant (see Results for details)

				ANOVA test statistics (including colonies with data available)							
				Pesticide treatment				Number of workers at start			
Dependent variable	Control colonies Mean (± S.E.) $n = 11$	Pesticide colonies Mean (± S.E.) $n = 9$	Trend	F	d.f.	Error d.f.	P	F	d.f.	Error d.f.	P
Number of workers produced	196 (± 35)	184 (± 47)	–	0·136	1	17	0·717	5·879	1	17	**0·027***
Average dry mass of workers (g)	0·066 (± 0·002)	0·055 (± 0·002)	–	9·846	1	17	**0·006****	0·075	1	17	0·787
Total dry mass of workers (g)	13·221 (± 2·520)	10·624 (± 3·004)	–	0·684	1	17	0·420	3·904	1	17	0·065
Number of males produced†	207 (± 47)	192 (± 54)	–	0·022	1	17	0·884	7·138	1	17	**0·016***
Average dry mass of males (g)	0·109 (± 0·008)	0·128 (± 0·007)	+	2·915	1	17	0·106	1·124	1	17	0·304
Total dry mass of sexual offspring (g)†	28·057 (± 7·296)	27·059 (± 8·911)	–	0·017	1	17	0·898	5·357	1	17	**0·033***
Worker mortalities†	57 (± 13)	57 (± 20)	0	0·306	1	17	0·587	3·569	1	17	0·076

†Data were $\log_{10}$-transformed prior to analysis. 'Trend' indicates whether the pesticide treatment had a negative or positive (but not necessarily significant) effect on each variable.
Significant p-values are shown in bold: *$P < 0·05$, **$P < 0·01$.

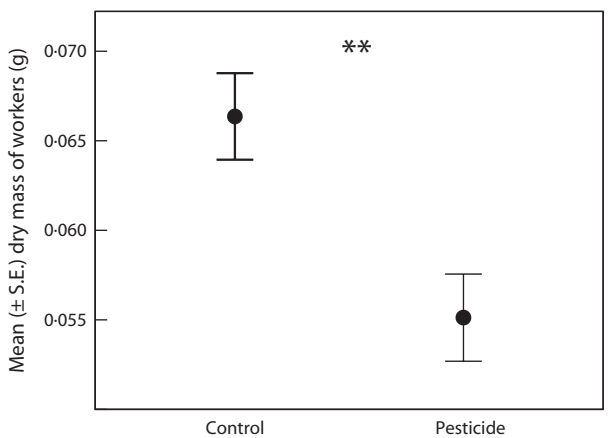

Fig. 1. Mean dry mass of *Bombus terrestris* workers subsampled from colonies treated with a control or pesticide (λ-cyhalothrin). ** indicates significant difference ($P = 0·006$).

sizes for other variables measured (see Appendix S5, Supporting information) have much larger confidence intervals which cross zero, suggesting that larger samples may be needed to definitively ascertain the impact of pesticide treatment.

Pollen consumption increased in both treatment groups over the first 8–9 weeks as colonies grew and then decreased as they began to senesce (mixed-design ANOVA, $F_{2·268,\ 45·361} = 51·970$, $P < 0·005$). Pesticide treatment did not significantly affect pollen consumption in the first 9 weeks (mixed-design ANOVA, $F_{1,\ 20} = 0·053$, $P = 0·821$) or the full 14 weeks of the experiment (mixed-design ANOVA, $F_{1,\ 21} = 0·331$, $P = 0·571$). There was no significant effect of whether the pollen was treated (with acetone or λ-cyhalothrin) or untreated on average daily consumption (mean ± S.E. (g) pesticide treated = 5·77 ± 0·94; pesticide untreated = 5·97 ± 0·94; control treated = 6·72 ± 1·24; control untreated = 6·21 ± 1·28: repeated measures ANOVA, $F_{1,21} = 0·001$, $P = 0·972$) when the total number of bees produced by each colony was controlled for.

Pesticide treatment did not affect workers susceptibility to *C. bombi*, or the intensity of infections (see Appendix S6, Supporting information).

Worker survival was not significantly affected by pesticide treatment (GLMM, $F_{1,89} = 0·006$, $P = 0·936$), parasite treatment (GLMM, $F_{1,89} = 1·371$, $P = 0·245$) or the interaction between these factors (GLMM, $F_{1,89} = 0·391$, $P = 0·532$) (Fig. 2). Similarly, male survival was not significantly affected by pesticide treatment (mean ± S.E. (days) pesticide = 32 ± 1 days; control = 31 ± 2: GLMM, $F_{1,7} = 0·352$, $P = 0·555$).

Discussion

In this experiment, chronic exposure to λ-cyhalothrin resulted in the production of smaller workers by *B. terrestris* colonies. However, there were no significant impacts on the production of gynes or males, the susceptibility of individual workers to *C. bombi*, or any interactive effects of the pesticide and parasite on worker survival.

Whilst the smaller size of workers in pesticide-treated colonies did not result in any effects on sexual offspring production in this study, this is unsurprising, as previous laboratory studies also using *ad libitum* food showed that

Table 2. Colony development data from 24 *B. terrestris* colonies treated with either the pesticide λ-cyhalothrin or a control solution, used in statistical analysis which did not include worker mass as a variable. Data shown are colony means (± S.E.) and *n* indicates the number of colonies per treatment group. Test statistics are from individual ANOVAS for the variable in each row. The overall MANOVA was significant (see Results for details)

				ANOVA test statistics (including all colonies)							
				Pesticide treatment				Number of workers at start			
Dependent Variable	Control colonies Mean (± S.E.) $n = 11$	Pesticide colonies Mean (± S.E.) $n = 13$	Trend	F	d.f.	Error d.f.	P	F	d.f.	Error d.f.	P
Queen longevity (days from treatment start)‡	59 (± 5)	50 (± 6)	–	2·465	1	21	0·131	1·656	1	21	0·212
Number of workers produced†	196 (± 35)	165 (± 33)	–	1·517	1	21	0·232	3·798	1	21	0·065
Number of males produced	207 (± 47)	239 (± 49)	+	0·035	1	21	0·854	9·413	1	21	**0·006****
Average dry mass of males (g)	0·109 (± 0·008)	0·124 (± 0·005)	+	2·085	1	21	0·163	0·294	1	21	0·593
Total dry mass of sexual offspring (g)	28·057 (± 7·296)	31·457 (± 7·162)	+	0·035	1	21	0·853	5·289	1	21	**0·032***
Worker mortalities†	57 (± 13)	70 (± 16)	–	0·084	1	21	0·775	8·024	1	21	**0·010***

†Data were $\log_{10}$-transformed.
‡Data were transformed with a reciprocal transformation prior to analysis. 'Trend' indicates whether the pesticide treatment had a negative or positive (but not necessarily significant) effect on each variable.
Significant *P*-values are shown in bold: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

Table 3. Gyne production data from *B. terrestris* colonies treated with either the pesticide λ-cyhalothrin or a control solution. The bootstrapping column shows the significance and confidence intervals after bootstrapping the data 1000 times. 'Trend' indicates whether the pesticide treatment had a negative or positive (but not necessarily significant) effect on each variable

				Bootstrapping		
					95% Confidence Intervals	
Dependent Variable	Control colonies Mean (± S.E.)	Pesticide colonies Mean (± S.E.)	Trend	P	Lower	Upper
Number of gynes produced	9 (± 7) $n = 11$	1 (± 1) $n = 13$	–	0·380	−25·143	1·408
Average dry mass of gynes (g)	0·302 (± 0·030)	0·240 (± 0·041)	–	0·181	−0·271	0·014
Total dry mass of gynes (g)	8·951 (± 6·480)	1·285 (± 0·689)	–	0·422	−33·882	1·739

bumblebee colonies are able to compensate under such conditions (e.g. Müller & Schmid-Hempel 1992). However, a reduction in worker size is likely to have impacts on colony productivity in the field. Larger workers have greater visual acuity (Spaethe & Chittka 2003), higher antennal sensitivity (Spaethe *et al.* 2007), are better able to fly under lower light conditions (Kapustjanskij *et al.* 2007), and are more efficient foragers (Goulson *et al.* 2002; Spaethe & Weidenmüller 2002). Consequently, a colony producing smaller workers may be less able to collect sufficient food resources, which will impact on the production of sexual offspring, and make the colony more vulnerable to the costs associated with an energy shortfall (Cartar & Dill 1991).

The mechanism underlying the reduced mass of workers produced by λ-cyhalothrin-treated colonies is unknown, but could be due to differences in larval feeding. In bumblebees the size of an adult worker is determined by how much it is fed during development (Sutcliffe & Plowright 1988), and so a difference in larval feeding between treatment groups might account for the difference in adult worker mass. The results of our pilot study (Appendix S2 and Table S1, Supporting information) indicate that *B. terrestris* workers readily forage on λ-cyhalothrin-treated pollen and feed it to larvae. Furthermore, there was no significant effect of pesticide treatment on pollen consumption by colonies, indicating that if reduced feeding of larvae occurred, it was not due to any repellent or antifeedant effect of the pesticide. Previous research has identified behavioural changes in worker honeybees and bumblebees after exposure to a range of doses of pesticides (Gill, Ramos-Rodriguez & Raine 2012; Henry *et al.* 2012; Schneider *et al.* 2012) suggesting we could also see behavioural changes relating to within nest tasks, like brood care, potentially resulting in reduced larval feeding by workers. Interestingly, the mass of males and gynes produced during the current experiment was not significantly affected by the pesticide treatment, possibly

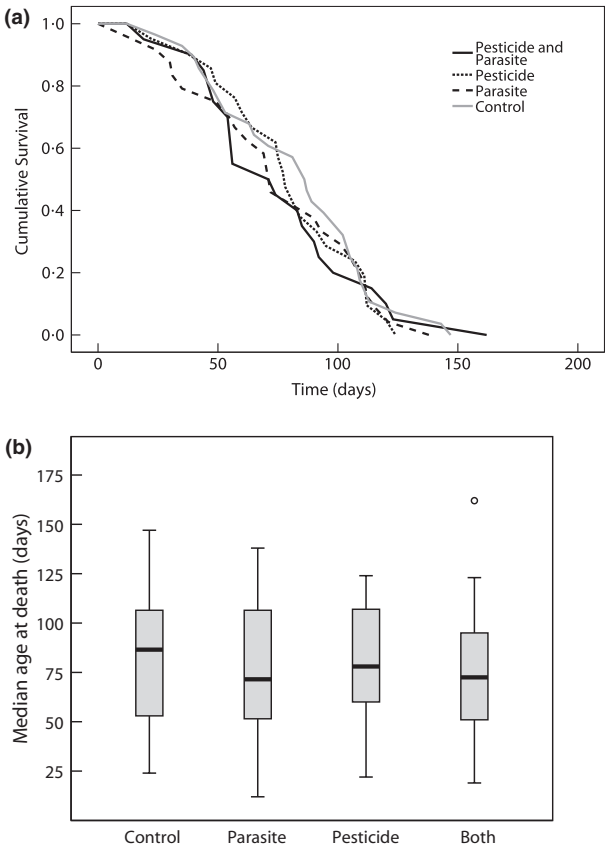

Fig. 2. The cumulative survival (a) and median age at death (b) of *Bombus terrestris* workers exposed to a pesticide (λ-cyhalothrin), a parasite (*Crithidia bombi*), both pesticide and parasite, or neither (control). In the box and whisker plots, the thick horizontal bar is the colony median, the top and bottom of the box indicate the first and third quartile, and the whiskers show the minimum and maximum values.

suggesting that the pesticide had a stronger effect earlier in colony development, when most larvae developed into workers. The ratio of workers to brood is lower earlier in the colony cycle (Duchateau & Velthuis 1988), and so male and gyne larvae could have been buffered from any pesticide induced reduction in larval feeding, as there would have been more workers available for brood care.

Gill, Ramos-Rodriguez & Raine (2012) found that some impacts of pesticide exposure on bumblebee colonies only became apparent several weeks after exposure began, highlighting a need for longer-term studies into chronic exposure to pesticides (EFSA 2012). However, the profile of pesticide exposure bees experience in the field remains unknown. Lambda-cyhalothrin is applied to a wide range of crops in the spring and summer (Garthwaite *et al.* 2012a,b), on several of which bumblebees are known to forage (Thompson & Hunt 1999). Bumblebees are likely to be exposed to this pesticide on a range of crops which flower at different times. There is a paucity of data on how compounds such as λ-cyhalothrin persist in floral tissue such as pollen, which makes it difficult to predict how long bee colonies may be exposed to residues. Furthermore, it is unknown whether bumblebees will actually take contaminated pollen back to the colony – acute effects of the pesticide may cause death of workers in the field. However, this compound has been detected in stored pollen in honeybee hives (Mullin *et al.* 2010) and pollen collected from foraging honeybees (Choudhary & Sharma 2008), showing that honeybees collect pyrethroid contaminated pollen and may subsequently be exposed to residues in the hive for some time. In addition, our data show that bumblebee workers will collect pollen treated with pesticide at the dose provided in our experiment with no significant impact on mortality. Individual crops can be treated up to four times during flowering (Syngenta Crop Protection UK, 2011), and it is likely that different crops will be sprayed at different times dependent on the pest being targeted. Consequently, the 14-week exposure period used in this study explores a potential worst-case scenario. Interestingly, the significant effect of pesticide exposure (a 16% reduction in worker mass) occurred during the first 5–6 weeks of the experiment. Not only does this correspond to an ecologically realistic timeline, it coincided with one of the most vulnerable stages of colony development. This suggests that assessments of colony-level impacts should match field-relevant pesticide exposure with appropriate developmental stages of the focal species' life cycle.

Despite the extensive period of exposure in our experiment, the impacts on colony development and reproductive output under laboratory conditions were minimal. However, interpretation of the effect size and confidence intervals for the variables measured in this study (Fig. S2 and Appendix S5, Supporting information) suggest that larger sample sizes may be required to fully understand any impacts of λ-cyhalothrin exposure on some aspects of colony development (e.g. worker mortality) and reproductive output of colonies. In addition, our study only takes into account pesticide exposure of bees and brood within the colony via contaminated food resources. There is also a chance that foraging bees may encounter pyrethroids at higher doses outside the colony, for example if they are sprayed during pesticide application, and these impacts should be taken into account when considering the potential risks of pyrethroid use to wild bees.

In order to fully understand the pesticide impacts on beneficial arthropods in the wild, it is crucial to understand how pesticides interact with other stressors such as parasites. This is the first study to address this question in bumblebees using a pyrethroid pesticide. We found no effect of pesticide treatment during larval development on the susceptibility of adult workers to *C. bombi* infection, or on the intensity of infection. Larval exposure of workers to λ-cyhalothrin did not have an impact on adult survival even under subsequent challenge with *C. bombi*. Individuals in this study were provided with *ad libitum* food, and different results may be found if individuals are placed under nutritional stress (Brown, Loosli & Schmid-Hempel 2000). Additionally, there was no impact of larval

λ-cyhalothrin exposure on male survival. Previous studies on honeybees have found that several pesticides interact synergistically with *N. ceranae* resulting in an increased worker mortality (Alaux *et al.* 2010; Vidau *et al.* 2011; Aufauvre *et al.* 2012), although these studies exposed adult workers directly to an acute dose of pesticide. Given the differential susceptibility of bumblebees and honeybees to pesticides and differences in parasite virulence, our results suggest that the simple extrapolation of studies across taxa, across stressors or between exposure scenarios is unwarranted.

The growing evidence that neonicotinoid pesticides have a detrimental impact on bumblebees (Cresswell *et al.* 2012; Gill, Ramos-Rodriguez & Raine 2012; Laycock *et al.* 2012; Whitehorn *et al.* 2012; Bryden *et al.* 2013) and other non-target organisms (Goulson 2013), and the recent moratorium on the use of three major neonicotinoid pesticides in Europe is likely to result in an increase in demand for alternative crop protection products such as pyrethroids. If this shift in pesticide usage is to take place, it is important that we understand potential impacts on essential wild pollinators. Our study shows that field research into the exposure profile and impacts on vulnerable life stages of these pollinators is urgently needed. Such studies should inform risk assessments and policy guidelines for the future application and usage of pesticides.

Acknowledgements

We thank Lisa Evans, Matthias Fürst, Dave Garthwaite, Richard Gill, Andrew Jackson, Ainsley Jones, Catherine Jones, Tammy Mak Tin-Mei, Inti Pedroso, Oscar Ramos-Rodriguez and Karen Smith for comments and technical assistance, The Crown Estate for permission to collect wild bumblebees at Windsor Great Park, Syngenta Bioline Bees for supplying colonies and the Editor and three anonymous reviewers for additional comments. The study was funded jointly by two Grants (BB/I000178/1 and BB/1000151/1) from BBSRC, Defra, NERC, the Scottish Government and the Wellcome Trust, under the Insect Pollinators Initiative, and a NERC studentship to GB.

Author Contributions

GB carried out the experiment and statistical analyses; GB, MJFB and NER designed the experiment and wrote the paper, and MJFB and NER conceived the project.

References

Alaux, C., Brunet, J.-L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M. *et al.* (2010) Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environmental Microbiology*, **12**, 774–782.

Aufauvre, J., Biron, D.G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M. *et al.* (2012) Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee. *Scientific Reports*, **2**, 326.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T. *et al.* (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351–354.

Breeze, T.D., Bailey, A.P., Balcombe, K.G. & Potts, S.G. (2011) Pollination services in the UK: how important are honeybees? *Agriculture Ecosystems & Environment*, **142**, 137–143.

Brown, M.J.F., Loosli, R. & Schmid-Hempel, P. (2000) Condition dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos*, **91**, 421–427.

Brown, M.J.F. & Paxton, R.J. (2009) The conservation of bees: a global perspective. *Apidologie*, **40**, 410–416.

Brown, M.J.F., Schmid-Hempel, R. & Schmid-Hempel, P. (2003) Strong context-dependent virulence in a host-parasite system: reconciling genetic evidence with theory. *Journal of Animal Ecology*, **72**, 994–1002.

Bryden, J., Gill, R.J., Mitton, R.A.A., Raine, N.E. & Jansen, V.A.A. (2013) Chronic sublethal stress causes bee colony failure. *Ecology Letters*, **16**, 1463–1469.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F. & Griswold, T.L. (2011) Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 662–667.

Cartar, R.V. & Dill, L.M. (1991) Costs of energy shortfall for bumble bee colonies: predation, social parasitism, and brood development. *Canadian Entomologist*, **123**, 283–293.

Carvell, C., Roy, D.B., Smart, S.M., Pywell, R.F., Preston, C.D. & Goulson, D. (2006) Declines in forage availability for bumblebees at a national scale. *Biological Conservation*, **132**, 481–489.

Chauzat, M.P., Carpentier, P., Martel, A.C., Bougeard, S., Cougoule, N., Porta, P. *et al.* (2009) Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. *Environmental Entomology*, **38**, 514–523.

Choudhary, A. & Sharma, D.C. (2008) Dynamics of pesticide residues in nectar and pollen of mustard (*Brassica juncea* (L.) Czern.) grown in Himachal Pradesh (india). *Environmental Monitoring and Assessment*, **144**, 143–150.

Cole, R.J. (1970) Application of triangulation method to purification of *Nosema* spores from insect tissues. *Journal of Invertebrate Pathology*, **15**, 193–195.

Colla, S.R., Otterstatter, M.C., Gegear, R.J. & Thomson, J.D. (2006) Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biological Conservation*, **129**, 461–467.

Coors, A., Decaestecker, E., Jansen, M. & De Meester, L. (2008) Pesticide exposure strongly enhances parasite virulence in an invertebrate host model. *Oikos*, **117**, 1840–1846.

Corbet, S.A., Williams, I.H. & Osborne, J.L. (1991) Bees and the pollination of crops and wild flowers in the European community. *Bee World*, **72**, 47–59.

Cresswell, J.E. (2011) A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology*, **20**, 149–157.

Cresswell, J.E., Page, C.J., Uygun, M.B., Holmbergh, M., Li, Y., Wheeler, J.G., Laycock, I., Pook, C.J. & Hempel de Ibarra, N. (2012) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). *Zoology*, **115**, 365–371.

Dicks, L.V., Abrahams, A., Atkinson, J., Biesmeijer, J., Bourn, N., Brown, C. *et al.* (2013) Identifying key knowledge needs for evidence-based conservation of wild insect pollinators: a collaborative cross-sectoral exercise. *Insect Conservation and Diversity*, **6**, 435–446.

Duchateau, M.J. & Velthuis, H. (1988) Development and reproductive strategies in *Bombus terrestris* colonies. *Behaviour*, **107**, 186–207.

Durrer, S. & Schmid-Hempel, P. (1994) Shared use of flowers leads to horizontal pathogen transmission. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **258**, 299–302.

EFSA Panel on Plant Protection Products and their Residues (PPR) (2012) Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). *EFSA Journal*, **10**, 2668.

vanEngelsdorp, D., Hayes, J., Underwood, R.M. & Pettis, J. (2008) A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *PLoS ONE*, **3**, e4071.

Fauser-Misslin, A., Sadd, B.M., Neumann, P. & Sandrock, C. (2014) Influence of combined pesticide and parasite exposure on bumblebee colony traits in the laboratory. *Journal of Applied Ecology*. Doi:0.1111/1365-2664.12188.

FERA (2012) *Pesticide Usage Survey Statistics*. The Food and Environment Research Agency, York. Retrieved from https://secure.fera.defra.gov.uk/pusstats on 28/2/13.

Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A. *et al.* (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, **339**, 1608–1611.

Garthwaite, D.G., Barker, I., Parrish, G., Smith, L., Chippindale, C. & Pietravalle, S. (2010) *Arable crops in the United Kingdom 2010*. Pesticide Usage Survey Reports, DEFRA, 235.

Garthwaite, D.G., Hudson, S., Barker, I., Parrish, G., Smith, L. & Pietravalle, S. (2012a) *Arable crops in the United Kingdom*. Pesticide Usage Survey Reports, DEFRA, 250.

Garthwaite, D.G., Hudson, S., Barker, I., Parrish, G., Smith, L. & Pietravalle, S. (2012b) *Soft fruit in the United Kingdom 2012*. Pesticide Usage Survey Reports, DEFRA, 251.

Gill, R.J., Ramos-Rodriguez, O. & Raine, N.E. (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature*, **491**, 105–108.

Goulson, D. (2013) An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, **50**, 977–987.

Goulson, D., Peat, J., Stout, J.C., Tucker, J., Darvill, B., Derwent, L.C. & Hughes, W.O.H. (2002) Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Animal Behaviour*, **64**, 123–130.

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J.F., Aupinel, P., Aptel, J., Tchamitchan, S. & Decourtye, A. (2012) A common pesticide decreases foraging success and survival in honey bees. *Science*, **336**, 348–350.

Imhoof, B. & Schmid-Hempel, P. (1998) Patterns of local adaptation of a protozoan parasite to its bumblebee host. *Oikos*, **82**, 59–65.

Johnson, R.M., Ellis, M.D., Mullin, C.A. & Frazier, M. (2010) Pesticides and honey bee toxicity - USA. *Apidologie*, **41**, 312–331.

Kapustjanskij, A., Streinzer, M., Paulus, H.F. & Spaethe, J. (2007) Bigger is better: implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. *Functional Ecology*, **21**, 1130–1136.

Kiesecker, J.M. (2002) Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 9900–9904.

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

Knight, M.E., Osborne, J.L., Sanderson, R.A., Hale, R.J., Martin, A.P. & Goulson, D. (2009) Bumblebee nest density and the scale of available forage in arable landscapes. *Insect Conservation and Diversity*, **2**, 116–124.

Laycock, I., Lenthall, K.M., Barratt, A.T. & Cresswell, J.E. (2012) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology*, **21**, 1937–1945.

Logan, A., Ruiz-González, M.X. & Brown, M.J.F. (2005) The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. *Parasitology*, **130**, 637–642.

Lopez-Vaamonde, C., Raine, N.E., Koning, J.W., Brown, R.M., Pereboom, J.J.M., Ings, T.C., Ramos-Rodriguez, O., Jordan, W.C. & Bourke, A.F.G. (2009) Lifetime reproductive success and longevity of queens in an annual social insect. *Journal of Evolutionary Biology*, **22**, 983–996.

Meeus, I., Brown, M.J.F., De Graaf, D.C. & Smagghe, G. (2011) Effects of invasive parasites on bumble bee declines. *Conservation Biology*, **25**, 662–671.

Müller, C.B. & Schmid-Hempel, P. (1992) Variation in life-history pattern in relation to worker mortality in the bumblebee, *Bombus lucorum*. *Functional Ecology*, **6**, 48–56.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simons, R., van Engelsdorp, D. & Pettis, J.S. (2010) High levels of miticides and agrochemicals in north american apiaries: implications for honey bee health. *PLoS ONE*, **5**, e9754.

Ollerton, J., Winfree, R. & Tarrant, S. (2011) How many flowering plants are pollinated by animals? *Oikos*, **120**, 321–326.

Osborne, J.L., Williams, I.H. & Corbet, S.A. (1991) Bees, pollination and habitat change in the European community. *Bee World*, **72**, 99–116.

Pettis, J., van Engelsdorp, D., Johnson, J. & Dively, G. (2012) Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften*, **99**, 153–158.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010a) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, **25**, 345–353.

Potts, S.G., Roberts, S.P.M., Dean, R., Marris, G., Brown, M.A., Jones, R., Neumann, P. & Settele, J. (2010b) Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research*, **49**, 15–22.

Ruiz-González, M.X. & Brown, M.J.F. (2006) Honey bee and bumblebee trypanosomatids: specificity and potential for transmission. *Ecological Entomology*, **31**, 616–622.

Ruiz-González, M.X., Bryden, J., Moret, Y., Reber-Funk, C., Schmid-Hempel, P. & Brown, M.J.F. (2012) Dynamic transmission, host quality, and population structure in a multihost parasite of bumblebees. *Evolution*, **66**, 3053–3066.

Schmid-Hempel, P. (1998) *Parasites in Social Insects*. Princeton University Press, Princeton, New Jersey.

Schneider, C.W., Tautz, J., Grünewald, B. & Fuchs, S. (2012) RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS ONE*, **7**, e30023.

Shykoff, J.A. & Schmid-Hempel, P. (1991) Incidence and effects of four parasites in natural-populations of bumble bees in Switzerland. *Apidologie*, **22**, 117–125.

Spaethe, J. & Chittka, L. (2003) Interindividual variation of eye optics and single object resolution in bumblebees. *Journal of Experimental Biology*, **206**, 3447–3453.

Spaethe, J. & Weidenmüller, A. (2002) Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insectes Sociaux*, **49**, 142–146.

Spaethe, J., Brockmann, A., Halbig, C. & Tautz, J. (2007) Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften*, **94**, 733–739.

Stout, J.C. & Morales, C.L. (2009) Ecological impacts of invasive alien species on bees. *Apidologie*, **40**, 388–409.

Sutcliffe, G.H. & Plowright, R.C. (1988) The effects of food-supply on adult size in the bumble bee *Bombus terricola* Kirby (Hymenoptera, Apidae). *Canadian Entomologist*, **120**, 1051–1058.

Syngenta Crop Protection UK (2011) *Hallmark with zeon technology, product label*. Syngenta Crop Protection, UK.

Thompson, H.M. (2001) Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie*, **32**, 305–321.

Thompson, H.M. & Hunt, L.V. (1999) Extrapolating from honeybees to bumblebees in pesticide risk assessment. *Ecotoxicology*, **8**, 147–166.

Thomson, D. (2004) Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology*, **85**, 458–470.

Vanbergen, A.J., Baude, M., Biesmeijer, J.C., Britton, N.F., Brown, M.J.F., Brown, M. *et al.* (2013) Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, **11**, 251–259.

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Vigues, B., Brunet, J.-L. *et al.* (2011) Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS ONE*, **6**, e21550.

Westerkamp, C. & Gottsberger, G. (2000) Diversity pays in crop pollination. *Crop Science*, **40**, 1209–1222.

Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2003) Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters*, **6**, 961–965.

Whitehorn, P.R., O'Connor, S., Wackers, F.L. & Goulson, D. (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, **336**, 351–352.

Williams, P.H. (1982) The distribution and decline of British bumble bees (*Bombus* Latr.). *Journal of Apicultural Research*, **21**, 236–245.

Williams, P.H. (1986) Environmental-change and the distributions of British bumble bees (*Bombus* Latr.). *Bee World*, **67**, 50–61.

Williams, P.H. & Osborne, J.L. (2009) Bumblebee vulnerability and conservation world-wide. *Apidologie*, **40**, 367–387.

Yourth, C.P., Brown, M.J.F. & Schmid-Hempel, P. (2008) Effects of natal and novel *Crithidia bombi* (Trypanosomatidae) infections on *Bombus terrestris* hosts. *Insectes Sociaux*, **55**, 86–90.

Yourth, C.P. & Schmid-Hempel, P. (2006) Serial passage of the parasite *Crithidia bombi* within a colony of its host, *Bombus terrestris*, reduces success in unrelated hosts. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 655–659.

Received 3 April 2013; accepted 11 December 2013
Handling Editor: Juliet Osborne

Supporting Information

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

Appendix S1. Pesticide residue analysis of pollen samples.

Appendix S2. Pilot study to assess the foraging and larval feeding by workers provided with λ-cyhalothrin-treated pollen.

Appendix S3. Explanation of subsampling procedure for measuring the average mass of workers.

Appendix S4. Methods for data analysis

Appendix S5. Discussion of power analysis of colony development data.

Appendix S6. Infection success and intensity after exposure of *B. terrestris* workers to *C. bombi*.

Fig. S1. Mean worker mass estimates from random data samples.

Fig. S2. Percentage effect size (± 95% C. I.) of variables measured in λ-cyhalothrin-treated and control-treated *B. terrestris* colonies.

Table S1. Summary of observational data from two microcolonies of *B. terrestris* containing brood and ten workers, after provision of λ-cyhalothrin-treated pollen and λ-cyhalothrin-untreated pollen.

Table S2. Numbers of workers and males from either λ-cyhalothrin-treated colonies or control-treated colonies which were removed from their colonies and included in a survival experiment. Workers were either infected with the parasite *Crithidia bombi*, or uninfected.

Table S3. The timing of key events in colony development measured in *B. terrestris* colonies treated with either the pesticide λ-cyhalothrin or a control solution.

